4 Liquid-phase Microextraction Techniques

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4.1 Introduction

4.1.1 History

Every analytical chemist knows that “the best sample preparation is the one that does not exist”, however, it is considered a utopia because samples usually need to be adapted to the measurement instrument. Sample preparation has always been considered the Achilles heel of the analytical procedure due to its drawbacks such as tediousness, high degree of manipulation, risk of losses and contamination, the employment of large amounts of sample, solvents and sorbents, and therefore, generation of large amounts of wastes. For this reason, many efforts in recent decades have been focused on the reduction of this negative impact over the analytical procedure.

Sample preparation includes extraction and/or preconcentration of the target compound, interferences separation from the target compound and other operations such as derivatization or dilution. Nowadays, there are many sample preparation strategies available for these purposes, with liquid-liquid extraction (LLE) and solid-phase extraction (SPE) being the most commonly employed techniques for many years. However, these classical techniques present numerous disadvantages. For example, LLE requires large volumes of toxic organic solvents and samples, involves high degrees of sample manipulation such as glassware transfers, provides limited enrichment factors (EFs) and utilizes tedious procedures. On the other hand, SPE is also a tedious procedure, sorbents are limited and although the volume of toxic organic solvents is reduced, those employed in the procedure are still essential. For the reasons described above, these techniques have been replaced in the last two decades by their miniaturized techniques, maintaining their advantages and reducing or eliminating the drawbacks. Solid-phase microextraction (SPME) is the miniaturized technique of SPE developed by Pawliszyn in 1990 (Arthur & Pawliszyn, 1990). Otherwise, liquid-phase microextraction (LPME) or solvent microextraction (SME) are the given names to the miniaturized LLE technique introduced in the mid-1990s (S. Liu & Dasgupta, 1995; H. Liu & Dasgupta 1996; Jeannot & Cantwell, 1996). The great success of both techniques in the last two decades may be attributed to their fundamental properties, which have helped to overcome the shortcoming of sample preparation. In addition, microextraction techniques cover most of the twelve prin-
ciples of green analytical chemistry (GAC) (Gąłuszka et al., 2013) and have helped to recognize the importance of extraction technology on the generation of quality analytical information (Raynie, 2006).

LPME can be defined as a miniaturization of LLE technique where the volume of the extractant phase is equal or below 100 μL (Kokosa, 2013). The main advantages of LPME techniques are the rapidity and easiness, low cost, low sample volume, extremely low or even no solvent consumption, reduced generation of wastes, high EFs and its affordability to any laboratory. Numerous developments and applications about LPME techniques have appeared in the last two decades as reflected in the excellent book (Kokosa, Przyjazny, & Jeannot, 2009) and numerous reviews published on this topic (Psillakis & Kalogerakis, 2002, 2003; Raynie, 2004, 2006; Pawlisyn & Pedersen-Bjergaard, 2006; L. Xu et al., 2007; Nerín et al., 2009; Jeannot et al., 2010; Pena-Pereira et al., 2010b, 2011; Sarafraz-Yazdi & Amiri, 2010; Jain & Verma, 2011; Ramos, 2012; Andruch et al., 2013b; Kocúrová et al., 2013; Kokosa, 2013; Szreńiawa-Sztajnert et al., 2013; Spietelun et al., 2014).

Most of the scientific literature gives the year 1996 as the starting point of LPME, however, the history of LPME really starts in 1995 when S. Liu and Dasgupta (1995) introduced the first droplet-based analysis systems. This system proposed the exposition of a microdrop at the end of a capillary tube to collect NH$_3$ and SO$_2$ contained in gaseous samples. The same year, Cardoso and Dasgupta (1995) substituted the spherical drop with a liquid film (14–57 μL) to determine NO$_2$. One year later, Liu and Dasgupta develop the well known drop-in-drop system, where a droplet of chloroform (1.3 μL) is suspended inside a bigger aqueous drop (H. Liu & Dasgupta, 1996). At the same time, Jeannot and Cantwell (1996) introduced another miniaturized system where a small drop (8 μL) of a water-immiscible organic solvent is located at the end of a Polytetrafluoroethylene (PTFE) rod which is immersed in a stirred aqueous sample solution. After the extraction, the organic phase is sampled with a microsyringe and injected into the gas chromatograph for quantification. Since the use of the PTFE rod presented some inconvenience, the same authors described in 1997 the use of a microsyringe not only as a holder of a microdrop, but also for injection into the analytical instrument (Jeannot & Cantwell, 1997). At this point, this configuration is known as single-drop microextraction (SDME) technique. The same year, He and Lee introduce the terms ‘static’ and ‘dynamic’ for LPME techniques (He & Lee, 1997). Both systems employ a conventional microsyringe. The term static LPME is given to the technique developed by Jeannot and Cantwell (Jeannot & Cantwell, 1997). Dynamic LPME changes the exposition of the droplet to the sample by introducing the sample into the syringe, which contains the extractant phase. The microsyringe is used as a microseparation funnel for extraction as well as for injection into the analytical system. The sample is introduced in the microsyringe for a controlled time, and then discharged to the sample. This procedure is repeated many times and provides better EFs, but relatively poorer precision (12.8%) due to manual operation. For this reason, Myung et al. (2003) proposed an automated procedure.
To date, two-phase systems (i.e., aqueous sample and organic extractant phase) had been considered; however, Ma and Cantwell proposed a three-phase system where the final extractant phase is an aqueous droplet (Ma & Cantwell, 1998, 1999). The configuration commonly termed liquid-liquid-liquid microextraction (LLLME) employed a 30 µL \textit{n}-octane liquid membrane confined inside a small PTFE ring, layered over 1.60 mL of aqueous sample buffered at pH = 13. The acceptor phase was a 1 µL aqueous drop buffered at pH = 2.1 which was suspended in the 30 µL membrane directly from the tip of a microsyringe needle (Ma & Cantwell, 1999).

Since the use of a droplet caused some inconvenience (e.g., instability), Pedersen-Bjergaard and Rasmussen integrated the basic principle of a supported liquid membrane (SLM) into a simple extraction unit for LLLME using a polypropylene porous hollow fiber (HF) to protect the extractant phase (Pedersen-Bjergaard & Rasmussen, 1999). They presented the three-phase system where the pores of the fiber were impregnated with an organic solvent and the lumen contained an acceptor aqueous solution. The two-phase system was developed one year later (Rasmussen et al., 2000) and an electric field has been used to assist the extraction (Pedersen-Bjergaard & Rasmussen, 2006), leading to a technique known as electromembrane extraction (EME).

In 2000, Liu and Lee (2000) introduced the continuous-flow microextraction (CFME) mode where an organic drop (1–5 µL) is held at the outlet tip of a polyetheretherketone (PEEK) connecting tubing which is immersed in a continuously flowing sample solution and acts as the fluid delivery duct and as a solvent holder. Once the extraction is finished, the organic drop is withdrawn by a microsyringe and injected into the analytical instrument.

Until now, the developed LPME systems had maintained the droplet or film of extractant phase (i.e., organic or aqueous) in direct contact with the sample or through a SLM. The direct contact presents more instability problems due to the stirring, solvent solubility or dirty matrices. For this reason, Theis et al. (2001) and Tankeviciute et al. (2001) proposed in 2001 the headspace SDME (HS-SDME) mode where the droplet is exposed in the headspace above the sample. This mode allows the extraction of volatile and semi-volatile analytes contained in complex and dirty matrices while avoiding instability problems.

In 2004, Jiang and Lee (2004) proposed a modification of HF-LPME termed solvent-bar microextraction (SBME). This extraction mode fills the lumen of a short piece of a porous HF with organic solvent and seals the HF at both ends. The solvent bar is introduced into the sample solution together with a stirring bar and after extraction for a prescribed period of time the solvent bar is taken out and the extractant phase analyzed. A three-phase system was proposed by Melwanki and Huang (2006).

Since SDME and HF-LPME presented some drawbacks such as drop instability, slow kinetics and the use of holders (i.e., microsyringes, hollow fibers, capillaries), Rezaee et al. (2006) and Yangcheng et al. (2006) proposed the elimination of any holder, disposing the extractant phase (i.e., organic solvent) directly into the sample. These modalities of extraction were named dispersive liquid-liquid microextrac-
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Liquid-phase microextraction (LPME) involves techniques such as dynamic LPME (DLLME) (Rezaee et al., 2006) and directly suspended droplet microextraction (DSDME) (Yangcheng et al., 2006). The former technique is based on a ternary component solvent system where a dispersing agent is used to disperse the extractant phase, infinitely increasing the surface area of the interface. A cloudy solution is formed and the phase separation is carried out by centrifugation. This technique provides more simplicity of operation, decreased equilibration time, higher recovery and therefore, higher EFs. All these advantages make DLLME the most and widely used technique today. On the other hand, the DSDME technique avoids the use of the disperser agent and the drop maintains its integrity along the extraction procedure. After the extraction, the droplet, floating on the water surface, is removed with a syringe for analysis. The difficulty on the manipulation of the floating droplet promotes the development of another technique named solidification of floating organic drop microextraction (SFODME) (Khalili Zanjani et al., 2007). The difference with DSDME is that this technique employs an ice bath for 5 min to solidify the floating drop once the extraction is finished; meanwhile the sample maintains its liquid state. The solidified solvent is transferred into a conical vial where it melts immediately before analysis.

The timeline of the LPME techniques is shown in Figure 4.1. Since its appearance, LPME techniques have undergone important modifications where different solvents (i.e., ionic liquids, surfactants, etc.), dispersion modes (i.e., ultrasound (US) energy, vortex, etc.), energy and radiation (i.e., US, microwave, vortex, etc.), analytical detection systems or automated procedures have been employed. These modifications carry out the appearance of new names with new acronyms when each scientist tends to vary a general method (Kokosa, 2013). This turns into a negative fact because the LPME literature contains a confusing array of more than 100 acronyms (Kokosa, 2013). Hence, classifying all the developed methods in a few groups turns up a difficult task. Therefore, in this chapter LPME techniques have been classified in three main groups (Figure 4.2): (i) Single-drop microextraction (SDME); (ii) hollow fiber liquid-phase microextraction (HF-LPME); and (iii) dispersive liquid-liquid microextraction (DLLME). It is important to point out that some techniques that employ a
film of extractant phase (i.e., LLLME or dynamic-LPME) have been included in the SDME group since these are not appropriate to be included in the HF-LPME or DLLME groups.

4.1.2 Solvents

Organic solvents and aqueous solutions had been the main extractant phases until 2003, when ionic liquids (ILs) were proposed as extractant phases in SDME technique (Liu et al., 2003). ILs are a group of organic salts with melting points below 100 °C. They possess unique properties such as their immeasurable low vapor pressure, good chemical and thermal stability, nonflammability, high ionic conductivity, and a wide electrochemical potential window. These properties make them widely used in every chemistry field. However, the negligible volatility and the adjustable hydrophobicity, polarity and selectivity propose them as excellent “green” solvents to replace harmful organic solvents in extraction and separation techniques. It is important to remark that some studies about the “green” aspect of ILs have proven their toxicity in aqueous media (Pham et al., 2007). For this reason, more criticism should be shown when the green aspect of a new developed method/technique that uses ILs is highlighted. However, the use of ILs in LPME techniques allows bigger and more stable droplets and higher stirring speeds, what results in higher extraction efficiency (EE) and therefore, better analytical parameters. In addition, ILs are also called “designer” solvents due to the $10^{18}$ possible combinations (Carmichael & Seddon, 2000), what
provides ILs with different properties designed for many applications. These different properties enable the extraction of organic compounds, both polar and non-polar, as well as metals. From an analytical point of view, the use of ILs in extraction techniques provides more pros than cons but additional larger studies related to toxicity are needed before firm conclusions can be drawn. Since 2003, the number of publications related to ILs and LPME has increased exponentially. This fact is reflected in the numerous and excellent reviews recently published (Koel, 2005; Liu et al., 2005; Zhao et al., 2005; Pandey, 2006; Han & Armstrong, 2007; R. Liu et al., 2009; Soukup-Hein et al., 2009; Aguilera-Herrador et al., 2010; Poole & Poole, 2010; Sun & Armstrong, 2010; Zhao & Anderson, 2011; Joshi & Anderson, 2012; Patel & Lee, 2012; Tan et al., 2012; Vičkačkaitė & Padarauskas, 2012; Escudero et al., 2013; Kokosa, 2013). In addition, the use of ILs as extractant phases in LPME has helped not only to overcome problems associated with LPME techniques using organic solvents (i.e., instability, volatility and irreproducibility) and has allowed researchers to extract a wider range of analytes (Aguilera-Herrador et al., 2010), but also has enabled the development of new methods, including temperature-controlled IL dispersive liquid-liquid microextraction (TC-IL-DLLME) (Zhou et al., 2008), and in situ IL-DLLME (Baghdadi & Shemirani, 2009).

