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Review

Green aspects, developments and perspectives of liquid phase microextraction techniques

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ABSTRACT

Determination of analytes at trace levels in complex samples (e.g. biological or contaminated water or soils) are often required for the environmental assessment and monitoring as well as for scientific research in the field of environmental pollution. A limited number of analytical techniques are sensitive enough for the direct determination of trace components in samples and, because of that, a preliminary step of the analyte isolation/enrichment prior to analysis is required in many cases. In this work the newest trends and innovations in liquid phase microextraction, like: single-drop microextraction (SDME), hollow fiber liquid-phase microextraction (HF-LPME), and dispersive liquid–liquid microextraction (DLLME) have been discussed, including their critical evaluation and possible application in analytical practice. The described modifications of extraction techniques deal with system miniaturization and/or automation, the use of ultrasound and physical agitation, and electrochemical methods. Particular attention was given to pro-ecological aspects therefore the possible use of novel, non-toxic extracting agents, inter alia, ionic liquids, coacervates, surfactant solutions and reverse micelles in the liquid phase microextraction techniques has been evaluated in depth. Also, new methodological solutions and the related instruments and devices for the efficient liquid phase micoextraction of analytes, which have found application at the stage of procedure prior to chromatographic determination, are presented.

Abbreviations: AALLME, air-assisted liquid–liquid microextraction; BSED, bell-shaped extraction device; BTEX, benzene toluene ethylbenzene and xylene; CAE, coacervative extraction; CE, capillary electrophoresis; CIAME, cold-induced aggregation microextraction; CPE, cloud point extraction; D-μ-SPE, dispersive micro solid-phase extraction; DD-SDME, drop to drop-single drop microextraction; DLLME, dispersive liquid–liquid microextraction; DLLME-SFO, dispersive liquid–liquid microextraction based on the solidification of a floating organic drop; DLPNE, dynamic liquid phase nanoextraction; DSDME, directly suspended droplet microextraction; DSSBME, dual solvent-stir bars microextraction; EME, electro membrane extraction; EMI, electro membrane isolation; GC, gas chromatography; HF, hollow fiber; HF-LPME, hollow fiber liquid-phase microextraction; HF-SLPME, hollow fiber solid–liquid phase microextraction; HFM-LLLME, hollow fiber membrane liquid–liquid–liquid microextraction; HPLC, high performance liquid chromatography; HS-SDME, headspace single drop microextraction; IL-DMME, ionic liquid-linked dual magnetic microextraction; IL-USA-DLLME, ionic liquid based ultrasound-assisted dispersive liquid–liquid microextraction; IL-USAEME, ionic liquid-based ultrasound-assisted emulsification microextraction; IP-SAME, ion pair based surfactant assisted microextraction; ISFME, in situ solvent-formation microextraction; LLE, liquid–liquid extraction; LLLME, liquid–liquid–liquid microextraction; LPME, liquid phase microextraction; MADLLME, microwave-assisted dispersive liquid–liquid microextraction; MS, mass spectrometry; MWCNT, multi-walled carbon nanotube; PAH, polycyclic aromatic hydrocarbon; SA-DLLME, surfactant-assisted dispersive liquid–liquid microextraction; SBME, solvent bar microextraction; SC-DHF-LPME, solvent cooling assisted dynamic hollow-fiber liquid phase microextraction; SD-DLLME, solvent demulsification dispersive liquid–liquid microextraction; SDCME, single-drop coacervative microextraction; SDME, single drop microextraction; SFOD, solidification of a floating organic drop; SFOME, solidified floating organic drop microextraction; SFVCDME, solidified floating vesicular coacervative drop microextraction; SI-DLLME, sequential injection dispersive liquid–liquid microextraction; SM-DLLME, supramolecular based dispersive liquid–liquid microextraction; SM-LLME, stir membrane liquid–liquid microextraction; SPME, solid-phase microextraction; SPMTE, solid phase membrane tip extraction; SS-BVMME, supramolecular solvent-based vortex-mixed microextraction; ST-DLLME, solvent terminated dispersive liquid–liquid microextraction; SUSME, supramolecular solvent-based microextraction; TILDLME, temperature-controlled ionic liquid dispersive liquid phase micro-extraction; UA-IL-DLLME, ultrasound-assisted ionic liquid dispersive liquid–liquid microextraction; UA-IL-DLPME, ultrasound-assisted ionic liquid dispersive liquid-phase microextraction; UASEME, ultrasound-assisted surfactant-enhanced emulsification microextraction; USAEME, ultrasound-assisted emulsification-microextraction; USAEME-SFO, ultrasound-assisted emulsification microextraction with solidification of floating organic droplet; US-DLLME, ultrasound dispersion liquid–liquid microextraction; VALLME, vortex-assisted liquid–liquid microextraction; VSLLME, vortex-assisted surfactant-enhanced-emulsification liquid–liquid microextraction; VSLLME-SFO, vortex-assisted surfactant-enhanced-emulsification liquid–liquid microextraction with solidification of floating organic droplet

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Contents

1. Introduction

Liquid–liquid extraction (LLE) is one of the oldest extraction techniques used most frequently in the case of aqueous samples with complex matrix composition. The technique is based on sequential treatment of the same sample with fresh portions of a solvent or with a series of solvents of increasing polarity. As a result, various extract fractions are obtained which are enriched with a different analyte or a group of analytes. However, multi-stage analytical procedures are highly time-consuming and labor intense which in consequence results in long exposure of laboratory personnel to harmful vapors from chemical reagents, particularly organic solvents. Moreover, the risk of losing analytes or of sample contamination increases with the increasing number of operations performed on the same sample. Therefore the application of alternative pro-ecological, automated [1], solvent-free extraction techniques or techniques employing a minimal amount of solvents (liquid-phase microextraction techniques, LPME), and those which use safe and non-toxic extractants (e.g. ionic liquids, supercritical liquids, surfactant solutions [2], and supramolecular solvents [3]) has become one of the most popular research topics in analytical chemistry in recent years [4–16]. A definition of liquid microextraction is all modes of sample preparation technique used solvent in volumes of 100 μL or less for analytes extraction [17], allowed the integration of extraction and enrichment of analytes to the level above the method detection limit, as well as the analyte isolation from sample [18].

The use of alternative microextraction techniques for sample preparation reduces the number of errors that commonly result from multi-stage procedures, and limits the negative impact on the environment and the health of analytical chemists performing laboratory work. The reduction of the amount of organic solvents employed during the extraction process translates into lowered utilization costs of waste treatment and spent solvents, which in turn allows the cost reduction of analytical procedures as well as saving money on the purchase of high purity solvents. So, it is clear that the improvement of sample microextraction techniques could be of interest for environment ethic considerations and business opportunities. This new green approach is often described in literature as the three R's, which stands for replace, reduce and recycle (replacement of toxic solvents with green solvents, reduction of solvent consumption and waste production, and solvent recycling) [19]. However, we cannot renounce to the use of high sensitive analytical techniques suitable to obtain multiparametric information about complex samples through the use of chromatography after an appropriate dissolution of samples and preconcentration of the target analytes and in this case, the techniques discussed in this paper offer a good alternative to the most commonly employed long and tedious procedures used for sample preparation and sample clean-up, including analyte preconcentration. The use of ultrasonic irradiation [20], microwaves [21], green extraction medium [22–24], and use of electrochemical support and commercial available autosamplers, are opening alternatives for a fast, nondestructive and low cost processes and for improving the available methodologies, also opening the way for automation and integration of sample treatments and analytical measurements and not only in this way but also improving the extraction processes.

In recent years, one of the most investigated topics in analytical chemistry has been the use of ionic liquids, due to their valuable characteristics: (i) very low vapor pressure, (ii) high viscosity, (iii) high thermal stability, (iv) non-flammability, (v) capable to dissolve a wide spectrum of organic and inorganic compounds and (vi) specific electrochemical characteristics [25–28]. An attempt was also made to use ionic liquids as universal solvents in chromatographic, electrochemical and extraction techniques [29–33]. Due to their capacity to dissolve different organic compounds, ionic liquids are a real alternative to conventional solvents used in liquid–liquid extraction techniques [34]. Therefore investigations of the properties of ionic liquids in relation to their application as solvents are of utmost importance for elaborating efficient extraction procedures [35].

Toxic organic solvents used in liquid extraction techniques can be also substituted by surfactant-based coacervate [2,36] in micellar-mediated extraction techniques, e.g. cloud point extraction CPE [37–41] and coacervative extraction (CAE) [42–44]. Coacervates are large colloidal micelles (drop-shaped microscopic structures) that self-assemble in colloidal systems. Coacervate based on anionic or cationic surfactants is produced by cooling the solution below its cloud point, while in the case of non-ionic surfactants the solution has to be heated above its cloud point [45,46]. Thanks to the semipermeable barrier around the coacervates, the extraction of analytes can take place inside the micelles. Non-polar and low-solubility analytes in an aqueous micellar solution dissolve inside the micelles and aggregate into the surfactant-rich phase, while the remaining aqueous sample contains the diluted surfactant as monomers or dimers at a concentration that approximates its critical micelle concentration. Similarly, in organic solutions, the presence of reverse micelles increases the solubility of hydrophilic substances. After the extraction, the solution is centrifuged, cooled (to increase the micellar phase viscosity), and decanted to be later dispensed into a measuring instrument. Micelle-based extraction techniques are simple, inexpensive and eliminate the need for use of toxic solvents, the problem of the formation of emulsions and lack of sensitivity for more volatile analytes, appearing in the standard liquid–liquid extraction techniques. High capacity to concentrate a wide range of analytes makes the surfactant-rich phases are multipurpose solvents, allowing for a high recoveries and high concentration factors to obtained.

In this paper, microextraction techniques and the application of alternative solvents are discussed in detail, with special emphasis

on strategies for reducing, or even eliminating of use of organic solvent. This deep review based on the most relevant, representative, and the most latest references. This knowledge of the details will be very helpful in making a decision concerning the choice of a particular solution in order to use on the sample preparation step and to make analytical methods greener.

2. Novel solutions in the field of SDME

One of the most popular techniques in which the use of solvents has been significantly reduced (down to a droplet from few nanoliters [47] to microliters) compared to classical liquid–liquid extraction is a single-drop microextraction (SDME). This method, developed in 1996, was originally known under the name Solvent Extraction in a Microdrop [48], or Solvent Microextraction into a Single Drop [49], and previously as other combinations of miniaturized liquid–liquid extraction systems [50–52]. In SDME, the extraction takes place via dissolution of target analytes in a drop of liquid suspended at the end of a microsyringe needle which has been immersed in the sample (extraction medium cannot be miscible with the sample) or extraction could be performed from a headspace above the sample (HS-SDME) [53]. In order to stabilize the suspended drop, different shaped needle tips are applied, i.e. bell-mouthed device [54], flange rod [55], stainless steel net [56] and brass funnel [57].

Information about two other variants of SDME can be found in the published literature, namely, drop to drop-SDME (DD-SDME) and liquid–liquid–liquid microextraction (LLLME). The drop to drop variant, in which analytes are extracted from the sample into a drop of solvent (up to 10 μL), is characterized by high extraction rate, and it eliminates the sample mixing [58] (Fig. 1). In the case of LLLME, the analytes are extracted from an aqueous phase into an organic phase, and then back-extracted into a drop of an aqueous phase. In order to achieve a high level of analyte enrichment, the acceptor and donor phases with proper pH are selected, while basic and acidic properties of the extracted

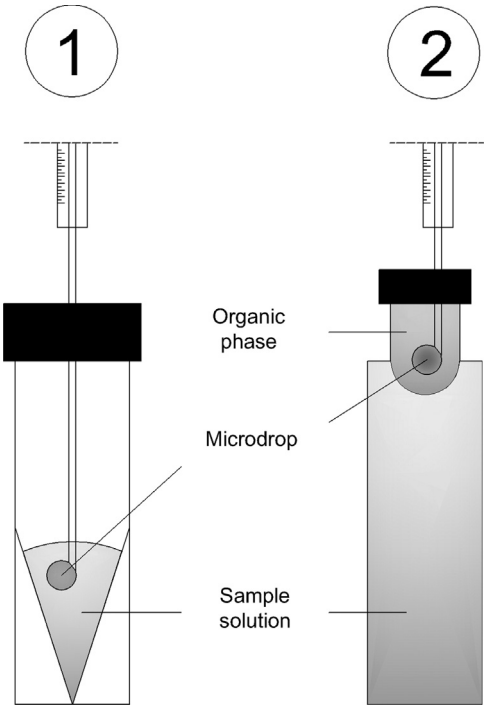

Fig. 1. Extraction of analytes by DD-SDME (1) and LLLME (2).

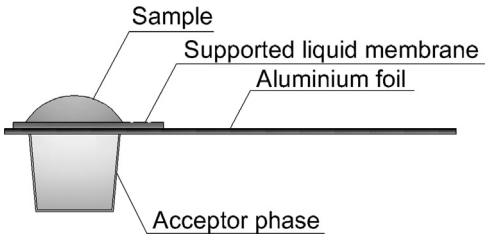

Fig. 2. Schematic diagram of a device for extracting analytes by droplet-membrane-droplet-LPME.

analytes are also considered. Due to these restrictions the LLLME technique is only suitable for ionizable compounds [59]. After the analyte extraction, the drop of liquid sorbent is withdrawn back into the syringe by pulling the plunger and then dispensed into a measuring device for final determination. The analyte extraction by SDME is characterized by short extraction time, low cost, simplicity of operation, and it does not require the use of complex equipment. To increase the mass transfer, a dynamic version of SDME [60,61] and its nano-scale variant, namely, dynamic liquid phase nanoextraction (DLPNE) [62] were also developed. In 2007, SDME was fully automated and became commercially available in the form of an autosampler [63] and by coupling with commercially available sequential injection system [64,65].

The SDME technique was also applied in the miniaturized version of lab-on-a-chip, known under the name of droplet-membrane-droplet-LPME, in which analytes are extracted through a supported liquid membrane into few microliters of an acceptor phase (Fig. 2). A chip used for this extraction method is built from aluminum foil as a small well, containing ca. 10 μL of the acceptor phase. The well is covered with a piece of microporous polypropylene membrane that has been impregnated with the solvent. One drop (10–15 μL) of sample is placed on the membrane. After the extraction, the acceptor phase is withdrawn back with a syringe and dispensed into a measuring device for final determination (capillary electrophoresis with laser-induced fluorescence detection). Also, electrochemically-assisted droplet-membrane-droplet-LPME coupled on-line with microchip capillary electrophoresis was proposed; the method is based on commercially available glass microchips [66].