Surfactants, often presented as safer and more economical alternatives to hazardous and expensive organic solvents, have also been used as extractant phases in LPME techniques (Ballesteros-Gómez et al., 2010). The first use of surfactants was introduced by Kanyanee et al. who proposed soap bubbles of Triton-X 100 to extract \( \text{SO}_2 \) (Kanyanee et al., 2006). However, the terms surfactant and LPME technique were not jointed until 2008 when López-Jiménez et al. (2008) employed coacervates as the extractant phase in SDME. Coacervates made of surfactant aggregates were first used as solvents and proposed for the extraction and concentration of chlorophenols prior to liquid chromatography (LC). Since then, many other applications have appeared and three reviews can be found in the literature (Ballesteros-Gómez et al., 2010; Yazdi, 2011; Moradi & Yamini, 2012b). As the authors claim, these solvents are a valuable strategy to extend the applicability of LPME to areas where the use of conventional water immiscible organic solvents is less effective or not adequate, e.g., when LC is used for the separation of analytes or when the extraction of polar or ionic organic compounds is of interest (López-Jiménez et al., 2008). These solvents present the ability to extract a wider range of compounds than organic solvents and with different configurations as aqueous micelles, reverse micelles and vesicles have been tested (Ballesteros-Gómez et al., 2010; Yazdi, 2011; Moradi & Yamini, 2012b).

Water solutions as extractant phases have been employed since the development of the three-phase system (Ma & Cantwell, 1998, 1999). These solutions can be considered as the greenest solvents compared to the others (i.e., ILs, organic solvents), however, their extraction is limited to ionizable compounds. The different pH values between the sample and extractant phase are responsible for the extraction. Analytes should be undissociated in the sample to diffuse towards the organic solvent layer.
and then to the droplet or film, where the pH is adjusted to ionize the analytes, being back-extracted. Although aqueous solutions as extractant films (Ma & Cantwell, 1998) or droplets (Ma & Cantwell, 1999) had been published previously, Zhang et al. later introduced the term headspace water-based LPME (Zhang et al., 2005). In comparison with previous works, a drop of 5 μL of NaOH solution (1 M) was disposed in the headspace to extract five phenols contained in water samples. The analytical instrumentation was capillary electrophoresis (CE) and the authors presented the systems as an entire analytical process that was completely organic solvent-free. However, one year before Fragueiro et al. (2004) had already introduced the use of a Pd(II)-containing aqueous drop to determine arsine. The same research group has extracted methylmercury (Gil et al., 2005) and selenium (Figueroa et al., 2005, 2006) with noble metal-containing aqueous drops.

ILs combined with other reagents or materials, such as surfactants or quantum dots (QDs), have been proposed as extractant phases in SDME. Carrillo-Carrión et al. (2012) proposed the use of [C₆mim][PF₆] IL-modified with CdSe/ZnS QD for the combination of IL-HS-SDME and QD-based fluorimetric detection. Trimethylamine from fish samples was extracted and preconcentrated directly onto a (QD)IL microdrop. Then, the microdrop was transferred to a microcuvette with 300 μL of acetonitrile and the fluorescence was recorded. In another study, Yao et al. (2010) studied micellar ILs as extraction solvents in HS-SDME to perform the extraction of 17 aromatic compounds. Two different micellar solutions were formed by dissolving [C₁₀mim][Br] IL and the traditional surfactant sodium dodecyl sulphate in [C₄mim][Cl] IL. EE increased with the micellar systems compared to the net [C₄mim][Cl].

4.1.3 Separation and Detection Systems

The use of organic solvents in LPME techniques established gas chromatography (GC) coupled to different detectors (e.g., flame ionization detector (FID), electron capture detector (ECD) or mass spectrometry (MS)) as the most suitable analysis system. Afterwards, the use of aqueous solutions as extractant phases extended the use of its homologous LC technique coupled to UV-Vis spectrophotometry or spectrofluorimetry. In addition, the compatibility of ILs and surfactants with LC has increased its number of applications. On the other hand, Fang et al. introduced in 2006 the first use of CE as analytical system for LPME (Fang et al., 2006), and three recent reviews summarize the developments reached with LPME and CE (Arce et al., 2009; Xie & He, 2010; ALOthman et al., 2012).

Apart from the wider range of compounds that are extractable by ILs and surfactants, their compatibility with spectroscopic techniques (e.g., UV-Vis spectrophotometry, spectrofluorimetry, electrothermal atomic absorption spectrometry (ETAAS), flame atomic absorption spectrometry (FAAS), inductively coupled plasma-optical emission spectroscopy (ICP-OES) or ICP-mass spectrometry (ICP-MS)) proposes these
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as the optimum systems for analysis (Pena-Pereira et al., 2010a; Andruch et al., 2012b; Dehghani et al., 2012; Miró & Hansen, 2013). UV-Vis spectrophotometry had been used mainly coupled to a separation technique (e.g., LC), however, the direct measurement of the extractant phase by a UV-Vis spectrophotometer, avoiding a dilution process, was a difficult task until Pena-Pereira et al. (2009a) introduced the cuvetteless microspectrophotometer. This instrument enable the analysis of a 1–2 μL drop held during measurements between a pair of sample pedestals made of stainless steel and quartz fiber by surface tension only. The same research group has also reported the use of a cuvetteless microvolume fluorospectrometer for formaldehyde determination (Sáenz et al., 2011). An excellent review summarized all the developed works that combine SDME and DLLME with UV-Vis and other related detection techniques until 2012 (Andruch et al., 2012b). Microvolume measurements have also been carried out with FAAS after LPME, where a hand-made system was employed for the determination of lead in water samples (Naseri et al., 2008).

The low vapor pressure of ILs limits their direct analysis by GC, and different systems were developed for that purpose (Aguilera-Herrador et al., 2008; Zhao et al., 2008a, 2008b; Chisvert et al., 2009). The coupling of ILs with GC not only improves analytical parameters (i.e., limits of detection), but also avoids the interference of broad organic solvent peaks, allowing the separation and identification of a wide range of analytes with different polarities and boiling points (Aguilera-Herrador et al., 2010). The first system designed by Aguilera-Herrador et al. (2008) in 2008 proposed a removable interface that enabled the introduction of the extracted analytes, contained in IL, into the GC while preventing the IL from entering the column. The system developed by Zhao et al. (2008a) proposed the exposition of the IL drop in the injection port and then it was retracted into the syringe. For this system an improvement in the injection system was required and the upper diameter of the split inlet liner of the GC instrument had to be enlarged (Zhao et al., 2008). Another modification was carried out by the same research group by placing a small glassy tube in the sample-injection part of the GC to avoid the IL entering the column (Zhao et al., 2008). An external option for direct coupling was proposed by Chisvert et al. (2009). The approach was based on thermal desorption of the analytes from the IL droplet to the GC system, by using a robust and commercially-available thermodesorption system. For this purpose, a two-glass-tube concentrically disposed system was designed.

The vast majority of the conventional detection systems (i.e., LC-UV/MS, FAAS, ETAAS, GC-MS or ICP-OES/MS) are slow, expensive and bulky, so analytical instrumentation used for detection has not achieved miniaturization to the same extent as sample preparation methods. For this reason, alternative detection systems have been proposed. Fernández et al. (2014) have presented the use of electrochemical sensors as an attractive detection option for IL-LPME techniques. Screen-printed electrodes (SPELs) (Metters et al., 2011) are inexpensive, mass-produced, disposable devices, which are ideal for low volume sample analysis. The combination of IL-LPME with SPEL for 2,4,6-trinitrotoluene extraction is shown in Figure 4.3. Ahmar et al. also pro-
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posed the use of SPELs after EME (Ahmar et al., 2013, 2014; Fakhari et al., 2014). The research group of Valcárcel (Aguilera-Herrador et al., 2009; Márquez-Sillero et al., 2011) proposed the combination of IL-LPME with ion mobility spectrometry (IMS). IMS is an analytical technique that characterizes molecules by the gas phase mobility of their ions formed at ambient pressure in a weak electric field (Eiceman, 2005). This system suffers from the limitation that liquids cannot be directly injected in IMS detector. Therefore, an injection unit was designed where the IL was trapped and the analytes were volatilized and transported to IMS. On the other hand, a tungsten coil electrothermal atomic absorption spectrometer (W-coil ETAAS) has also been coupled to LPME (Wen et al., 2013a, 2013b). The system enabled the determination of different metals by employing ILs as extractant phases. Due to the viscosity of the ILs, a dilution process with tetrahydrofuran was needed before the deposition on the tungsten coil for analysis.

These alternative detection systems together with the cuvetteless micro-spectrophotometer provide simplicity, portability, sensitivity, fast response and relatively low cost to any LPME methodology.

Figure 4.3 In situ IL-DLLME coupled with screen-printed graphite electrodes. Reprinted from Fernández et al. (2014) with kind permission from Springer Science and Business Media. Copyright (2014) Springer.
4.1.4 Energy and Radiation

Over the past two decades, different energy and radiation sources such as US energy, microwave radiation and vortex agitation have been employed to enhance the efficiency of LPME techniques. Microwave radiation has mainly been applied for heating of the sample in the HS-SDME technique. It is well known that extraction is an exothermic process and heating of the droplet should be prevented to avoid EE decreases. Conventional heating systems are slow and heat the entire system, including the sample, headspace and droplet, meanwhile microwave radiation focuses the heating just on the sample. The developed microwave heating system introduces the sample inside the microwave cavity disposing the droplet, suspended on the tip of the syringe, outside the cavity at room temperature. This system was firstly used by Deng et al. for the distillation and extraction of volatile compounds from Chinese herbs employing a droplet of dodecane (Deng et al., 2007), and by Vidal et al. who used an IL droplet to extract chlorobenzene compounds from water samples (Vidal et al., 2007a). US energy produces a cavitation phenomenon that accelerates mass transfer enhancing EE, therefore, US energy has also been used for treating the sample and maintaining the droplet outside of the ultrasonic system (H. Xu et al., 2007).

In addition, cavitation can be used to produce very fine emulsions from immiscible liquids, which result in very large interfacial contact areas between the liquids. For this reason, US energy has been employed to assess the dispersion of the extractant phase in DLLME techniques. In addition, vortex agitation has been used for the same purpose, and two recent publications review the developments about SME techniques that employ ultrasonic irradiation and vortex agitation (Andruch et al., 2013b; Szreniawa-Sztajnert et al., 2013). The first research about using US energy to assist the dispersion of the extractant phase was published by Huang et al. (Huang et al., 2006). Since then, different modalities of extraction such as ultrasound-assisted emulsification microextraction (USAEME), ultrasound-assisted dispersive liquid-liquid microextraction (UA-DLLME) or vortex-assisted liquid-liquid microextraction (VALLME), among others, have been developed. US energy was also used in HF-LPME technique for the extraction of benzene and toluene in beverage samples (Yang et al., 2010).

4.1.5 Optimization Strategies

The parameters that mainly affect the extraction are stirring speed, extraction time, sample and headspace volumes, pH, ionic strength of the sample, temperature, disperser solvent, centrifugation time and speed, drop volume and extractant solvent (Pena-Pereira et al., 2010a). Two different strategies can be followed to optimize these parameters (Stalikas et al., 2009): (i) step-by-step and (ii) experimental design. The former approach is most commonly used and studies the variation of one parameter while all the others kept constant. This approach is inefficient, since the interac-
tion between the parameters and the statistical significance of the parameters are excluded. Furthermore, it requires a large number of experiments, which leads to higher sample and reagent volumes and longer experiment times. The second strategy has increased in use during recent years due to its numerous advantages over the step-by-step approach. This strategy involves the variation of all the parameters in the different experiments to study their effect upon the extraction and provides information such as interactions between parameters and the statistical significance. In addition, this approach reduces the number of experiments and, therefore, reduces experiment time, effort, the amount of reagents and samples required, energy and costs. For the reasons described above, experimental design should be the unique strategy chosen for optimization following the guidelines of environmental friendly of microextraction techniques according to the 12 principles of GAC (Gałuszka et al., 2013).

The authors would like to highlight that due to the numerous articles in LPME field, applications have generally been excluded, except those that report novel and unique developments.

### 4.2 Single-drop Microextraction

Single-drop microextraction (SDME), as described above, was the first LPME technique developed by S. Liu and Dasgupta (1995). Since then, SDME has undergone different modifications and the system introduced by Jeannot and Cantwell (1997) that employs a conventional GC syringe as a support of the drop and for the injection in the analytical system has been the most widely used. More recent techniques avoid any holder for the drop, disposing it directly into the sample (Khalili Zanjani et al., 2007; Yangcheng et al., 2006). In general terms, SDME employs just a few microliters of solvent as a single drop disposed either to the headspace or directly immersed in the sample. The analytes within the sample partition to the drop and after the extraction, the drop is transferred to the analytical instrument for analysis. Initially, depending on the position of the drop, the SDME technique was defined as: (i) headspace SDME (HS-SDME) (Tankeviciute et al., 2001; Theis et al., 2001) or (ii) direct immersion SDME (DI-SDME) (Jeannot & Cantwell, 1997). However, evolution of the technique has provided different direct immersion modalities which are termed drop-to-drop/drop-in-drop, CFME, LLLME, DSDME and SFODME. Therefore, DI-SDME is considered as an individual technique where the droplet is held on the tip of the microsyringe in a two-phase system. Since the boundary between the different direct immersion techniques is not clearly defined, drop-to-drop/drop-in-drop, CFME, LLLME, DSDME and SFODME are included in the direct immersion section. On the other hand, the authors of this chapter consider both DSDME and SFODME as SDME techniques bearing in mind that the extractant phase maintains its integrity as a single drop throughout the extraction procedure.
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The volume of the drop typically used with organic solvents is around 1–3 μL, however, viscosity, surface tension and density of ILs and surfactants can enable bigger drops (e.g., 3–10 μL). The syringe usually employed with ILs or surfactants is a LC syringe with flat tip, in contrast to the bevel tip of the GC syringes mainly used with organic solvents. Different systems (Figure 4.4) employing a short 3 mm tube (Liu et al., 2003), a PTFE flange (Batlle et al., 2008; López-Jiménez et al., 2008) a bell-mouthed (Ye et al., 2007) or a brass funnel (Qian & He, 2006) have been developed since the flat tip prevents the formation of the drop or to maximize the contact area between the needle and the drop allowing bigger volumes (e.g., 20 μL). These arrangements augment the adhesion force between the drop and the improvised device, increasing drop stability over a longer duration and allowing use of higher stirring rates.

Organic and inorganic compounds, small and large molecules, polar and non-polar analytes and charged and non-charged compounds have successfully been extracted by the SDME technique due to the different properties of solvents used. As a representative example of the wide range of extraction ability, organic compounds (Tobiszewski et al., 2009; Poole & Poole, 2010; Martín-Calero et al., 2011; Tankiewicz et al., 2011; Ruiz-Aceituno et al., 2013), inorganic compounds (Pena-Pereira et al., 2009b; Dadfarnia & Shabani, 2010; Martinis et al., 2010; Martín-Calero et al., 2011; Hu et al., 2013) and emerging pollutants (Mahugo-Santana et al., 2011) have successfully been extracted. Regarding sample matrices, numerous simple and complex matrices have been studied along these almost twenty years, including environmental (Tankiewicz et al., 2011; Han & Row, 2012; Ruiz-Aceituno et al., 2013), food (Asensio-Ramos et al., 2011b; Martín-Calero et al., 2011; Ruiz-Aceituno et al., 2013) and biological samples (Han & Row, 2012; Escudero et al., 2013).

Simplicity in operation, time savings, low cost, virtually organic solventless nature, freedom from analyte carryover, and compatibility of microextraction for direct analysis by a large variety of instrumental techniques make SDME one of the preferred techniques for sample preparation.

Figure 4.4 Devices used as holders for SDME.
4.2.1 Headspace Single-drop Microextraction

The headspace single-drop microextraction (HS-SDME) technique disposes the drop to the headspace of the sample thus avoiding direct contact with the sample. This technique limits the extraction to volatile and semi-volatile compounds, but enables the extraction from complex and dirty samples. For instance, this method allows rapid stirring of the sample solution with no adverse impact on the stability of the droplet. Additionally, non-volatile matrix interferences are reduced, if not eliminated. The system is made up of a conventional syringe, a vial containing the sample, a stirring bar and a cap to avoid losses of the analytes and solvent (Figure 4.5A). This technique can be considered a three-phase system because analytes are distributed among the liquid sample, headspace and drop of solvent. The analytes should be transferred from the sample to the headspace and then to the drop. The rate-determining step of the extraction is the transfer from the sample to the headspace since the diffusion in a gas phase is faster than in a liquid medium. Hence, a high stirring speed of the sample solution is required to facilitate mass transfer among the three phases.

Organic solvents were the first solvents of choice for HS-SDME. However, these solvents presented a limitation related to the evaporation problems. This limitation was solved by ILs and surfactants (section 4.1.2.) and by dynamic methods. The first dynamic method was proposed by Shen and Lee (Shen & Lee, 2003). Extraction took place within the microsyringe barrel as in dynamic LPME and the spherical drop form is transformed in a film. The organic solvent film formed in a microsyringe barrel was used as an extraction interface in headspace liquid-phase microextraction (HS-LPME) of chlorobenzenes. The extraction consisted of drawing 2 µL of organic solvent into the microsyringe, then the microsyringe needle was passed through the vial septum and kept the needle suspended over the liquid sample. Next, 5 µL of gaseous sample was withdrawn and maintained for a short period of time, and then the plunger was depressed back to the original mark. The same process was repeated 25 times. Finally, the syringe needle removed from the vial was injected into the GC for analysis. In general, the reasons for successful extraction were the very small space within the microsyringe barrel and the fast equilibrium between gaseous analytes and the organic solvent film. Both of these factors significantly reduced the risk of solvent loss during extraction due to evaporation or dislodgement of the drop. Thus, dynamic HS-LPME was shown to be an inexpensive, fast, and simple sample preparation method for volatile compounds, improving efficiency and avoiding evaporation problems.

Since the manual operation of dynamic HS-LPME presents high imprecision, Saraji proposed a technique called semiautomatic dynamic HS-LPME (Saraji, 2005). The author proposed the system to improve ease of operation and to achieve greater reproducibility in the sample extraction. A variable speed stirring motor was used for automation of sample extraction step to ensure uniform movement of syringe plunger through the barrel. In comparison to manually operated extraction, semiautomation
of the method led to a better precision. Ouyang et al. (2005, 2007) went a step further and developed fully-automated SDME methods using the Combipal autosampler (CTC Analytics, Zwingen, Switzerland) (Kokosa, 2007). In the first work (Ouyang et al., 2005), the authors automated HS-LPME, named static and dynamic HS-LPME. The latter was operated with exposition of the droplet to the headspace instead of withdrawing the headspace gaseous sample into the syringe barrel. The 1-octanol droplet of 1 μL volume was withdrawn in and out of the barrel for a repeated number of times. Agitation of the sample was carried out when the drop was inside the barrel to avoid instability problems. All the operational parameters involved in this process were precisely and conveniently controlled by the autosampler. The same research group automated all the SDME modes and the HF-LPME technique. SDME included static HS-LPME, dynamic HS-LPME (exposed and unexposed drop) and DI-LPME (static and dynamic) (Ouyang et al., 2007).

Related to automation, Vallecillos et al. have developed a fully automated IL-HS-SDME procedure to preconcentrate trace amounts of ten musk fragrances from wastewater (Vallecillos et al., 2012b) and sewage sludge (Vallecillos et al., 2012a) samples prior to analysis by GC and ion trap tandem mass spectrometry. A CTC Combipal autosampler was also used for the fully automated IL-HS-SDME. The [C₈mim][PF₆] IL was exposed in the headspace of a 10 mL sample. The main problem associated with the use of ILs in automated processes is its high viscosity, therefore, to easily take 1 μL of IL, the fill and ejection speed of the syringe during all the HS-SDME procedure was 1 μL s⁻¹. A large internal diameter (3.4 mm) GC inlet liner with a piece of glass wool was used to avoid the introduction of the ILs to the GC column and a guard column.

Figure 4.5 SDME modes. (A) HS-SDME and (B) DI-SDME.
was used to prevent analytical column damage. The authors also highlighted that the non-modification of the GC injector permitted the development of a completely automated, simple, and environmentally friendly method. On the whole, automated systems are preferred because they offer important advantages, such as minimizing the errors associated with manual handling, reducing time and improving sensitivity and precision.

Microwave radiation and US energy, as described in section 4.1.4., have been employed for the enhancement of the extraction, speeding up the sample heating and maintaining the drop at room temperature. Vidal et al. proposed a one step, in situ sample preparation method coupling microwave radiation and HS-SDME (Vidal et al., 2007a). A homemade experimental setup with a domestic microwave oven was used for the extraction of chlorobenzenes from water samples. The chlorobenzenes were extracted directly onto a \([C_6\text{mim}][PF_6]\) IL single-drop in the vial headspace under the aid of microwave radiation. Limits of detection one order of magnitude below those obtained without microwave heating (Vidal et al., 2007b) demonstrated the favorable effect of the microwave radiation upon SDME. On the other hand, H. Xu et al. (2007) proposed the extraction of chlorophenols from water samples assisted by US energy. The vial containing the sample was introduced in the ultrasonic cavity while the extractant phase was suspended on the bottom of a cone-shaped polychloroprene rubber (PCR) tube outside of the cavity. Ice was placed around the bulge section of PCR tube to maintain the relatively low temperature of extractant. EE was found to be 21 times higher in US-assisted HS-LPME than with the conventional HS-LPME. Part of this improvement was related to the larger volume (20 μL) of solvent suspended on the bottom of the cone-shaped PCR tube.

### 4.2.2 Direct Immersion

#### 4.2.2.1 Direct Immersion Single-drop Microextraction

Direct immersion single-drop microextraction (DI-SDME) was the term given to the direct immersion of the drop into the sample to differentiate from HS-SDME. The DI-SDME technique is used when the solvent drop is held from the tip of a syringe or capillary in a two-phase system (Figure 4.5B). The main drawback of this technique is that the drop at the needle or capillary tip limits the use of extended extraction times, high stirring rates and sample temperatures, and samples should be filtered before use to avoid drop instability by suspended particulate matter.

For the reasons above, dynamic mode is preferred because the inconvenience of accidently losing the drop is eliminated and also results in higher enrichment of analytes and much faster extractions are achieved (He & Lee, 1997; Wang et al., 1998). In dynamic mode, the solvent is moved inside the needle and the barrel of a syringe, and the sample solution is withdrawn and ejected. Pulling up the syringe plunger permits the formation of a renewable microfilm along the inside walls of the microsyringe into
which the analytes are transferred quickly from the aqueous sample. After a dwell time of several seconds to achieve equilibrium, the aqueous sample is discharged and during this step the microfilm is merged with the organic solvent. The same process is repeated several times on fresh portions of aqueous sample, always retaining the organic solvent in the syringe. Finally, the solvent is transferred to the instrument for analysis (Jain & Verma, 2011). The parameters that mostly affect the extraction are the plunger withdrawal speed, sample volume and the number of cycles. As in dynamic HS-SDME, the manual operation reduces the precision, hence, the semiautomation of the technique was also developed (Hou & Lee, 2002). A syringe pump was used to automate the repetitive procedure of filling a microsyringe barrel with fresh aliquots of sample and expelling them after extraction. The number of cycles carried out were 40 during 20 min, obtaining an EF higher than 280 for the extraction of polycyclic aromatic hydrocarbons (PAHs). Fully static (Ouyang et al., 2007) and dynamic DI-LPME (Myung et al., 2003; Ouyang et al., 2007) have been automated. The developed homemade dynamic LPME device was a programmable automated syringe dispenser to overcome deteriorating precision and difficulties in manually manipulating the plunger repeatedly (Myung et al., 2003). The authors claim that accuracies had a high percentage bias, but the precision, which is one of major problems in manual LPME, showed better results than manual dynamic LPME. Thus, the automation greatly simplified the operations and produced better sensitivity and precision than manual DI-SDME.

On the other hand, Pena-Pereira et al. (2008) and Anthemidis and Adam (2009) have proposed an on-line semi-automated system based on sequential injection analysis (SIA) coupled to atomic techniques. The former hyphenates the DI-SDME technique and an SIA system for ultratrace quantification Cr(VI) by ETAAS (Pena-Pereira et al., 2008). The extraction is carried out in a homemade flow-through extraction cell connected to the SIA valve. The sampling capillary with the inserted needle is fitted with an autosampler arm and connected to a high-precision microsyringe. The capillary filled with 10 μL is immersed at a 5 mm depth into the microextraction cell containing the sample and a 3 μL drop volume is suspended at the tip. After a microextraction time in the range of 5–20 min, depending on the sought preconcentration factor, the drop is retracted back into the sampling capillary, the furnace autosampler arm is directed to the graphite tube and the whole volume present inside the PTFE capillary is injected into the instrument. Finally, the extraction cell is emptied and washed. Anthemidis and Adam also proposed the on-line extraction of metals in a homemade flow-cell connected directly to an SIA manifold (Anthemidis & Adam, 2009). The system employed a flow-through cell made of a polyethylene and glass capillary tube to suspend the microdrop of extraction solvent. After the extraction is completed, the drop was retracted into the holding coil and subsequently delivered into a graphite tube for analyte atomization and quantification. The efficiency of this system was demonstrated for cadmium determination using ammonium diethyldithiophosphate as a chelating agent.
Even when ILs are employed, the volume is restricted to the stability of the drop on the needle or capillary tip. Therefore, Cruz-Vera et al. eliminated the syringe and proposed the use of ILs in dynamic liquid-phase microextraction (dLPME) for the efficient extraction of non-steroidal anti-inflammatory drugs (Cruz-Vera et al., 2008) and phenothiazine derivatives (Cruz-Vera et al., 2009a) in urine. The extraction was carried out in a Pasteur pipette where the volumes and flow rates of different solutions involved in the extraction process were controlled by an automatic flow configuration. First, 100 μL of the mixture IL:acetonitrile (50:50, v/v) were picked up in the Pasteur pipette at a flow rate of 0.5 mL min⁻¹. In a second step, the pipette was immersed in the sample vial to draw in a fixed volume of sample at a flow rate of 0.5 mL min⁻¹. The sample was continuously introduced in the system and passed through the IL plug, allowing a dynamic extraction of the analytes since the extractant remained in the lower part of the extraction unit due to its higher density. In this cycle of sample loading, the flow rate of the syringe emptying step was fixed to 5 mL min⁻¹. Finally, the extract was delivered to an Eppendorf vial, diluted with acetonitrile and analyzed by LC. The overall extraction process takes place in less than 35 min. Since a new Pasteur pipette was used for each extraction, no analyte carry-over was observed. This technique also avoids repeated filling and emptying of a syringe with sample.