In the SDME technique, mainly organic solvents (e.g. 1-octanol, toluene, dodecanol or undecanol) are used as extractants because they are compatible with gas chromatography [67]. Additional solvents (e.g. dichloromethane, trichloromethane, and carbon tetrachloride) can also be employed. However, due to their high toxicity their use is becoming limited. In recent publications on novel methodological solutions in the field of SDME, the application of aqueous β-cyclodextrine solution as an extractant was proposed for the headspace extraction of polycyclic aromatic hydrocarbons (PAH) [68]. The limitations of SDME in connection to instability of the drop and the constant evaporation of solvent, due to its volatility and low viscosity, forced scientists to search for replacement solvents. Ionic liquids are the obvious alternative to organic solvents thanks to their high viscosity and surface tension which helps to form a stable drop of a much larger volume [28]. This in turn allows the application of this technique for extracting the analytes from the sample headspace as well as by immersing the drop directly in the sample.

First report about the use of ionic liquids in SDME was published in 2003. The study demonstrated that the extraction of PAH analytes directly from a sample and its headspace is characterized by a three-times increase in the enrichment coefficient as compared to the extraction with an organic solvent (1-octanol) [69]. This particular technique was used for extracting chlorinated anilines from aqueous samples [70] and PAH, benzene,

toluene, ethylbenzene and xylene (BTEX), phthalates, aromatic amines and herbicides [71]. The SDME technique employing ionic liquids as extractants was also used for extracting pesticides [72], phenols [73,74], trihalomethanes [75,76], aromatic amines [77,78], BTEX [79] and PAH compounds [80], residues of cosmetics from urine samples [81] as well as inorganic compounds of mercury from aqueous samples [82] and lead from food samples [83]. Instability of the ionic liquid drop at the end of a needle still remains the most significant limitation of SDME in relation to coupling this technique to high performance liquid chromatography (HPLC). Small volume of solvent (microliter level) is not sufficient for achieving high sensitivity of liquid chromatography determinations [84]. Therefore in 2009, the application of ionic liquids in the dynamic version of liquid phase microextraction (dLPME) was proposed for the efficient extraction of non-steroidal anti-inflammatory drugs [85] and phenothiazine derivatives [86] from urine samples. An automatic flow control device was specifically designed to control the volume of a single liquid drop and the flow rate of analytes. Thanks to this solution, the volume of the ionic liquid drop increased to 50 μL which resulted in the increased sensitivity of final determinations of the aforementioned exogenous compounds. Moreover, a negligible vapor pressure and high thermal stability enable thermal desorption of the analytes from the ionic liquid drop directly inside the gas chromatograph injector [87]. In this case, after the extraction, the syringe with a drop of ionic liquid was placed directly inside the GC injector; the drop was withdrawn back into the syringe after finalizing the thermal desorption of analytes. In order to avoid detachment of the drop from the needle end, it was necessary to enlarge the diameter of the injector insert [88] or to place a piece of glass tubing inside the injector to prevent the transfer of ionic liquid into the chromatography column [89,90].

In 2006, a new modification of the SDME techniques was proposed; a drop of water-insoluble solvent with a density lower than that of water was placed directly onto the surface of an aqueous sample. After the extraction, solvent was withdrawn back into a microsyringe and introduced into the injector of a measuring instrument. This technique has been named directly suspended droplet microextraction (DSDME) [91,92]; it was used for extracting PAH [93], BTEX [94] and tricyclic antidepressants [95] in aqueous samples, and polyphenols in food samples [96]. Liquid-phase microextraction based on the solidification of a floating organic drop/solidified floating organic drop microextraction (SFOD/SFOME) [97,98] is another modified version of the microextraction technique. Here, similarly as in DSDME, an extractant which is less dense than water, immiscible with water and which melts at room temperature, is used as a sorption medium. A drop of extractant is placed on the surface of an aqueous sample, and the sample is stirred throughout the extraction at constant temperature. After the extraction, the vessel containing the sample and extractant is cooled on ice bath until the extraction medium solidifies. Next, the sorptive phase with the absorbed analytes is transferred to another vessel in which it immediately melts. Liquid extraction medium is later dispended into a measuring instrument for the final determination of analytes [99]. The SFOD/SFOME technique has been used for extracting analytes of varying volatility and polarity e.g. pesticides [100,101], esters [102], phenolic compounds [103], and metals from aqueous samples [104–108].

Supramolecular assembly-based coacervates (e.g. surfactant micelles) due to their unique array of physicochemical properties, that render them very attractive to replace organic solvents, are often applied in analytical techniques to extract a variety of organic compounds prior to their separation by LC. Vesicular-based coacervate prepared by mixing of decanoic acid in tetrabutyl ammonium hydroxide and distilled water, was also used as solvent in the SDME technique. This technique is known as single-drop coacervative microextraction (SDCME) [109], and its combination with SFOD technique is known as solidified floating vesicular coacervative drop microextraction (SFVCDME) [110].

In 2012, another modification of SDME was reported. In this case, the extraction of analytes was conducted inside a small-diameter glass tubing (120 cm × 5 mm). A membrane was attached at one end of the tube; the sample, together with a solvent drop and air bubble, was delivered into the tube through the membrane. The extraction of analytes took place after placing the tube in a vertical position, which forced the solvent and air bubble to move towards the upper tube end. When the solvent was approaching the upper end, the whole system was turned upside down to repeat the travel of the drop through the tube. This operation was done a number of times. The aforementioned technique was used to extract pesticides from aqueous samples [111].

3. Novel solutions in the field of HF-LPME

The application of a single drop microextraction carries a risk of detachment of the extractant drop during the extraction process. Moreover, in the case of direct extraction from an aqueous sample, the number of suitable solvents is limited. One way to overcome these drawbacks is to introduce the liquid extractant inside a porous, semipermeable polymeric membrane. The technique in which this solution has been used is known as hollow fiber liquid-phase microextraction (HF-LPME) [112,113]. It requires a small amount of extraction medium (a few microliters) trapped inside the porous polypropylene tube that is attached to the needle of a syringe; the tube is immersed in the sample [114]. The analytes can also be extracted from the sample headspace with the proper attachment of the HF-LPME device above the sample surface. The HF-LPME technique can be used in a two-phase system, i.e. when organic solvent is used to fill both the wall pores and the HF lumen. In a three-phase system option, the hollow fiber lumen is filled with a different solvent than that impregnating the HF wall pores. After the extraction, the extraction phase is drawn inside the syringe by pulling the plunger and then injected into a measuring instrument for the determination of analytes. Hollow fiber can be also modified by coating its inner surface to increase the selectivity of the HF. In the latest reported work, molecularly imprinted polymers were synthesized and coated on the surface of a porous hollow fiber [115,116].

In 2007, a new variant of LPME was proposed. A small amount (ca. 200 μL) of extractant was placed inside the cone-shaped membrane that had been impregnated with a solvent. After the extraction, analyte determinations were performed by using micro-liquid chromatography [117]. A wide choice of available membranes [118] and solvents allows for achieving high selectivity; it also enables HF-LPME to be used for extracting analytes from samples that are contaminated or have complex matrix composition. In addition, efforts were made to employ an ionic liquid in HF-LPME [28,119–121] and in 3-phase extraction technique this technique is known as hollow fiber membrane liquid–liquid–liquid microextraction (HFM-LLLME), which was used for extracting aromatic and aliphatic hydrocarbons [122].