The most recent approach in DI-SDME has been the use of a microfluidic system where a new chip-based liquid–liquid extraction technique is coupled to GC (Peroni et al., 2012). Extraction is performed in a segmented flow system with additional mixing provided by an etched channel structure (Figure 4.6). The dimensions of the device were optimized to benefit from the advantages of chip technology without suffering from the limitations of over-miniaturization. Depending on the sample volume, two different instrumental modes were used, continuous introduction LLE and plug-injection LLE. The results obtained for selected test analytes show that the extraction is quantitative (recoveries = 92–110%, RSD < 6%) for a wide range of hydrophobicities (Log K_{ow} = 0.86–4.79). The authors highlighted that the instrumental set-up is simple and mechanically strong. This simplicity and robustness, combined with the stability typical of segmented flow, enables automated operation and/or use in the field.

### 4.2.2.2 Drop-in-drop and Drop-to-drop

Drop-in-drop system was the pioneering LPME technique developed by H. Liu and Dasgupta (1996) where an organic microdrop was suspended inside a flowing aqueous sample drop from which analyte was extracted. The analytical system was validated for sodium dodecyl sulphate by its ion-pair formation with methylene blue. After the sampling/extraction period, a wash solution replaced the sample/reagent in the aqueous layer, resulting in a clear outer aqueous drop housing a colored organic drop containing the extracted analyte. This also resulted in an automatic backwash. The color intensity of the organic drop, related to the analyte concentration, was
monitored by a light-emitting diode-based absorbance detector. This system had the advantage of performing both processes of extraction and analysis in the same chamber.

Drop-to-drop is an analogous technique where the sample is a static microdroplet of just a few μL in volume (Wu et al., 2006). The proposed system reduces the size of the sample to typically 7–8 μL, into which was placed less than 1 μL of extraction solvent drop hanging at the needle tip of microsyringe (Figure 4.7). Once the extraction is finished, the drop is withdrawn back into the syringe and injected into the analytical instrument. Equilibrium between the sample and solvent is established quickly due to the small sample and solvent volume. Stirring is not necessary, and this also contributes to drop stability and simplifies the experimental setup. However, the larger ratio of volumes of organic microdrop to aqueous sample reduces the EF. Another application of drop-to-drop has been the determination of trimiprazine involving its extraction in 0.6 μL of toluene from 8 μL of urine and blood samples of rats (Agrawal & Wu, 2007). Caffeine in beverages (Shrivas & Wu, 2007) and nicotine in nightshade vegetables (Shrivas & Patel, 2010) have also been extracted by this technique.
US energy has also been used in the drop-to-drop technique to determine benzene, toluene and xylene in water samples (Zhang et al., 2010). This method maintains the low volumes of extractant solvent (3 μL) and sample (20 μL), however, the extractant solvent is completely dispersed by means of the US energy and the spherical drop loses its integrity, being more DLLME than drop-to-drop approach.

### 4.2.2.3 Continuous Flow Microextraction

Continuous flow microextraction (CFME) is a technique developed by Liu and Lee where the microdrop of organic solvent is in contact with a continuously flowing aqueous sample solution (Liu & Lee, 2000). The organic drop (1–5 μL) is held at the outlet tip of a PEEK connecting tubing that is immersed in a continuously flowing sample solution and acts as the fluid delivery duct and as a solvent holder. A pump was used to introduce the aqueous sample into the extraction chamber at a constant flow rate via a line made of PEEK tubing and terminating at the center of the chamber. The solvent drop emerges from the PEEK tubing and stays at the end whilst the sample solution keeps flowing around the drop. Once the extraction is finished, the organic drop is picked up with a GC microsyringe and injected into GC. EFs from 260- to 1600-fold were achieved with 10 min of extraction time. Two different modifications of this system have been proposed. One modification proposed the use of a unique syringe to act as a holder of the drop and for injection in the analytical instrument. The syringe was placed just above the tube outlet in the extraction chamber.
(Xiao et al., 2006). The other proposed modification, named cycle-flow microextraction, returns the sample from the extraction chamber back to the sample reservoir and it is used repeatedly for extraction (Xia et al., 2004). The re-circulation of sample solution permitted the analysis of a smaller sample size (1–2 mL) and avoided inadvertently running the sample reservoir dry.

Another dynamic system named IL-based cycle-flow SDME was developed by Xia et al. for preconcentration of Co, Hg and Pb from biological and environmental samples (Xia et al., 2008). This system extracted analytes by exposing an IL droplet (2.5 μL) to a flowing stream of sample. [C<sub>4</sub>mim][PF<sub>6</sub>] IL was the extraction solvent and 1-(2-pyridylazo)-2-naphthol was used as complexing agent and chemical modifier. The EE and EF were higher than in static conditions since continuous contact between the IL phase and the fresh flowing sample solution was achieved.

### 4.2.2.4 Liquid-liquid-liquid Microextraction

Liquid-liquid-liquid microextraction (LLLME) is a three-phase mode best suited for the extraction of hydrophilic organic compounds, such as phenols, fatty acids or amines. 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 (Jeannot et al., 2010). The organic solvent, with lower density than water, 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 accumulated in the aqueous acceptor solution (Jeannot et al., 2010). The first system employed a PTFE ring (Ma & Cantwell, 1998, 1999), however, other systems avoiding the PTFE ring (He & Kang, 2006) or employing a volumetric flask have been used (Sarafraz-Yazdi et al., 2005; Fan & Liu, 2008). The latter system placed a layer of an organic solvent on top of the aqueous sample and a microdrop of the acceptor solution was immersed into the organic solvent layer with a syringe (Figure 4.8).

Choi et al. have proposed an automated three-phase SDME coupled to CE using a commercial instrument (Choi et al., 2009). The CE instrument provided adjustable forward and backward pressures and a single drop of an aqueous acceptor phase covered with a thin layer of an organic phase was formed at the capillary tip. Acidic analytes from an acidic donor phase were concentrated into a basic acceptor phase yielding 2000-fold enrichment in 10 min with agitation of the donor phase using a microstirrer. The end surface of the fused silica capillary was hydrophobically treated by silanization to increase drop adhesion, and therefore, the failure rate of drop
formation was negligible (Choi et al., 2009). Comparison studies with a two-phase system and without stirring were carried out, obtaining lower EF.

The most recent approach combines LLLME with DSDME. One proposal avoids the use of the microsyringe as a support of the aqueous drop and a large aqueous droplet is freely suspended at the top-center position of a layer of immiscible organic solvent, which is laid over the aqueous sample solution while being agitated (Sarafraz-Yazdi et al., 2009). The procedure involved the stirring of the sample with the organic solvent for a period of time before the acceptor phase was delivered at the top-center position of the immiscible organic solvent layer. Then, the three phases were stirred for 6 min, and finally, the aqueous droplet was withdrawn into the LC microsyringe and then injected into the LC system. Another combination uses a syringe as a holder of the aqueous droplet but the organic phase is a spherical suspended drop instead of a layer (Gao et al., 2011). This system also employs a two times stirring procedure, being the organic drop and the sample stirred before the aqueous drop is disposed inside the organic drop. After a second stirring and once the extraction is finished, the aqueous back-extractive phase was retracted into the syringe and transferred into a microvial (Figure 4.9).
4.2.2.5 Directly Suspended Droplet Microextraction

The use of a holder (i.e., microsyringes, capillaries) presents drawbacks such as drop instability and slow kinetics. For this reason, different approaches proposed the elimination of the holder and the droplet was directly suspended on the sample. One of these techniques, named directly suspended droplet microextraction (DSDME), was introduced by Yangcheng et al. (2006). In this technique a free microdroplet (5–100 μL) of solvent is delivered to the surface of an immiscible aqueous sample while being agitated (typically at 1000 rpm) by a stir bar placed on the bottom of the sample cell (Figure 4.10). After extraction, the microdroplet of solvent is withdrawn by a syringe and analyzed. Under the proper stirring conditions, the suspended droplet can remain in a top-center position of the aqueous sample. The droplet can become partly engulfed within the sample while maintaining a stable shape with mechanical equilibrium and the mass transfer could be effectively intensified.

Dislodgement of the drop, small organic solvent volumes and limited stirring speed are not drawbacks in DSDME. Relatively high stirring rates, limited by the disintegration of the droplet, can be used since the drop freely rotates on the top centre of the sample. This technique usually employs volumes higher than 4 μL due to the difficulty of withdrawing the drop after the extraction. However, the technique presents a limitation related to the solvent properties, which should be poor water miscibility, density lower than water and low vapor pressure. Other limitations that the authors noted were the size and shape of the stir bar used, which had a distinct effect on the shape of the drop, which in turn can lead to difficulty in sampling. In addition, they considered that analyte adsorption on the surface of the stir bar was inevitable.
Therefore, a modified method of DSDME was proposed in which the extraction vial was rotated to produce a vortex in the aqueous sample instead of using a stirring bar (Mingyuan et al., 2009). During extraction, the rotating vial provided a very stable flow field, and solvent spreading along the parabolic surface of the aqueous phase reduced mass transfer resistance. A modified two-way magnetic stirrer device was used for rotating the vial. A hole was drilled through the tray of the magnetic stirrer, and a rotating shaft was inserted. A circular deck made of PTFE with a circular groove was fixed onto the rotating shaft with a screw. A cylindrical extraction vial was snugly placed inside the groove such that the vial rotated along with the deck. For sampling convenience, a hole was made at the centre of the extraction vial lid through which a needle could be inserted. A piece of sealing film was placed inside of the lid to prevent the release of volatile compounds. Potential emulsification was avoided using the centrifugation effect of the rotating vial. During sampling, the shape of the organic solvent droplet changed upon insertion of a needle, causing the droplet height to increase approximately 3–4-fold. This increase in droplet height made solvent sampling much more convenient and allowed for the use of smaller volumes, which enhanced mass transfer and enrichment. In addition, the absence of a stir bar enables more stable and reproducible drop shapes providing better precision values and lower drop disintegration.

Figure 4.10 DSDME procedure.
Solidification of Floating Organic Drop Microextraction

Khalili Zanjani et al. (2007) proposed the technique named solidification of floating organic drop microextraction (SFODME) to eliminate or reduce the transfer of some aqueous sample when the organic solvent is being withdrawn by a syringe in DSDME. The difference with DSDME technique is that once the extraction is finished, the directly suspended solvent drop is solidified by introducing the vial into an ice bath for approximately 5 min. Then, the solidified organic solvent is transferred into a small conical vial using a microspatula \( \text{(Figure 4.11)} \). The solid organic solvent known as the ‘solid drop’ melts quickly at room temperature. Finally, it is retracted by a microsyringe and injected into an analytical instrument for final analysis. This method has initially been tested with PAHs using 1-undecanol, and the extract was analyzed by GC-FID (Khalili Zanjani et al., 2007). Preconcentration factors in the range of 560–1940 were obtained which was better than some other extraction methods. Different applications published since then are discussed in two recent reviews (Ganjali et al., 2010; Ghambarian et al., 2013).