Information about another consecutive variant of HF-LPME is available. In this case, the membrane pores were filled with a solvent in which multiwalled carbon nanotubes (MWCNT) were dispersed. The technique was named as hollow fiber solid–liquid phase microextraction (HF-SLPME). It is characterized by high selectivity and good extraction efficiency in case of organic analytes extracted from aqueous samples. In this system, the analytes diffuse from an aqueous sample via membrane, and are simultaneously retained by both sorptive phases, i.e. carbon nanotraps and an organic solvent. Next, the analytes are back-extracted into an aqueous acceptor phase inside the HF lumen [123]. The extraction process can also be conducted in a two-phase system in

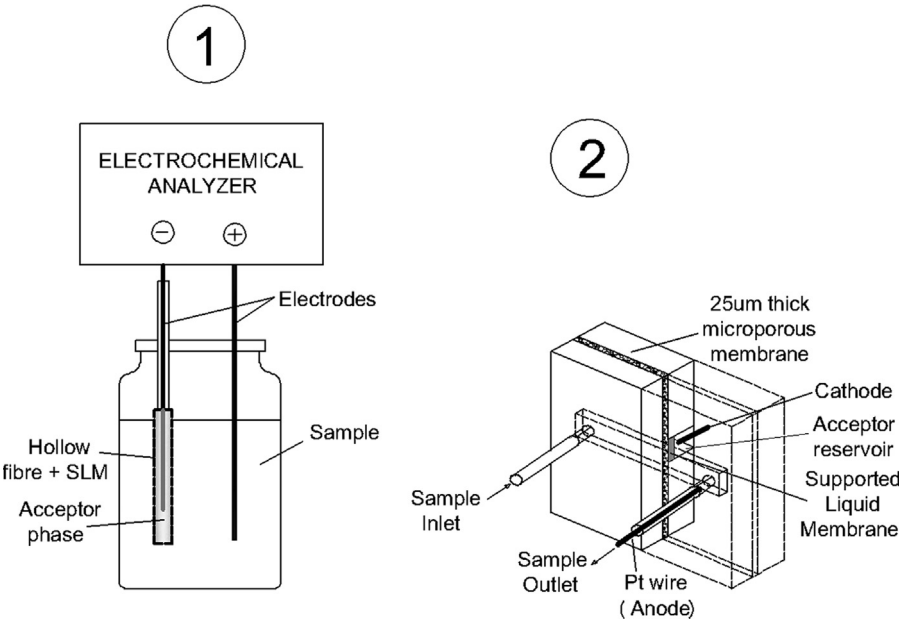

Fig. 3. Schematic diagram of devices employed for extracting analytes by EME (1) and on-chip EME (2).

which the suspension of carbon nanotubes fills the HF lumen and wall pores. In the proposed solution a hollow fiber, enclosed at both ends with magnetic stoppers, was employed as pseudo-stirring system [124]. Another dynamic variant used MWCNTs enclosed in a cone shaped hollow fiber is known as solid phase membrane tip extraction (SPMTE). In this technique membrane was attached to a pipette tip, extraction was performed by withdrawing manually of the aqueous sample through the membrane tip containing MWCNTs, and, after this was released back into the sample. After extraction adsorbed analytes were removed by ultrasonication in acetonitrile [125].

Based on the principles of HF-LPME, a technique named solvent bar microextraction (SBME) [126] was developed in 2004. In SBME, an extractant is immobilized inside the pores of a polypropylene tube (membrane) that is closed at both ends and filled with an acceptor phase (liquid–liquid–liquid system). In another version, the extractant also fills the tube lumen (liquid–liquid system). Such sorptive system either moves freely in the solution stirred with a magnetic stirrer or two closed membranes attached to the magnetic stirrer are propelled by it (dual solvent-stir bars microextraction (DSSBME) [127]). After the extraction, the acceptor phase is removed from the membrane system with a microsyringe and then injected into a GC [128] or HPLC [127]. An attempt was made to use ionic liquids in the SBME technique; in a three-phase system, an ionic liquid was immobilized inside the polypropylene membrane pores, while the membrane lumen was filled with an acceptor phase. Such sorptive system was used for extracting phenolic compounds [129].

The automated dynamic version of the HF-LPME technique have been successfully realized, in this system commercial autosampler was used [130–132]. Moreover, in the dynamic version of HF-LPME it is possible to simultaneously extract analytes from multiple samples [133]. Based on the principles of dynamic-HF-LPME, a novel design, named solvent cooling assisted dynamic hollow-fiber-supported headspace liquid phase microextraction (SC-DHF-HS-LPME), was developed. In this technique, the cooled extractant (at temperature as low as -1 °C) is pumped through a porous polymeric membrane which reduces the solvent loss (due to lowered vapor pressure), extends the extraction time and, as a result, improves the extraction efficiency [134].

A modified version of HF-LPME, in which extraction is electrochemically aided, was also developed. The method is known under two names electro membrane isolation (EMI) [135,136] and electro membrane extraction (EME) [137], and its nano-version with reducing volume of the acceptor phase, from microliters to a few nanoliters [138]. In this technique, an electrode is placed inside the membrane of a classical HF-LPME device (Fig. 3). The extraction proceeds in a three-phase system. Analytes migrate from the aqueous sample, in which the electrode is immersed, through a liquid membrane immobilized in the pores of a polypropylene tube and they enter the aqueous acceptor phase inside the tube where the counter electrode is present. After the extraction, the acceptor phase is withdrawn with a microsyringe to be further analyzed by capillary electrophoresis. In the case of electrochemically aided extraction, the transport of analytes is caused by the potential difference between the acceptor phase and the donor phase. Thus, it is possible to control the technique's selectivity and extraction efficiency by properly choosing the liquid membrane, applied potentials and the pH of the donor and acceptor phases. Electro membrane extraction is fast (the extraction time is 16–17 times shorter than in the classical HF-LPME [136]), very efficient and selective in samples with complex matrix composition as well as biological and environmental samples [135]. At present, EME is widely used for isolating medicines and narcotics [139–144], chlorophenols [145] and peptides [146,147] mostly from biological samples.

In 2010, information about the miniaturized to the microchip level version of EME appeared. In this particular case, the extraction took place in a microchannel (50 μm deep and 2 mm wide) cut in one of the two joined poly(methyl methacrylate) plates that had been sandwiched with a 25 μm-thick porous polypropylene membrane impregnated with the acceptor phase. A platinum wire, used as anode, was attached at the end of a microchannel into which the sample was being pumped in. The cathode was placed in the opening of the other plate containing a small amount (couple of microliters) of the acceptor phase. After the extraction and the disconnection of the applied voltage, the acceptor phase, i.e. 2-nitrophenyl octyl ether or dodecyl acetate was withdrawn with a micropipette to be analyzed by capillary electrophoresis with UV detection [148] or coupled to electrospray ionization mass spectrometry [149]. The most recent publication describes the system which is analogous to those presented earlier.