The main limitation of SFODME is that not only requires an extractant phase less dense than water, but also the melting point should be near room temperature (in the range 10–30 °C). The different solvents available for this technique are: 1-undecanol, 1-dodecanol, 2-dodecanol, 1-bromohexadecane, 1-hexadecane, 1,10-dichlorodecane and 1-chlorooctadecane, with 1-undecanol and 1-dodecanol being the most commonly used. Moreover, the complete removal of trace water is hard to accomplish and an extra centrifugation step of the melted drop is sometimes needed (Zheng et al., 2011). Therefore, the several steps needed to perform the microextraction process make SFODME a tedious and time-consuming technique. In addition, automation of DSDME and SFODME is hardly feasible.
4.3 Membrane-based Liquid-phase Microextraction

4.3.1 Hollow Fiber Liquid-phase Microextraction

SDME gained a widespread interest since its appearance and obtained an undoubted relevance as a starting point toward miniaturized liquid-phase extraction techniques. However, some problems related to the instability of the hanging drop, which can be easily dislodged from the holder during extraction, needed to be solved. In 1999, Pedersen-Bjergaard and Rasmussen (1999) introduced the HF-LPME technique which easily overcame this shortcoming through the stabilization of the extractant phase using a porous membrane. In HF-LPME, an organic solvent is immobilized in the pores of a polymeric HF, normally made of polypropylene, and forming a SLM. The final acceptor phase is introduced within the lumen of the fiber, being protected and physically separated from the sample by the SLM. HF-LPME possesses some remarkable advantages (Pedersen-Bjergaard & Rasmussen, 2008; Ghambarian et al., 2012), namely: (i) high stirring rates can be employed because the problem of the dislodgment of the drop is avoided, although undesirable air bubbles can be generated on the surface of the fiber at very high stirring rates; (ii) the contact area between the extractant phase and the sample is higher, favoring mass transfer; (iii) the HF acts as a protection barrier for the extractant phase, therefore, dirty samples can be analyzed while obtaining very clean extracts; (iv) the fiber can be disposable after an only one use due to its low cost, avoiding carry-over effects between extractions; and (v) the technique presents automation options. Fundamental theory and basic equations about HF-LPME have been presented in detail in the literature (Ghambarian et al., 2012; Gjelstad et al., 2012). As major shortcomings, the manual preparation of the fiber can lead to deviations between extractions and the relatively long extraction times. Ultrasound energy has been proposed to reduce extraction times by accelerating mass transfer rate of analytes in a methodology called US-enhanced HF-LPME (Yang et al., 2010).

Two extraction modes, two-phase and three-phase, are described for HF-LPME. In two-phase HF-LPME (Rasmussen et al., 2000), the same organic solvent is immobilized in the pores of the fiber and introduced in its lumen (Figure 4.12A). Thereby, analytes are extracted from the donor phase through the SLM into the organic solvent in the lumen of the fiber (acceptor phase). Two-phase mode is suitable for hydrophobic analytes with a substantial solubility in organic solvents, since high partition coefficients are essential for an efficient extraction. GC followed by different detectors (e.g., MS, FID, ECD) is normally selected as the quantification technique due to its compatibility with the organic acceptor phase. Two-phase HF-LPME can be performed in two different ways: direct immersion (Rasmussen et al., 2000) or headspace (Jiang et al., 2005). Strictly from the point of view of phases, headspace two-phase HF-LPME is not a two-phase mode due to the donor aqueous phase, the gaseous phase and the acceptor organic phase which are involved in the extraction process. However, if an
organic solvent fills the pores and lumen of the fiber, the methodology is included in the two-phase classification. Both conventional sample heating using hot plates (Jiang et al., 2005) and microwave radiation (Shi et al., 2008) have been employed to assist headspace extractions.

In three-phase HF-LPME (Pedersen-Bjørgaard & Rasmussen, 1999) analytes are extracted from the aqueous sample (donor phase) through the immobilized organic solvent in the pores of the fiber, into another aqueous solution (acceptor phase) within the lumen of the fiber (Figure 4.12B). Three-phase HF-LPME is normally coupled with LC and CE, usually followed by UV or MS detectors, due to the aqueous nature of the acceptor phase. Three-phase HF-LPME is intended for the extraction of hydrophilic or ionizable compounds by three different mechanisms, namely: (i) pH gradient (ii) carrier mediated and (iii) EME. The solubility of basic and acid analytes in donor and acceptor aqueous solutions can be adjusted by pH changes. For example, the extraction of basic compounds implies the basification of the sample solution to decrease the solubility, and the acidification of the acceptor phase to promote it. Conversely, for the effective extraction of acid compounds, the pH of the donor phase is adjusted into the acid range whereas the acceptor phase is basified. Thereby, basic and acid analytes can be easily extracted into the immobilized organic phase, diffuse through the SLM and, finally, be back-extracted into the acceptor phase without returning into the sample. Highly hydrophilic compounds are poorly extracted by partitioning and diffusion mechanisms and require an active transport to cross the SLM and reach the acceptor phase. The use of carriers can enhance EEs in these cases (Ho et al., 2003). Carriers form hydrophobic ion-pairs with analytes, which are extracted into the immobilized organic solvent and diffuse through the SLM. In the contact region with the acceptor phase, analytes are released from the ion-pairs into the acceptor phase,
and carriers form new ion-pairs with other counter ions present in the acceptor phase. The new ion-pair complexes are back extracted into the sample, thus completing the cycle (Rasmussen & Pedersen-Bjergaard, 2004). Three-phase HF-LPME assisted by an electric field (i.e., EME) will be discussed separately in another section of this chapter.

Although classical modes of two-phase and three-phase HF-LPME are described above, some authors have proposed alternative methodologies. For example, Bedendo et al. (2012) modified classical two-phase HF-LPME by disposing organic solvent in the sample and an empty fiber. Pesticides from orange juice were firstly extracted into the organic solvent, which were then absorbed into the fiber due to their high affinity, forming a renewable SLM and providing a good sample clean-up. A final solvent desorption step is included in the procedure to extract analytes into methanol-acetone mixture for its injection in a LC system. Three-phase HF-LPME generally involves an aqueous acceptor phase, but the use of two immiscible organic solvents in the pores and lumen of the fiber has also been presented. Ghambarian et al. (2010) devised a new approach compatible with GC in which n-dodecane was immobilized in the pores of the fiber whereas methanol or acetonitrile was introduced into the lumen for the extraction of chlorophenols from honey and water samples. Zhang et al. (2006) proposed a new organic solvent-free three-phase mode (named liquid-gas-liquid microextraction) in which the pores of the hydrophobic HF, not filled with a solvent, separated donor and acceptor aqueous phases. Therefore, analytes crossed the fiber by gas diffusion from a heated sample. This work included a comparison with classical three-phase HF-LPME, obtaining better EF for the most volatile compounds studied (phenols). Finally, Carletto et al. (2009) employed a method called HF renewal liquid membrane based on three-phase HF-LPME for the extraction of cadmium from water samples. The authors added organic solvent to the donor phase as well as in the pores of the fiber for the continuous renewal of SLM and replenishment of lost solvent, obtaining a high stability. Complexes of cadmium were extracted into the organic solvent in the donor phase and carried to the fiber. The organic solvent was then solubilized into the fiber forming a homogeneous phase. Cadmium finally reached the acceptor solution where the complex was released at a high pH.

Three general configurations of fiber exposure to the sample have been proposed in the literature. The first work about HF-LPME (Pedersen-Bjergaard & Rasmussen, 1999) employed a configuration based on an U-shaped fiber with each end connected to a needle, one for the injection of the acceptor phase, and the other for its collection after extraction (Figure 4.13A). A rod-like configuration appeared later, becoming the most interesting and widely employed option, especially when the fiber is held by a syringe (Zhu & Lee, 2001) (Figure 4.13B). In this configuration, the acceptor phase is introduced and removed through the same side whereas the other one is sealed. The syringe is employed not only as a holder but also to add and withdraw the acceptor phase, and directly inject it into the analytical system, thus simplifying the procedure. The length of the fiber is smaller than in the U-shaped configuration, therefore the volume of the acceptor phase is reduced and the EFs are enhanced. On the other
hand, the fiber can be directly introduced into the sample with both sealed ends, leading to a methodology called SBME (Jiang & Lee, 2004) (Figure 4.13C).

The extraction systems described up to now are all static systems where the donor and acceptor phases are stagnant, with the exception of stirring the sample to enhance mass transfer. HF-LPME can also be performed in a dynamic mode where the lower end of the rod-like fiber configuration is opened. In dynamic two-phase HF-LPME (Zhao & Lee, 2002), the acceptor phase is repeatedly drawn into the barrel and injected into the lumen of the fiber using a syringe. While drawing in sample in the direct immersion mode, aqueous sample fills the lumen and analytes are extracted into a thin film of organic solvents built up in the HF. During injection, sample is driven out of the fiber, the organic film recombines with the organic solvent and analytes distribute into the bulk solution. Dynamic two-phase HF-LPME has also been performed in the headspace mode where the gas phase enters and leaves the lumen of the fiber during sucking and injection cycles (Jiang et al., 2005). In dynamic three-phase HF-LPME (Hou & Lee, 2003), the syringe is first filled with the acceptor aqueous phase and then with an organic solvent. The organic solvent is injected into the HF and withdrawn in the consecutive cycles of sucking and injection, following a similar procedure than in dynamic two-phase, while the final aqueous acceptor phase
remains inside the syringe. Dynamic HF-LPME improves extraction speed and efficiency compared with static systems but the extraction setup is more complex. Automated or semi-automated dynamic HF-LPME systems are generally employed due to the obvious tediousness and irreproducibility of manual operation. In these systems, a syringe pump is fixed to the syringe that supports the fiber and is programmed to eject and withdraw the acceptor phase at a selected speed. Related to this, Pezo et al. (2007) devised an automatic multiple dynamic HF-LPME system in which several samples were simultaneously extracted with obvious benefits in throughput and operation time. In spite of reaching different degrees of automation, the developed methods using dynamic HF-LPME are off-line systems since sample preparation and instrumental analysis are carried out separately. Esrafili et al. (2012) have recently proposed an on-line three-phase HF-LPME which performed sample extraction and acceptor phase injection into a LC system automatically, without the need of an operator. The method employed a syringe pump for loading solvents, a platform lift for moving the sample vial and a sampling loop for on-line injection of the acceptor phase into the LC. Another novel on-line method for LC, named as push/pull perfusion hollow-fiber liquid-phase microextraction (PPP-HF-LPME), has recently been presented (Chao et al., 2013). PPP-HF-LPME uses a push/pull syringe pump with two opposing syringes as the driving source to perfuse the acceptor phase into the fiber. Therefore, the authors avoided losses or gains of solvent across the porous membrane that normally appear as a consequence of flowing the acceptor phase only by push or pull perfusions. In addition, this method employed US energy to accelerate mass transfer of neutral analytes leading to short extraction times.

HF-LPME has been employed in environmental, bioanalytical and food fields in its different modes and configurations (Lee et al., 2008; Ghambarian et al., 2012). This technique has found its major applications in organic analysis (Ghambarian et al., 2012; Lee et al., 2008), although the determination of metals and their speciation has also been studied in different methods using LC-UV, ETAAS, ICP-MS or ICP-OES systems (Hu et al., 2013). Parameters affecting HF-LPME such as the type of immobilized organic solvent, extraction time, stirring rate and salting out effect are commonly optimized in the different applications of two-phase and three-phase modes. The selection of the appropriate solvent within the pores of the fiber is a critical part of the technique. Some important aspects of the solvent to consider include (Ghambarian et al., 2012): (i) Immiscibility with water to prevent losses by dissolution; (ii) ability to be strongly immobilized in the pores of the fiber; (iii) low volatility to avoid losses by evaporation; (iv) good extractability and high partition coefficients of the target analytes; (v) low viscosity to ensure high diffusion coefficients across the liquid membrane; and (vi) compatibility with the selected analytical technique in two-phase HF-LPME. Organic solvents such as toluene, 1-octanol, undecane or dihexylether fulfill all these requirements and have successfully been employed in both modes of HF-LPME (Ghambarian et al., 2012). The fiber type and its dimensions are also important points to consider. Polypropylene fibers are usually chosen due to
their hydrophobicity and compatibility with a broad range of organic solvents. Polypropylene fibers have been modified with carbon nanotubes (CNTs) with the aim of enhancing EE (Es’haghi et al., 2010). CNTs immobilized into the fiber act as solid sorbents, and lead to a methodology called hollow fiber solid–liquid phase microextraction. The dimensions of the fiber have to ensure a good contact surface area, appropriate donor/acceptor volume ratio and mechanical stability. The length of the fiber is chosen by the user and in most cases is conditioned by the employed configuration (i.e., U-shaped, rod-like or SBME), whereas commercially available fibers commonly have an internal diameter of 600 µm and a 200 µm wall thickness. Finally, the pore size (typically of 0.2 µm) should allow the penetration of target analytes while providing good filtration and sample clean-up.

As mentioned above, organic solvents such as toluene, 1-octanol, undecane and dihexylether have successfully been employed in HF-LPME. However, their losses by evaporation are a disadvantage of the technique and an important source of irreproducibility between extractions. ILs have recently been presented as an interesting and useful alternative to those traditional solvents for the impregnation of the fiber in HF-LPME. Previous studies (Fortunato et al., 2004, 2005) demonstrated the feasibility of using ILs based on 1-alkyl-3-methylimidazolium cations to obtain stable SLMs, even under vigorous stirring conditions due to properties such as relative high viscosities, interfacial tensions and low solubility in water depending on the anion. The applicability of a SLM based on ILs in HF-LPME was demonstrated by Peng et al. (2007) who employed [C₈mim][PF₆] as intermediary solvent in three-phase HF-LPME for the extraction of chlorophenols from different water samples. Afterwards, Basheer et al. (2008a) presented a three-phase HF-LPME in which [C₄mim][PF₆] IL was employed as intermediary solvent whereas an organic solvent was the final acceptor phase. Thereby, aromatic and aliphatic hydrocarbons could be determined in storm water using a GC-MS system. The use of ILs in two-phase HF-LPME was successfully presented by Abulhassani et al. (2010) who employed [C₆mim][PF₆] for the extraction of ammonium pyrrolidine dithiocarbamate complexes of lead and nickel from water and biological samples, using ETAAS as the detection system.