However instead of the potential difference the difference in pH between the acceptor phase and the sample was used to force the extraction of analytes [150]. The microscale EME technique was applied to extract pharmaceuticals in a fast, selective and efficient way.

4. Novel solutions in the field of DLLME

In 2006, the use of a novel liquid phase microextraction technique was reported. It involved the extraction of analytes from aqueous samples into a small volume of organic solvent dispersed in the sample. In the original version of dispersive liquid–liquid microextraction (DLLME) the dispersion of the extractant drops was achieved by using a third liquid phase which was immiscible with both, the sample and the extractant, and served as dispersant [151–153]. After the extraction, samples are shaked and later centrifuged in order to separate the extraction phase. The obtained extraction phase is dispensed to the appropriate measuring instrument. Because DLLME can be potentially used as a sample preparation technique a number of studies have been undertaken to increase the efficiency of analyte extraction of this method [154–156]. The resulting novel designs and methodological solutions are presented in the form of a schematic diagram in Fig. 4.

In the case of DLLME, organic solvents denser than water are most frequently used as extractants because they enable simple phase separation by sample centrifugation. However, the number of such solvents is limited. Moreover, the necessity to eliminate toxic solvents, such as chlorinated hydrocarbons, has aimed the search for alternative solvents to be applied in DLLME technique. Information on the use of solvents less dense than water as extractants in DLLME can be found in recently published studies. The application of such solvents enables extraction without the use of a dispersing agent; it also eliminates the sample centrifugation step. Special vessels suitable for conducting extractions with extractants less dense than water have been designed, namely, a vessel with the narrow neck in which an extraction phase is collected [157,158], a special extraction vessel for the USAEME technique [159], a specially designed flask for magnetic stirring-assisted extraction [160], an automated version of syringe with stirring bar placed in it for in-syringe DLLME [161–165], and a glass tube (120 cm × 5 mm *i.d.*) capped at the lower end with a membrane through which extractant and dispersing agent are injected [166]. There are a significant number of successful analytical applications of ionic liquid in DLLME technique by four modes: conventional, in situ, temperature-assisted, and microwave-/ultrasound-/vortex-assisted [167].

In 2007, a new technique was proposed which offers a combination of advantages displayed by DLLME and SFOD [97];

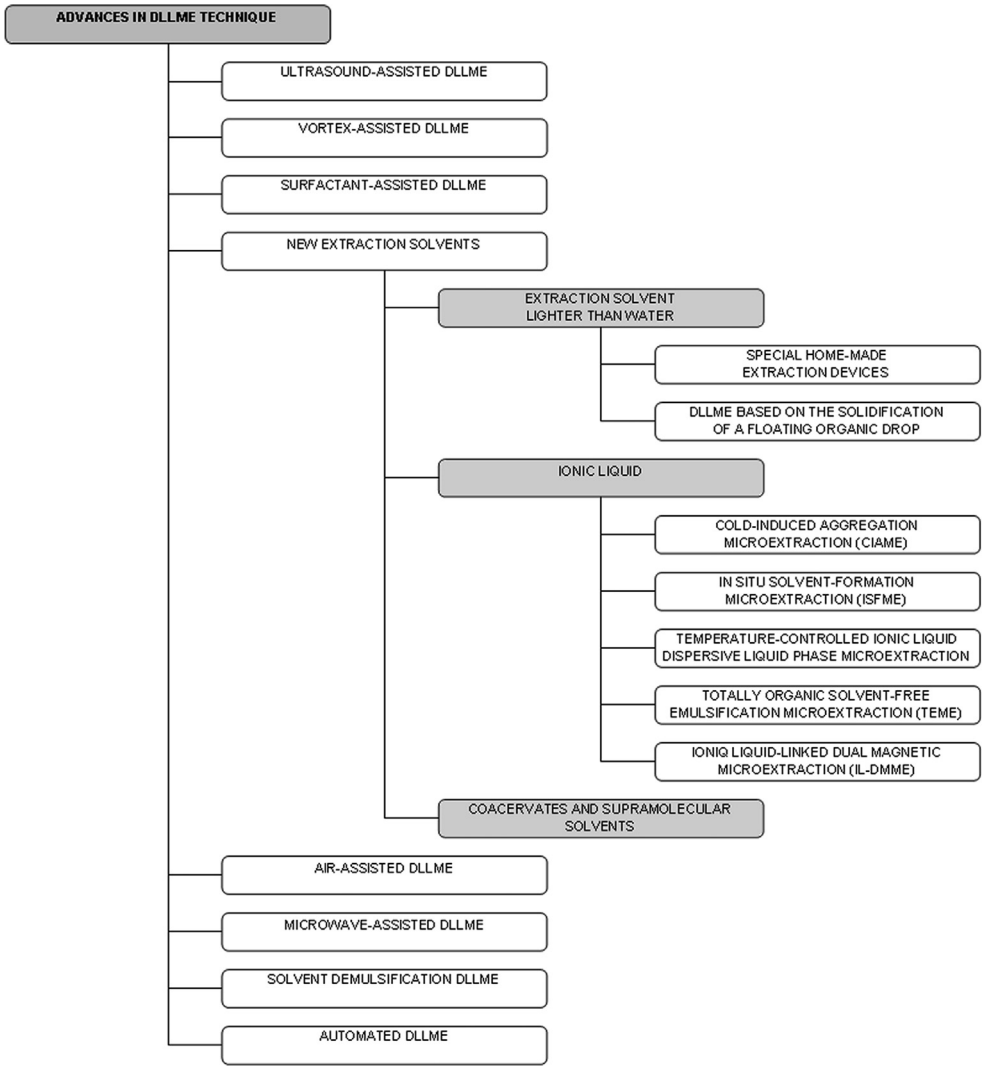

Fig. 4. Novel designs and innovative methodologies in the field of DLLME.

it is known as dispersive liquid–liquid microextraction based on the solidification of a floating organic drop (DLLME-SFO). Solvents less dense than water and characterized by a melting point close to room temperature are employed as extractants. In this technique, extraction takes place according to the scheme of a classical DLLME, i.e. a solvent (usually 1-undecanol or 1-dodecanol) together with a dispersing agent is injected into the analyzed sample; the sample is centrifuged and placed in an ice bath to solidify the extracting agent which had collected on the sample surface. The solidified extract is removed with a small spoon and then transferred into another vessel where it melts. Melted extract is analyzed by chromatography. The DLLME-SFO technique is used for extracting analytes of varied volatility and polarity e.g. PAH [168,169], PCBs [170], pesticides [171,172], and metals [173–178] from aqueous samples.

The application of a third, dispersion phase (usually 0.5–2 μL of methanol, acetone or acetonitrile) may decrease the value of the distribution ratio therefore, at present, the increasing trend is to aid the process of dispersion/emulsification by the ultrasound treatment (ultrasound-assisted emulsification-microextraction (USAEME) and ultrasound dispersion liquid–liquid microextraction (US-DLLME) [179–181]).Ultrasonic irradiation enhances the formation of the fine cloudy solution, speeds up the mass transfer between sample and extraction phases, and reduces the equilibrium time. Ionic liquid-based ultrasound-assisted emulsification microextraction technique (IL-USAEME) is also known, in this technique ionic liquids were used as extraction solvent [182]. It is also known as another variant of this technique, known in the literature as ultrasound-assisted emulsification microextraction with solidification of floating organic droplet (USAEME-SFO) using solidification of centrifuged emulsion droplet in an ice bath [183]. In this concept avoids the problem of the need to use high-density and toxic extraction solvents commonly used in the USAEME technique replacing them by solvents lighter than water having near room temperature melting points [184–186]. Another method of assisting the emulsification is the physical mixing by agitation (vortex-assisted liquid–liquid microextraction (VALLME) [187–190]). Using a vortex agitation is more cost-effective than an ultrasonic radiation, and the dispersion formed is thermodynamically unstable, causing the phase separation is easier. It has also been attempted to use ionic liquids as extracting solvents in VALLME [191]. Ultrasound- and vortex-assisted extractions enable the elimination of a dispersion agent, the coalescence effect and the stirring-induced heating effect, and also help with phase separation after centrifugation [192]. Moreover, the very small droplets of an extractant create a significantly larger interface area between the two immiscible liquids and improve the mass transfer between the phases, which in turn results in the improved analyte extraction from aqueous samples. In order to avoid the use of a toxic dispersing solvent, it was proposed to replace it by a surfactant solution; the two novel technique variants are known as surfactant-assisted dispersive liquid–liquid microextraction (SA-DLLME) [193,194] and ion pair based surfactant assisted microextraction (IP-SAME) [195]. Ultrasound-assisted surfactant-enhanced emulsification microextraction (UASEME) [196,197] and vortex-assisted surfactant-enhanced emulsification liquid–liquid microextraction (VSLLME) [198–200] (also in version with low-density solvent [201]) are the respective ultrasound- and agitation-assisted versions of the aforementioned techniques. Both techniques are also modified by combining the advantages of surfactant-enhanced emulsification microextraction and solidification of floating organic droplet methods: UASEME-SFO [202] and VSLLME-SFO [203]. In these techniques, low density solvents having near room temperature melting points was used as extraction solvent, solidified in ice bath after its centrifugation.

In 2012, a novel version of DLLME technique was proposed, known as air-assisted liquid–liquid microextraction (AALLME). In this concept, much less volume of an extraction solvent is used, and there is no need to apply of a disperser solvent [204]. Organic droplets were formed by sucking and injecting of the mixture of sample solution and extraction solvent with a syringe for several times in a conical centrifuge tube. After the extraction, samples are centrifuged in order to separate the extraction phase, and later were determined by GC-FID [205,206]. Simultaneous derivatization and extraction by the AALLME technique was also proposed [207].

It has been reported that in the case of DLLME it is possible to omit the following steps in the analytical procedure: sample centrifugation, mixing, emulsification in an ultrasound bath, the removal of the extraction phase by freezing, and the latter being an additional step called demulgation. Demulgation is conducted after the extraction by adding the additional portion of a dispersive solvent which plays a role of a demulgation agent. The emulsion quickly separates into two phases. Demulgation-based extraction techniques can be found in literature under the names solvent terminated dispersive liquid–liquid microextraction (ST-DLLME) [208] and solvent demulsification dispersive liquid–liquid microextraction (SD-DLLME) [209], the latter being performed by using Pasteur pipettes. In the two aforementioned techniques, solvents less dense than water are employed as an extraction phase which allows for withdrawing the extract from the sample surface with a syringe. The described demulgation-based techniques were applied to the extraction of PAHs [209], carbamate [208] and organochlorine pesticides [210] from aqueous samples.

A fully-automated version of DLLME, named sequential injection dispersive liquid–liquid microextraction (SI-DLLME), is also available. Here, samples of extraction phase are dispensed in an on-line mode into flame atomic absorption spectrometer [211] and electrothermal atomic absorption spectrometer [212,213] atomizers. In SI-DLLME, the dispersing solvent, extraction solvent and chelating agent are mixed with the aqueous sample stream. Next, the extraction phase containing a complexed analyte is retained on a filled microcolumn (separation based on relative retention), and later eluted with isobutyl methyl ketone into the atomizer of the atomic absorption spectrometer. In 2012, another automated version of the DLLME technique was developed in which all reagents in the holding coil of an *SIA system* are mixed in a cone-shaped vial. After the extraction and self-separation of phases, the extract is drawn into a micro-volume Z-flow cell to be spectrophotometrically analyzed [214]. The automated DLLME variant can be used for extracting metal ions only with final determinations performed by spectrophotometry, which is a significant limitation.

The use of coacervates and supramolecular systems for the analyte extraction by the DLLME technique has also been reported, this technique is known as supramolecular based dispersive liquid–liquid microextraction (SM-DLLME) [215,216]. In the case of SM-DLLME, coacervates consist of reverse micelles of decanoic acid dispersed in an aqueous solution of tetrahydrofuran, this solution is added to the sample. After the extraction, the analyte-enriched coacervates are separated from the sample by centrifugation. Fiber optic-linear array detection spectrophotometry [217] or flame atomic absorption spectrometry [218] have been used for the final determination of analytes. In the described system, tetrahydrofuran plays a double role, as a dispersant of the extraction phase and to stimulate the decanoic acid micelles to self-assemble. In contrast to the classical version of the DLLME technique, SM-DLLME is characterized by short extraction time (less than 1.5 min), can be used for extracting hydrophilic analytes (i.e. polar compounds with a wide range of polarity), and does not requires the use of toxic solvents and sample mixing. Another variant of DLLME technique with using supramolecular solvents and ionic liquid is known as supramolecular solvent-based vortex-

mixed microextraction (SS-BVMME) [219]. In this combination supramolecular solvent was formed by dispersion of ionic liquid in butanol. During extraction of analytes the mixture was shacking by vortex-agitator, subsequently supramolecular solvent was separated from the sample by centrifugation, mixed with acetonitrile and analyzed by HPLC. Very similar solution, supramolecular solvent-based microextraction (SUSME), consumes less organic solvents and provides very high preconcentration factors was proposed. In this technique supramolecular solvent with sample was magnetically stirred to disperse the supramolecular solvent in the aqueous suspension, thus accelerating the extraction of the target analytes, and finally, centrifuged again, and after separation of extraction phase was analyzed by the liquid chromatographic system [220]. Supramolecular solvent production was done in a specially-designed centrifuge cone by dissolving octanoic acid [221] or decanoic acid [222–227] in THF and then shaking to formation supramolecular solvent into the bulk solution.

In the case of DLLME an attempt was undertaken to apply ionic liquids as extractants because it is possible to change their properties (viscosity, surface tension, and hydrophilicity/hydrophobicity) by selecting an appropriate cation–anion system [28]. In the case of DLLME, ionic liquids were used, inter alia, for extraction of aromatic compounds [228,229], heterocyclic insecticides [230] and PAHs [231] from aqueous samples, non-steroidal anti-inflammatory drugs from urine [232], pesticides from food samples [233,234]. In DLLME, ionic liquids can also be used for extracting cadmium [235] and chromium [236] from aqueous media, followed by electrothermal atomic absorption spectrometry. For these analytes, it was necessary to use a complexing agent and a surfactant in order to eliminate the effects of adhesion between the ionic liquid and the vessel wall [237]. An ultrasound-assisted variant of the DLLME technique, which employs ionic liquids as extractants is also known. This technique can be found in literature as ionic liquid based ultrasound-assisted dispersive liquid–liquid microextraction (IL-USA-DLLME) or as ultrasound-assisted ionic liquid dispersive liquid-phase/liquid microextraction (UA-IL-DLPME/UA-IL-DLLME) and is used for extraction of e.g. metals [238–241] pesticides [242–244], aromatic amines [245] from water samples. Also microwave-assisted variant of DLLME technique with application of ionic liquid (MADLLME) was developed for the preconcentration of triazine herbicides from water samples [246].