In general, both organic and inorganic analytes have been extracted from aqueous samples using IL based HF-LPME, although more complex matrices such as biological tissues (Abulhassani et al., 2010), hair and tea (Zeng et al., 2011) have also been studied. The rod-like configuration is most commonly used, but SBME has been chosen by some authors (Zhang et al., 2013).

4.3.2 Electromembrane Extraction

Electromembrane extraction (EME) was presented for the first time in 2006. PederSEN-BJERGAARD AND RASMUSSEN (2006) proposed a novel technique based on three-phase HF-LPME in which analytes are extracted from the donor phase through
the SLM into the acceptor phase using an electrical potential difference as driving force. The authors extracted basic drugs from 300 µL of sample into 30 µL of acceptor phase, both of which were acidic to maintain analyte protonation. The solvent 2-nitrophenyl octyl ether (NPOE) was immobilized in the pores of the fiber, which used the rod-like configuration. A platinum electrode was immersed into the sample and another platinum electrode was placed into the acceptor phase. Both electrodes were connected to a power supply and an electrical field was created across the SLM. Thereby, charged analytes were extracted by electrokinetic migration and not by passive diffusion. A schematic illustration of the general EME setup is shown in Figure 4.14A. EME presents the following general advantages (Petersen et al., 2011b; Gjelstad & Pedersen-Bjergaard, 2013): (i) shorter extraction times (1–5 min) than in HF-LPME (typically between 10–60 min) due to the enhancement of mass transport by the force of the electrical potential; (ii) extraction selectivity can easily be modulated by changes in the magnitude and direction of the electrical potential, simply by manipulating the external power supply; (iii) efficient sample clean-up and feasibility of direct extraction from untreated complex matrices; and (iv) possibilities of downscaled format (i.e., microchip devices) and automation. As major drawbacks, the homemade extraction equipment exhibited bubble formation due to electrolysis reactions and sparking at high voltages or during the analysis of real samples with high ion concentrations.

Fundamental theory about EME has been discussed in the literature (Gjelstad et al., 2007). The proposed model describes the influence of the thickness of the fiber, voltage applied and coefficient of diffusion across the SLM over the flux of analytes. As in HF-LPME, commercial fibers with a 200 µm wall thickness are usually employed. The theoretical model predicts an improvement in the flux with thinner fibers but...
instability problems have to be considered. It has been theoretically proposed and experimentally proven that an increase in the applied voltage produces an increase in the flux across the SLM, thus affecting the recoveries. Potentials between 1 and 300 V are commonly employed. The coefficient of diffusion of analytes across the SLM depends on the viscosity of the immobilized solvent and is higher in those with low viscosity. Apart from these parameters, other considerations such as the pH of donor and acceptor phases, extraction time, the conductance of the immobilized organic solvent or the use of carriers if necessary, are essential for good EME performance. The acceptor phase volume, salt effects and sample stirring speed are also optimized in some applications.

EME has mainly been applied for the extraction of acidic and basic drugs from biological samples (e.g., blood, plasma, urine, breast milk, saliva or amniotic fluids). For the extraction of basic analytes, the anode is placed into an acidic donor phase whereas the cathode is placed into an acidic acceptor solution. SLMs based on NPOE have generally shown the best performance for the extraction of hydrophobic basic analytes. However, the use of ion-pair reagents which act as carriers seems to be necessary for enhancing mass transport across the SLM of more polar basic compounds (Gjelstad et al., 2006). Contrary to the extraction of basic molecules, the extraction of acidic drugs requires the basification of donor and acceptor phases in order to maintain their charge. The direction of the electrical field is reversed with respect to EME of basic compounds, therefore the cathode is placed into the donor phase and the anode is placed into the acceptor solution. SLMs containing 1-octanol have shown the best results for EME of acidic compounds. The simultaneous extraction of acidic and basic drugs has also been presented in the literature using a dual EME with a compartmentalized membrane (Basheer et al., 2010) or two separated hollow fibers (Seidi et al., 2012). The last configuration is simpler and robust and thus has found more use in subsequent works (Figure 4.14B). For the extraction of acidic analytes, the lumen of one fiber is filled with a basic solution and the anode is introduced in it. The other fiber is filled with an acid solution and contains the cathode for the extraction of basic analytes. The pH of the donor phase is kept neutral in order to have both acidic and basic compounds ionized. Using this configuration, analytes are simultaneously but separately extracted, therefore a further step of mixing the two acceptor solutions is necessary for a unique analysis. Peptides (Balchen et al., 2008) and metal ions (Basheer et al., 2008b) have been also target analytes in EME. On the other hand, apart from the excellent performance in biological analysis, EME has also been applied in the environmental field for the extraction of organic compounds (Xu et al., 2008) and metals (Kubáň et al., 2011) from different water samples.

CE and LC are commonly selected as separation techniques after EME. CE has allowed the chiral separation of different drugs in methods where enantiomers were equally extracted (Nojavan & Fakhari, 2010; Fakhari et al., 2013). CE-UV was employed in the first report (Pedersen-Bjergaard & Rasmussen, 2006) and the early years of EME. Later, CE coupled with a contactless conductivity detector (Xu et al.,
2008) appeared as a more universal detection method since it allows the conductometric analysis of all charged species. EME has recently been combined with GC in the classical three-phase mode (Davarani et al., 2012) and in a novel two-phase EME (Davarani et al., 2013) in which the aqueous acceptor phase is replaced by an organic solvent with sufficient electrical conductance (e.g., heptanol, octanol). On the other hand, EME has been combined with SPELs (Ahmar et al., 2013). Electrochemistry offers easy operation, high sensitivity and rapid response. In addition, SPELs present an interesting alternative to traditional bulky and expensive instrumentation and are perfectly compatible with the low-volume acceptor phase in EME. Thus, the use of SPELs in the detection stage could be a powerful tool in future investigations and applications of EME.

Different modifications to the electrical field application have been proposed. Traditionally, a constant voltage was applied across the SLM, however, a recent publication has proposed the use of pulsed voltages (Rezazadeh et al., 2012). In pulsed electromembrane extraction (PEME) the duration of the pulse is long enough for analyte extraction through the SLM, but it is short enough to avoid instability problems, which normally appear in EME at high potentials. Two-way PEME (Rezazadeh et al., 2013) was then introduced for the selective extraction of amino acids (AAs) utilizing their isoelectric pHs in the acceptor phase. Once the extraction of different AAs was achieved, the direction of the electrical field was reversed. The AAs in the zwitterionic form remain in the acceptor phase as they have no mobility in the electrical field, whereas the rest of AAs return to the donor phase. Another publication has suggested the use of voltage steps in PEME (Rezazadeh et al., 2014) and compared it with the application of constant pulses. Similar or higher recoveries were obtained but the step voltage approach required a lower energy supply. In another study, the application of constant voltage was compared with the application of constant current across the SLM (Slampová et al., 2012). The results demonstrated that the use of a stabilized constant current provided better repeatability in the extraction process.

Recent developments in EME membrane composition have also been reported. As in HF-LPME, CNTs have been inserted in the pores of the polypropylene HF in a method called CNTs assisted EME (CNTs-EME) (Hasheminasab & Fakhari, 2013). CNTs possess a high surface area with a high adsorption capacity for organic and inorganic species. In general, when using CNTs-EME, the mechanism of solute transfer involves both liquid and solid phase extractions, acting simultaneously to increase the analyte partition coefficient across the membrane. Therefore, the mass transport across the membrane is improved and better analytical results (lower limit of detection, higher preconcentration factor and recovery) were obtained in comparison with EME (Hasheminasab & Fakhari, 2013; Hasheminasab et al., 2013). CNTs-EME has also been employed in the two-phase mode compatible with GC (Hasheminasab et al., 2014).

EME has been downscaled to a chip format (Petersen et al., 2010). The microfluidic extraction device comprises a SLM with a flat configuration which separates the sample channel and acceptor reservoir. Continuous delivery of fresh sample to the
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Electromembrane provides higher recoveries compared with EME in which sample solution is stagnant. This miniaturized system was first coupled off-line with CE-UV (Petersen et al., 2010), and thereafter with both CE-UV (Petersen et al., 2011a) and electrospray ionization-MS (Petersen et al., 2011a, 2012) in two on-line systems with a flowing acceptor phase. Another on-line system using CE-UV has been employed in a novel nanoelectromembrane extraction (nano-EME) approach in which analytes were extracted from 200 µL of sample into approximately 8 nL of acceptor solution (Payán et al., 2013). Nano-EME provided a system with an excellent enrichment capacity since the acceptor solution is notably reduced in comparison with EME. Another downscale approach is the drop-to-drop system developed for extraction of basic drugs (Petersen et al., 2009). The extraction was performed through an organic solvent of NPOE immobilized as SLM in a flat polymeric membrane placed between 10 µL of sample and 10 µL of acidic aqueous drop based on a direct current electrical potential of 15 V (Figure 4.15). The extraction conditions allowed only cationic compounds with relatively low polarity to cross the SLM whereas neutral and cationic compounds of high polarity could not.

A recent method has introduced the concept of parallel EME (Eibak et al., 2014) in which eight human plasma samples were simultaneously extracted using flat membranes in a multiwell configuration and a single power supply. Thereby, time consumption per sample is reduced and the throughput of the method is notably improved.

Figure 4.15 Schematic illustration of the set-up for drop-to-drop EME. Reprinted from Petersen et al. (2009), Copyright (2009), with permission from Elsevier.
4.4 Dispersive Liquid-liquid Microextraction

4.4.1 Classical Dispersive Liquid-liquid Microextraction

Dispersive liquid-liquid microextraction (DLLME) was proposed for the first time in 2006 (Rezaee et al., 2006). Rezaee and co-workers presented a novel microextraction technique based on a ternary component solvent system in which a water-immiscible organic solvent that is denser than water is dispersed in fine drops into the aqueous sample with the aid of an organic disperser agent (Rezaee et al., 2006). A cloudy solution is formed due to the cosolvency of the dispersant with the other two phases and leading to a greater contact surface area. After extraction, phases are separated by centrifugation, and the enriched organic phase at the bottom of the centrifuge tube is collected to be analyzed. A scheme of the described procedure is shown in Figure 4.16.

This simple methodology presents the characteristics of LPME techniques such as ease of handling, low cost, low solvent and sample consumption, and having a negligible environmental and human health impact due to the reduction of wastes generated. In addition, DLLME offers the following remarkable advantages (Rezaee et al., 2010; Yan & Wang, 2013): (i) fast achievement of the equilibrium state due to the infinitely large contact surface between the fine drops of the extractant solvent and the aqueous phase; (ii) short extraction times; (iii) high EFs; and (iv) problems associated with other LPME techniques such as the dislodgement of the drop in SDME, and the manipulation of the fiber in HF-LPME are avoided.

Parameters affecting DLLME, such as the type and volume of extractant and dispersing solvents, sample volume, salting-out effect, pH, type and amount of chelating agent for metals determination, extraction time and centrifugation time and speed are typically optimized in different applications. Chlorinated solvents (from 20 to 100 µL) (e.g., chlorobenzene, chloroform, tetrachloromethane or tetrachloroethylene) have traditionally been selected as extractants due to their high density, whereas acetone, methanol, ethanol or acetonitrile (from 0.1 to 2 mL) are the most common dispersants.

GC was the analytical technique selected in the first applications of DLLME due to its compatibility with chlorinated solvents. However, polar and ionizable compounds cannot be determined by GC unless a derivatization process converts them into volatile species (Lin et al., 2013). The first use of LC after DLLME was proposed by Farajzadeh et al. (2007). In this work, an additional step of solvent evaporation was included after phase separation, and the solid residue was reconstituted in methanol for injection into the chromatographic system. Afterwards, Wei et al. (2007) demonstrated the feasibility of direct injection of chlorinated solvents in reverse-phase LC columns avoiding the time consuming evaporation steps. For inorganic analysis, ETAAS and FAAS are the most commonly chosen analytical techniques, although fiber optic-linear array detection spectrophotometry, UV-Vis spectrophotometry and ICP-OES or ICP-MS have also been utilized (Andruch et al. 2013a; El-Shahawi & Al-Saidi, 2013). ETAAS requires microvolume samples; therefore, it is perfectly compatible with low
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volume extracts obtained after DLLME. When using FAAS, larger sample volumes are necessary and further steps of dilution of the organic phase or evaporation and replacement of the extractant by a larger volume of other solvent may be necessary (Andruch et al., 2013a). The use of microsample introduction systems have also been reported (Naseri et al., 2008; Baliza et al., 2009). Finally, CE, followed in most cases by UV-Vis detectors, has been used after DLLME (Wen et al., 2012).