Considering theoretical basis of dispersive microextraction, three consecutive modifications of the technique, differing in the mode of extractant dispersion, were elaborated. Ionic liquids were also used as an extraction phase in all of them. In the technique known as cold-induced aggregation microextraction (CIAME), an ionic liquid is dissolved in the heated sample and then the vessel is cooled in an ice bath in order to obtain a cloudy solution. Next, the vessel is centrifuged and the analyte-enriched extractant sedimented at the bottom of the vial. In another technique, known as in situ solvent-formation microextraction (ISFME), extraction of hydrophobic species occurs during in-situ formation of fine droplets of the hydrophobic ionic liquid by addition of the hydrophilic ionic liquid and the ion-pairing agent to the sample. The extract is separated by sample centrifugation and later dispensed into a measuring instrument [247,248]. The dispersion effect can also be achieved by controlling the temperature. This phenomenon has been employed in the technique named temperature-controlled ionic liquid dispersive liquid phase micro-extraction (TILDLME). In TILDLME, the dispersing solvent is not necessary. A drop of ionic liquid dispensed into the sample dissolves completely due to heating of the sample. Next, the drop becomes analyte-enriched via sample cooling which results in the formation of visible extract drops. The TILDLME technique was used for extracting pyrethroid [249], organochlorine [250] and phosphate [251] pesticides from aqueous samples. Similar solution was applied in technique known as totally organic solvent-free emulsification microextraction procedure (TEME). In the conical tube sample and ionic liquid were subjected to ultrasonic treatment for cloudy solution formation; then heated in a temperature-controlled water bath, afterwards cooled with simultaneous ultrasonic treatment for full extraction the analytes from the very fine droplets. The mixture was then centrifuged and the IL extraction phase was analyzed by HPLC [252].

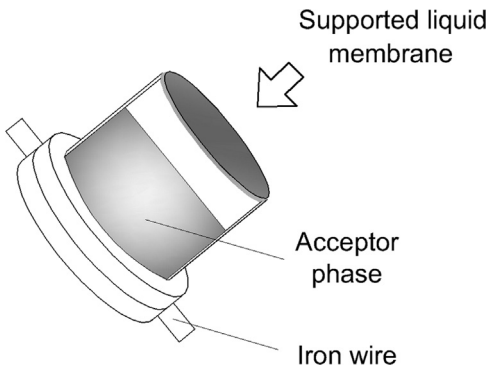

Fig. 5. Schematic diagram of a device for the analyte extraction by SM-LLME.

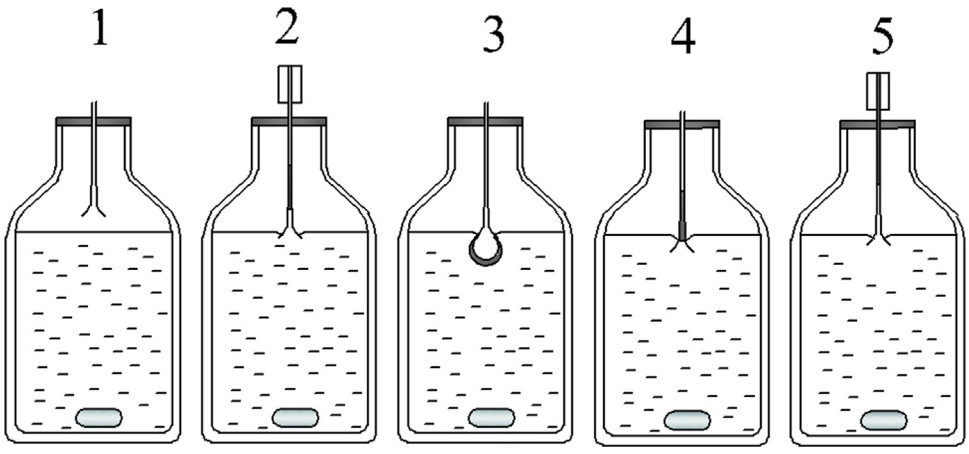

Fig. 6. Schematic diagram of a device for the analyte extraction by BSED-LPME. (1) placing a bell-shaped device in the vessel, (2) placing the funnel-shaped part of the device inside the sample and filling with a solvent, (3) forming a stable layer of solvent, (4) pushing the extraction phase toward the upper part of the device by immersing it deeper in the sample, and (5) withdrawing the extraction phase by microsyringe.

In 2012, a novel combination of DLLME and dispersive micro solid-phase extraction (D-μ-SPE) techniques was proposed, known as ionic liquid-linked dual magnetic microextraction (IL-DMME) [253]. In this technique ionic liquid is used as extraction medium, which was agitate by vortex with sample to accelerate the formation of the fine droplets, after extraction Fe_3O_4 magnetic nanoparticles were added to the tube and again vigorously shaken by the same vortex. The IL phase with analytes was successfully extracted on nanoparticles after then magnet was subsequently held around the vial to collect the nanoparticles at the bottom of the vial. Aqueous phase was removed, and IL phase with analytes were desorbed to solvent solution and the nanoparticles were isolated from solution with a magnet. This method showed high preconcentration factor and low detection limit and eliminates the need for application of toxic dispersive solvent used in conventional DLLME [254–256].

5. New methodological solutions in the field of liquid phase microextraction techniques

In recent years, additional efforts have been made to improve the liquid–liquid extraction techniques through the development of simple and original devices, suitable to be produced at a reduced cost; that improves the classical separation processes by avoiding drawbacks of the previously mentioned techniques.

Membrane techniques are finding an increasingly broader application in a classical version of liquid–liquid extraction. It is

Table 1
Advantages, disadvantages and trends of liquid phase microextraction techniques.