DLLME has gained rapid and widespread recognition, attracting the interest of the scientific community and even starting to dominate LPME research publications in recent years (Kokosa, 2013). Despite its wide acceptance, the original configuration of DLLME (Rezaeet al., 2006), termed as classical DLLME, suffers from some drawbacks or limitations that are in continuous revision, namely: (i) environmentally harmful organic solvents denser than water (e.g., chlorinated solvents) are employed as extractants; (ii) emulsification requires a dispersant solvent which competes with the extractant for the analyte, thus reducing EE; and (iii) centrifugation is necessary to separate phases after microextraction. Numerous modifications of classical DLLME have been proposed in order to overcome the above mentioned disadvantages of the technique and to develop efficient, easier and more environmentally friendly approaches. An overview of recent advances is shown in the scheme of Figure 4.17.

One of the most representative modifications is the employment of alternative extractant solvents such as those less dense than water (Kocúrová et al., 2012), ILs (Trujillo-Rodríguez et al., 2013) or supramolecular systems (Moradi & Yamini, 2012b). The use of less toxic non-chlorinated solvents that are less dense than water requires developing new strategies to collect the enriched organic phase after phase separation. Almost simultaneously, Leong and Huang (2009) and Xu et al. (2009) combined low-density solvent-based DLLME with the so-called solidification of floating organic

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**Figure 4.16** General procedure of classical-DLLME.
Dispersive Liquid-liquid Microextraction (SFOD) procedure (Khalili Zanjani et al., 2007). In this technique, the organic solvent, which remains in the upper layer after phase separation, was solidified in an ice-bath, separated from the aqueous phase with a microspatula and then melted at room temperature. Extractant solvents must have a melting point near room temperature (between 10 and 30 °C), be water-immiscible and compatible with the selected analytical technique, have a low vapor pressure to avoid losses by evaporation and a good extractability of the target analytes (Kocúrová et al., 2012). Solvents such as 1-undecanol, 1-dodecanol, 2-dodecanol and hexadecane satisfy these requirements and have successfully been employed in DLLME-SFOD for the determination of both organic and inorganic analytes (Kocúrová et al., 2012). However, the condition of having melting points near room temperature restricts the possibilities of DLLME-SFOD to a few non-chlorinated solvents. Some authors have explored the alternative of using special extraction devices, avoiding the restriction of melting points and the additional freezing step. Different models of homemade vessels have been proposed with the common characteristic of having a narrow upper neck in which the extractant phase is accumulated after centrifugation for its easy collection (Farajzadeh et al., 2009; Hashemi et al., 2009; Saleh et al., 2009). The main limitation of devices is that they are not yet commercially available which hinders their expansion and use in other laboratories. Other original alternatives have been presented in the literature such as the use of disposable polyethylene pipettes (Hu et al., 2010; Guo & Lee, 2011).

**Figure 4.17** Recent advances in DLLME.
and pipette tips (Moreno-González et al., 2012) as extraction units, a capillary tube to collect the extractant phase by simply dipping the tube into the floating organic drop (Farajzadeh et al., 2010), or a long-needle syringe to first evacuate the aqueous phase and then collect the organic phase settled in the conical bottom of the test tube (Bidari et al., 2011). Low-density solvents such as cyclohexane (Farajzadeh et al., 2009), toluene (Saleh et al., 2009; Moreno-González et al., 2012), $n$-hexanol (Hashemi et al., 2009), tri-$n$-butylphosphate (Hu et al., 2010), $n$-hexane (Guo & Lee, 2011), octanol (Farajzadeh et al., 2010) and naphthalene (Bidari et al., 2011) have been employed as extractants in the above mentioned approaches.

The use of ILs in classical DLLME (termed classical IL-DLLME) was described for the first time by Y. Liu et al. (2009). ILs present low miscibility with water, good extractability of organic and inorganic compounds and higher density than water, therefore, they appear to be good candidates to act as solvents in DLLME. The authors used $\text{[C}_6\text{mim]}\text{[PF}_6\text{]}$ as the extractant phase and methanol as the disperser solvent for the determination of heterocyclic insecticides in water samples. Since then, numerous works have employed classical IL-DLLME for the determination of both organic and inorganic analytes (e.g., Trujillo-Rodríguez et al., 2013). In the vast majority of the cases, analytes are extracted from aqueous samples, although more complex matrices such as urine, saliva and cosmetics, have also been explored (Trujillo-Rodríguez et al., 2013).

Classical DLLME requires relatively large amounts of an organic solvent (i.e., acetone, methanol, ethanol or acetonitrile) to disperse the extractant phase. The type and amount of dispersant play a crucial role in the EE. Low volumes prevent the effective formation of the cloudy state whereas high volumes decrease the partitioning of the analyte into the extractant phase, especially for polar compounds. Surfactants have been proposed as alternative disperser agents since they are amphiphilic compounds capable of lowering the surface tension between the two phases and thus facilitating the dispersion of the extractant into the sample (Saraji & Bidgoli, 2010). Cetyltrimethyl ammonium bromide, methyltrialkylammonium chloride, Triton-X or sodium dodecyl sulfate have been employed for the dispersion of both chlorinated (Saraji & Bidgoli, 2010; Deng et al., 2013) and low-density solvents (Moradi et al., 2010; Tehrani et al., 2012) in the so-called surfactant-assisted DLLME. Compared with traditional disperser solvents, lower volumes of surfactant are necessary to generate an effective dispersion and they are more environmentally benign. Nevertheless, the tendency of developing greener methodologies that reduce the use of chemicals and the production of residues, has led to the appearance of new emulsification systems that allow the exclusion of any disperser agent and avoid the competition for the analyte. US energy, vortex agitation, temperature changes, metathesis reactions and air-assisted methodologies have successfully been proposed to assist the dispersion. They are presented and discussed in next sections of this chapter.

The centrifugation step that is needed for phase separation is considered the most time-consuming part and one of the major weaknesses of DLLME. Some authors
have attempted to avoid this step by developing alternative strategies to break the emulsion or simply waiting for the self-separation of the phases (Cruz-Vera et al., 2009b; Andruch et al., 2012a; Farajzadeh et al., 2012). Chen et al. (2010) devised a methodology named solvent-terminated DLLME in which an organic solvent was used as a chemical demulsifier. The term solvent-based demulsification DLLME (SD-DLLME) (Zacharis et al., 2010) has been adopted in subsequent works as the name provides more information about the procedure. Briefly, after forming the cloudy state an appropriate volume of an organic solvent, normally the dispersant, is injected into the mixture and the emulsion is rapidly separated in two phases. SD-DLLME has generally been employed with low-density solvents, although a method using chloroform (Liang et al., 2013) has recently been proposed. SD-DLLME notably reduces operation time due to the elimination of the centrifugation step and presents a potential application in field analysis because electrical connections are not necessary. An important drawback to consider is the larger consumption of disperser solvent which can lead to dissolution of certain analytes and therefore decreasing EEs (Zacharis et al., 2010).

Despite of the enormous popularity of DLLME arising from its simplicity and excellent performance, one of the most concerning restrictions of the technique is the difficulty of complete automation, especially when centrifugation is employed for phase separation. Some authors have resolved this issue by developing different methods that attempt to demonstrate a feasible solution. Automated on-line systems based on sequential injection DLLME have been proposed for metals determination by ETAAS (Anthemidis & Ioannou, 2010) and FAAS (Anthemidis & Ioannou, 2009, 2011). In these approaches, phases were separated by the retention of the enriched phase in a packed microcolumn. Then, an appropriate solvent is employed for the elution and transportation of analytes to the detection system. Other methodologies maintaining the original concept of DLLME and avoiding the retention column have recently been introduced (Andruch et al., 2012a; Maya et al., 2012). However, more efforts are needed to address the challenge of an effective, simple and operational automated DLLME.

Finally, it should be mentioned that DLLME has found its major application in environmental water sample analysis (Yan & Wang, 2013). Organic and inorganic analytes have been directly extracted from relatively simple water matrices (i.e., river, tap, lake, sea and wastewaters). In the most complicated cases, simple pre-treatment operations such as filtration have been included to adapt the real-world matrices to the procedure. The interest of expanding the application of DLLME to bioanalytical (Yan & Wang, 2013) and food analysis (Yan & Wang, 2013; Viñas et al., 2014) fields is continuously growing. Sample pretreatments are then essential due to the incompatibility of the direct extraction of analytes from complex matrices and the difficulty of obtaining suitable extracts for analysis. Previous digestion or extraction steps, especially in solid samples, and operations such as dilution, filtration, degassing, centrifugation, protein precipitation or enzymatic hydrolysis are commonly included prior to DLLME of complex biological and food samples.
4.4.2 Ultrasound- and Vortex-assisted Dispersive Liquid-liquid Microextraction

Regueiro et al. (2008) proposed for the first time the use of US energy to assist the dispersion in DLLME. The effect of US energy in a mixture of two immiscible liquids is based on a number of inter-related complex processes which result in the formation of a stable emulsion and increase the mass transfer speed between the two phases (Regueiro et al., 2008). Thus, UA-DLLME allows the elimination of the disperser solvent which enhances EEs. In some studies, the dispersant, in lower concentrations than in classical DLLME, is still included to ensure the effective formation of the cloudy state.

Different sonication devices are employed for US irradiation in UA-DLLME, namely US baths (Regueiro et al., 2008), US probes (Cortada et al., 2011) or sonoreactors (Cabaleiro et al., 2011). US baths are the most employed option due to their lower cost and widespread availability in analytical laboratories. However, well-known disadvantages of US baths can be mentioned such as the lack of uniformity of US energy and the power declining with time, which lead to problems of reproducibility between extractions (Szreniawa-Sztajnert et al., 2013). In addition, US baths provide an indirect US irradiation since waves have to cross the walls of sample vessel. By contrast, US probes are directly immersed into the sample and supply a more powerful, uniform and efficient US energy.

UA-DLLME has been employed to assist the dispersion of chlorinated and low-density solvents and ILs (Andruch et al., 2013b; Picó, 2013; Szreniawa-Sztajnert et al., 2013). When using organic solvents lighter than water, both the use of special extraction devices (Saleh et al., 2009) and the combination of UA-DLLME with SFOD methodology (Mohamadi & Mostafavi, 2010) have been proposed. Surfactants (Wu et al., 2010) or hydrophilic ILs (Gao et al., 2012) have been included as an emulsifier in some UA-DLLME applications with the aim of improving the dispersion of the extractant phase, reducing extraction times and avoiding the use of traditional organic disperser solvents. Organic and inorganic analytes have been determined in a wide range of samples (e.g., water, cosmetics, fruit juices, soil, plant tissues or biological samples) using UA-DLLME (Andruch et al., 2013b; Picó, 2013; Szreniawa-Sztajnert et al., 2013).

US energy offers advantages when improving the speed and efficiency of the extractions in DLLME. However, the above mentioned drawbacks led to a search for alternative mixing modes, such as vortex agitation. Vortex-assisted dispersive liquid-liquid microextraction (VA-DLLME) avoids the generation of free radicals that normally appear when US energy is employed (Andruch et al., 2013b). In addition, the dispersion under vortex-mixing is unstable, therefore phase separation is easily accomplished. Finally, another important point to consider is the fact that vortex methodology is more cost-effective than US baths or US probes (Andruch et al., 2013b).

VA-DLLME was presented for the first time by Yiantzi et al. (2010). The performance of this novel methodology was illustrated with the determination of alkylphenols in water samples, using octanol as extractant phase and omitting the disperser agent. Since then, several approaches have employed vortex methodology with high-
density solvents (Zhang & Lee, 2012), low-density solvents (Jia et al., 2010), ILs (Asensio-Ramos et al., 2011a), surfactant-enhanced emulsification (Yang et al., 2011) and solvent demulsification (Seebunrueng et al., 2014). A novel mixed mode of US-vortex-assisted DLLME (Cinelli et al., 2013) has recently appeared in which the mixture of sample, extractant and disperser solvent is vortexed before sonication in a US bath. Organic and inorganic analytes have been determined in samples such as water, sediments, hair, urine, fruit juices, milk, beer and wine using VA-DLLME (Andruch et al., 2013b).

### 4.4.3 Temperature-assisted Dispersive Liquid-liquid Microextraction

Temperature-controlled IL dispersive liquid-liquid microextraction (TC-IL-DLLME) was presented by Zhou et al. (2008), demonstrating for the first time that ILs could be used in DLLME. This microextraction methodology is based on the use of a temperature increase for the complete dispersion or solubilization of the extractant phase into the sample. Thereafter, when the mixture is cooled in an ice-water or ice bath the solution becomes turbid and phases are finally separated by centrifugation. Temperatures between 50–90 °C are normally required for IL dissolution where the upper limit is established by the boiling point of water. Extraction time is generally considered as the cooling time, being the most time consuming part of the methodology. Cooling times between 10 and 30 min are usually employed, although shorter extraction times have been reported when disperser agents are included.