TECHNIQUE	ADVANTAGES	DISADVANTAGES
SDME[a]	Inexpensive, rapid, simple and almost solvent-free No especial equipment required Combined with many techniques for determination of the analytes Easy to operate Versatile (numerous solvents can be used) Possibility of in situ derivatization or complexation Variety of extraction modes High enrichment factor obtained	Instability of the drop Restrictions on the selection of extraction solvent Ease of dislodgment of the microdrop Limited drop volume Limited rate of agitation of the sample solution Average precision Limited drop surface (slow kinetics) Special equipment required (SFOME)
Development of SDME technique	Application of ionic liquids and cocervates as an acceptor phase Modifications of the SDME technique: dynamic-LPME, dynamic liquid phase nanoextraction (DLPNE), droplet-membrane-droplet-LPME, directly suspended droplet microextraction (DSDME), solidification of a floating organic drop microextraction (SFOD/SFOME), single-drop coacervative microextraction (SDCME), and solidified floating vesicular coacervative drop microextraction (SFVCDME)	
HF-LPME[b]	Inexpensive, simple, clean-up Supported of solvent on membrane pores Possibility of automation and miniaturization Combined with many techniques for determination of the analytes High versatility and selectivity Headspace and immersion modes Possibility of in situ derivatization	Memory effects when reusing membranes Pre-conditioning of membranes Average precision performed in manual mode Most of studies carried out in static mode Higher sampling time and temperature compared to SDME (lower evaporation rate)
Development of HF-LPME technique	Modifications of the HF-LPME technique: hollow fiber membrane liquid–liquid–liquid microextraction (HFM-LLLME), hollow fiber solid–liquid phase microextraction (HF-SLPME), solid phase membrane tip extraction (SPMTE), solvent bar microextraction (SBME), dual solvent-stir bars microextraction (DSSBME), solvent cooling assisted dynamic hollow-fiber-supported headspace LPME (SC-DHF-HS-LPME), dynamic-HF-LPME, electro membrane isolation (EMI), electro membrane extraction (EME), and on-chip EME	
DLLME[c]	Inexpensive, simple and fast Easy to operate Possibility of automation Enormous contact area between the acceptor phase and sample Combined with many techniques for determination of the analytes Fast extraction kinetics High enrichment factor obtained	Three solvents are needed Restrictions on the selection of extraction solvent Centrifugation/freezing/auxiliary solvent/demulsifier must be applied
Development of DLLME technique	Modification of the DLLME technique: ultrasound-assisted emulsification-microextraction (USAEME), ultrasound dispersive liquid–liquid microextraction (US-DLLME), vortex-assisted liquid–liquid microextraction (VALLME), in-syringe DLLME, surfactant-assisted-DLLME, ion pair based surfactant-assisted microextraction (IP-SAME), ultrasound-assisted surfactant-enhanced emulsification microextraction (UASEME), vortex-assisted surfactant-enhanced-emulsification liquid–liquid microextraction (VSLLME), solvent terminated-DLLME, solvent demulsification dispersive-DLLME, sequential injection-DLLME, supramolecular based dispersive liquid–liquid microextraction (SM-DLLME), air-assisted liquid–liquid microextraction (AALLME), DLLME variants with SFO, cold-induced aggregation microextraction (CIAME), in situ solvent-formation microextraction (ISFME), and temperature-controlled ionic liquid dispersive liquid phase microextraction (TILDLME), totally organic solvent-free emulsification microextraction procedure (TEME), ionic liquid-linked dual magnetic microextraction (IL-DMME), supramolecular based dispersive liquid–liquid microextraction (SM-DLLME), supramolecular solvent-based vortex-mixed microextraction (SS-BVMME), supramolecular solvent-based microextraction (SUSME), and microwave-assisted DLLME (MADLLME) Application of ionic liquids, coacervates, supramolecular systems and solvents with a density lower than water as extraction solvents	

[a] SDME – single drop microextraction.
[b] HF-LPME – hollow fiber liquid-phase microextraction.
[c] DLLME – dispersive liquid–liquid microextraction.

due to the fact that they reduce the amount of solvent used and eliminates the process of emulsion forming during the extraction. Polymeric membranes which are employed in these techniques are usually made of cellulose acetate, polyamides and polyethylene; they are characterized by diverse structure, high porosity, low production cost and simple production technology. By combining the advantages of membrane techniques and liquid–liquid microextraction, a technique called stir membrane liquid–liquid microextraction (SM-LLME) has been developed. Here, the device used for extraction consists of two coaxial cylinders sandwiched with a polytetrafluoroethylene membrane and a metal rod at the bottom; thanks to the rod the device spins during the extraction [257] (Fig. 5). After the extraction, the device is placed in a small amount (500 μL) of methanol in order to desorb the analytes retained on the membrane. The final analyte determination is performed by GC–MS. In a more recent publication, instead of desorbing the analytes with a solvent, an extraction mixture of solvents (50 μL) was employed for filling the space between the membrane and the outer cylinder wall. After the extraction, the mixture was withdrawn with a microsyringe [258]. The SM-LLME device was applied to efficiently extract PAH analytes [257] and chlorophenols [258] from aqueous samples.

In 2012, an original, novel and inexpensive design of the liquid-phase microextraction device was proposed which could be used for conducting the analyte isolation and enrichment in aqueous samples, namely, a bell-shaped extraction device-LPME (BSED) [259] (Fig. 6). The BSED device is made of transparent polypropylene. During the extraction, the funnel-shaped part of the device is placed halfway inside the sample, while its upper narrow part is filled with a solvent less dense than water by using a microsyringe. When the sample is stirred the solvent forms a stable layer which overlays the aqueous phase of the spinning sample. After the extraction, the device is immersed further in the sample which pushes the extraction phase toward the upper part of the device. The extraction phase is then withdrawn with a microsyringe and dispensed into the measuring instrument (GC–MS) for the final determination of analytes. The application of BSED-LPME at the sample preparation step prior to chromatographic analysis allows the elimination of the problems and inconveniences associated with the use of other liquid–liquid microextraction techniques, for example, slow analyte diffusion into the solvent via membrane in HF-LLME; and a limited number of suitable solvents and problems with maintaining stability of the solvent drop (SDME). In the case of BSED-LPME, a wide range of organic solvents can be employed. Therefore this technique can be used to sample organic contaminants of diverse volatility and polarity from aqueous environmental samples. The BSED was used to collect samples of volatile and semi-volatile organic compounds present in aqueous samples at trace level [259].

6. Conclusions

Environmental monitoring and up-to-date assessment of the state and the contamination level of specific environmental compartments as well as of the dynamics of man-induced changes in the natural environment often require the determination of analytes in samples with complex and varying matrix composition. Moreover, determination of analytes which are present in samples at a very low concentration level usually requires analytical procedures that include a preliminary step of the analyte isolation/enrichment. However, conventional multi-step liquid–liquid extraction procedures cannot be automated therefore they are labor-intensive. This, in turn, results in a long exposure time of the laboratory personnel to harmful vapors originating from organic solvents. Moreover, the risk of analyte loss and possible sample contamination is increased by subjecting the sample to a relatively big number of operations. Therefore the application of sample preparation techniques that require minimal amounts of solvents (i.e. microextraction techniques) or techniques employing safe and non-toxic extraction media as extractants, for example, ionic liquids, supercritical liquids, and supramolecular solvents is one of the most frequently studies topics in today analytical chemistry. Information on the disadvantages and advantages of the most popular liquid phase microextraction techniques (i.e. SDME, HF-LPME, DLLME) are compiled in Table 1. The table also contains literature data on the trends and novel modifications of these techniques that are based on different approaches like chip-level miniaturization and/or systems automation; the use of ultrasounds, mechanical agitation, and electrochemical procedures; or the solidification of extractants. Novel devices for liquid-phase extraction (i.e. HF-SLPME, SBME, DSSBME, SM-LLME and BSED) have been also presented. The application of microextraction techniques to analytical chemistry will result in large monetary savings in relation to purchasing high purity solvents and costs derived from collecting and utilizing spent solvents. On the other hand, the use of microextraction techniques will decrease the environmental impact of analytical chemistry laboratories as well as the exposure of the laboratory personnel to the vapors of harmful compounds. So, it can be concluded that on scaling down the size of the extraction processes, advantages can be obtained from the economical and environmental point of view, also improving the main analytical characteristics of the methods based on the strong reduction of the number and duration of analytical steps and the reduction of problems related to analyte losses and sample contaminations derived from long and intensive sample pretreatments.

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