Baghdadi and Shemirani (2008) proposed, almost at the same time as Zhou et al. (Zhou et al., 2008), an IL-based microextraction technique named cold-induced aggregation microextraction (CIAME). The general procedure and basic principles of CIAME are the same as in TC-IL-DLLME, although the authors named it differently. In both cases, temperature changes are employed to modify IL solubility and a cooling step is needed for the formation of the droplets of the extractant phase from a homogeneous solution.

TC-IL-DLLME and CIAME methodologies have generally been employed in water sample analysis for the determination of both organic and inorganic analytes (Trujillo-Rodríguez et al., 2013). Imidazolium- and hexafluorophosphate-based ILs have been selected as the extractant phase in vast majority of the reported works to date (Trujillo-Rodríguez et al., 2013).

### 4.4.4 In Situ Ionic Liquid Formation Dispersive Liquid-liquid Microextraction

Simultaneously, Baghdadi and Shemirani (2009) and Jao and Anderson (2009) proposed a novel IL-DLLME methodology based on the in situ formation of the extractant phase. Although the first authors named the technique as in situ solvent formation
microextraction (ISFME) (Baghdadi & Shemirani, 2009), the terms IL-based in situ DLLME or in situ IL-DLLME have been adopted more frequently. In situ IL-DLLME is based on the formation of the extractant phase in the sample solution via a metathesis reaction between a water-miscible IL and an ion exchange reagent to form a water-immiscible IL. Briefly, in the general procedure, the water-miscible IL is dissolved into the sample containing the analytes. Then, the ion exchange salt is added, the cloudy solution is immediately formed and the extraction of analytes occurs simultaneously with the ion exchange reaction. Finally, phases are separated by centrifugation and the enriched phase can be analyzed with the selected analytical technique. Imidazolium-based ILs with chloride or tetrafluoroborate anions have been employed as hydrophilic ILs, whereas hexafluorophosphate salts (i.e., NaPF$_6$, KPF$_6$ and NH$_4$PF$_6$) and lithium bis[(trifluoromethyl)sulfonyl]imide (LiNTf$_2$) have acted as ion-pairing agents. Among the advantages that the in situ IL-DLLME methodology offers we highlight that the dispersion of the extractant IL takes place via a metathesis reaction, therefore, the disperser agent is not needed. Thus, the competition between the IL and the disperser solvent is avoided. Moreover, additional devices such as vortex or US are not necessary to assist the emulsification.

In situ IL-DLLME has mainly been employed for the determination of organic and inorganic analytes in water samples (Trujillo-Rodríguez et al., 2013). More complex matrices such as cereals, marine sediments, fruits, tea and blood have also been explored with the combination of different pre-treatments (Trujillo-Rodríguez et al., 2013). Extraction of DNA from water matrices containing albumin protein or metal ions has been tested using in situ DLLME (Li et al., 2013), proving the advantages of this methodology over other DNA extraction protocols in terms of speed, low consumption of solvents and high EE. Recently, a novel methodology combining in situ IL-DLLME with electrochemical detection using SPELs has been presented (Fernández et al., 2014). Considering the electrochemical properties of ILs and the low volume of the IL phase formed during in situ IL-DLLME, SPELs were perfectly compatible candidates for analyzing the IL drop after microextraction without any further modification. Therefore, the authors employed miniaturized systems both in sample preparation and in the detection stage, and avoided the disadvantages of chromatographic techniques (e.g., special devices or interfaces in GC or shorter column life and resolution problems in LC). The feasibility of the proposed methodology was demonstrated through the determination of 2,4,6-trinitrotoluene in water samples.

### 4.4.5 Supramolecular-based Dispersive Liquid-liquid Microextraction

As mentioned before, exploring alternative extractant solvents has been a recurring theme in DLLME publications and supramolecular solvents (SUPRASs) have received special attention recently. SUPRASs, also named coacervates, are water-immiscible liquids made of large and three-dimensional assemblies of amphiphili-
lic structures (Yazdi, 2011; Moradi & Yamini, 2012b). Amphiphilic compounds form different kinds of aggregations above a critical concentration (e.g., reverse micelles or vesicles) (Moradi & Yamini, 2012b). SUPRASs are formed by the self-assembly of these aggregates induced by an external stimulus which promotes their separation from the bulk solution (Moradi & Yamini, 2012b). Considering this phenomenon, two microextraction modes based on SUPRASs of alkylcarboxylic acids have been proposed in the literature. In the first methodology, reverse micelles of carboxylic acids are formed in tetrahydrofuran and the subsequent mixture with the aqueous sample induces the formation of the separated SUPRAS phase. The addition of water causes partial desolvation of reverse micelles which facilitates their interaction and the formation of larger water-immiscible structures (Moradi & Yamini, 2012b). In the second methodology, coacervates of carboxylic acid vesicles are formed by the action of the tetrabutylammonium cation (TBA). In the aqueous phase, protonated and deprotonated carboxylic acids form small water-miscible vesicles. The addition of TBA causes the formation of larger water-immiscible vesicles based on hydrophobic interactions between the hydrocarbon chains, hydrogen bonding between carboxylic and carboxylate groups and the electrostatic interactions between the carboxylate and TBA groups (Moradi & Yamini, 2012b). In both described methodologies, extraction of analytes is accomplished after SUPRASs formation and phases are finally separated by centrifugation. Special extraction devices are employed due to the low density of the extractant phase, although an alternative using the SFOD methodology has recently been proposed (Moradi & Yamini, 2012a).

The term supramolecular-based DLLME (SM-DLLME) appeared for the first time in 2011 (Jafarvand & Shemirani, 2011). However, this work followed the same procedure and principles as other previous publications (Ballesteros-Gómez et al., 2007, 2008; García-Prieto et al., 2008a, 2008b; García-Fonseca et al., 2010; López-Jiménez et al., 2010). Terms such as supramolecular solvent-based microextraction (García-Fonseca et al., 2010; López-Jiménez et al., 2010; Caballo et al., 2013), reverse micelle-mediated dispersive liquid-liquid microextraction (Tayyebi et al., 2012) or vesicular coacervate phase microextraction (Moradi & Yamini, 2012a) have been adopted to describe an identical methodology, showing the lack of agreement to establish a unified terminology within the scientific community.

SM-DLLME has successfully been employed for the extraction of organic and inorganic analytes from water, food and biological samples (Yazdi, 2011). SUPRASs possess high concentrations of amphiphilies and regions of different polarities which results in a high number of binding sites with different interactions for the analyte (Yazdi, 2011). In addition, the type of interaction can be tuned by varying the hydrophobic or polar groups, and the most appropriate SUPRAS could be designed for a specific application (Yazdi, 2011). Therefore, SM-DLLME has a promising future for the effective extraction of a wide range of analytes, especially polar and ionizable compounds.
4.4.6 Air-assisted Liquid-liquid Microextraction

Air-assisted liquid-liquid microextraction (AALLME) (Farajzadeh & Mogaddam, 2012) is the most recently developed DLLME methodology, appearing in 2012. Farajzadeh and Mogaddam presented a novel approach in which the dispersion of the extractant solvent is obtained by the repeated drawing of sample and extractant mixture into a glass syringe, and subsequent injection into a conical centrifuge tube. The turbidity of the solution continuously increased in every cycle of aspiration and injection and after a certain number of cycles, phases are separated by centrifugation. The effect of the syringe needle dimensions and number of extraction cycles were investigated because were believed to affect the formation of the cloudy state and EE. Results showed that dimensions of syringe needle had negligible effect but EE increased with the number of cycles, remaining constant after a certain number of cycles. This first work also included a comparison with classical DLLME, showing better analytical parameters for AALLME, attributed to the absence of the disperser solvent (Farajzadeh & Mogaddam, 2012).

AALLME has mainly been applied for the determination of organic analytes in water samples (Farajzadeh & Mogaddam, 2012; Farajzadeh & Nouri, 2013; Farajzadeh et al., 2013b), food (Farajzadeh & Khoshmaram, 2013), personal care products (Farajzadeh et al., 2013a) and juices (You et al., 2013). Only one study described the use of AALLME for chromium determination by means of a single-valve sequential injection setup (Alexovič et al., 2013). High-density chlorinated solvents have generally been employed, although low-density solvent-based AALLME has been presented in one publication using a homemade extraction vessel (Farajzadeh & Khoshmaram, 2013).

The most advantageous characteristic of AALLME is the exclusion of the disperser agent without the need for additional devices such as vortex or US, which simplify the procedure and lead to an improvement in the EFs, extraction recoveries and limits of detection (Farajzadeh & Mogaddam, 2012; Farajzadeh et al., 2013b).

4.5 Conclusions

GAC is linked to 12 principles with the aim of reducing the negative impact of chemical analysis on the environment and to enable the implementation of sustainable development principles in analytical laboratories (Gałuszka et al., 2013). Most of the 12 principles negatively affect the quality of analytical parameters (i.e., accuracy, precision, selectivity, sensitivity); therefore, an important challenge is to reach a compromise between increasing the quality of results and improving the environmental friendliness of analytical methods (Gałuszka et al., 2013). Related to this, LPME techniques have strongly helped to achieve this compromise because their numerous advantages fulfill most of the 12 principles without sacrificing the quality of analytical parameters. Therefore, LPME techniques have become widely used since their appearance...
in 1995. Over the last two decades, many modifications and improvements have been made, converting SDME, HF-LPME and DLLME in the three main LPME groups. For the near future, research should be focused on the use of greener reagents (i.e., those obtained from renewable sources), increasing automation and in situ analysis, reducing the use of energy (e.g., more efficient and faster measurement systems) and avoiding toxic reagents that are still in use in order to develop complete sustainable analytical methods.

**Abbreviations**

- [C₄ mim][Cl] 1-butyl-3-methylimidazolium chloride
- [C₄ mim][PF₆] 1-butyl-3-methylimidazolium hexafluorophosphate
- [C₆ mim][PF₆] 1-hexyl-3-methylimidazolium hexafluorophosphate
- [C₈ mim][PF₆] 1-methyl-3-octylimidazolium hexafluorophosphate
- [C₁₀ mim][Br] 1-decyl-3-methylimidazolium bromide
- AALLME air-assisted liquid-liquid microextraction
- AAs amino acids
- CE capillary electrophoresis
- CFME continuous flow microextraction
- CIAME cold-induced aggregation microextraction
- CNTs carbon nanotubes
- DI-SDME direct immersion single-drop microextraction
- DLLME dispersive liquid-liquid microextraction
- dLPME dynamic liquid-phase microextraction
- DSDME directly suspended droplet microextraction
- ECD electron capture detector
- EE extraction efficiency
- EF enrichment factor
- EME electromembrane extraction
- ETAAS electrothermal atomic absorption spectrometry
- FAAS flame atomic absorption spectrometry
- FID flame ionization detector
- GAC green analytical chemistry
- GC gas chromatography
- HF hollow fiber
- HF-LPME hollow fiber liquid-phase microextraction
- HS-LPME headspace liquid-phase microextraction
- HS-SDME headspace single-drop microextraction
- ICP-MS inductively coupled plasma-mass spectrometry
- ICP-OES inductively coupled plasma-optical emission spectroscopy
- IL ionic liquid
Liquid-phase Microextraction Techniques

IMS  ion mobility spectrometry
ISFME  in situ solvent formation microextraction
LC  liquid chromatography
LLE  liquid-liquid extraction
LLLME  liquid-liquid-liquid microextraction
LPME  liquid-phase microextraction
MS  mass spectrometry
NPOE  2-nitrophenyl octyl ether
PAHs  polycyclic aromatic hydrocarbons
PCR  polychloroprene rubber
PEEK  polyetheretherketone
PEME  pulsed electromembrane extraction
PPP-HF-LPME  push/pull perfusion hollow-fiber liquid-phase microextraction
PTFE  Polytetrafluoroethylene
QDs  quantum dots
RSD  relative standard deviation
SBME  solvent-bar microextraction
SD-DLLME  solvent-based demulsification dispersive liquid-liquid microextraction
SDME  single-drop microextraction
SFOD  solidification of floating organic drop
SFODME  solidification of floating organic drop microextraction
SIA  sequential injection analysis
SLM  supported liquid membrane
SM-DLLME  supramolecular-based dispersive liquid-liquid microextraction
SME  solvent microextraction
SPE  solid-phase extraction
SPELs  screen-printed electrodes
SPME  solid-phase microextraction
SUPRASs  supramolecular solvents
TBA  tetrabutylammonium
TC-IL-DLLME  temperature-controlled IL dispersive liquid-liquid microextraction
UA-DLLME  ultrasound assisted dispersive liquid-liquid microextraction
US  ultrasound
USAEME  ultrasound-assisted emulsification microextraction
VA-DLLME  vortex-assisted dispersive liquid-liquid microextraction
VALLME  vortex-assisted liquid-liquid microextraction
W-coil ETAAS  Tungsten coil electrothermal atomic absorption spectrometer.
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