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Liquid - phase microextraction and electromembrane extraction in millifluidic devices : A Tutorial.

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Abstract

This tutorial discusses how to integrate different microextraction procedures into millifluidic platforms and the applicability of such systems for the determination of acidic and basic drugs. Sample preparation techniques have been downscaled into a millifluidic format and the replacement of conventional analytical systems by miniaturized alternatives has increased during recent years due to the small volume consumption of toxic solvents and sample required, shorter extraction times, simple-handling and low cost, among others. This review comprehensively summarizes the development of liquid-liquid extraction into a millifluidic device in a three-phase configuration, with focus on (a) historical development, (b) extraction mechanisms and performance, (c) operation modes and automatization, (d) operational parameters, (e) applications, and (f) future directions and perspectives.

Keywords

Millifluidic device, sample preparation, electromembrane, liquid phase microextraction, supported liquid membrane

1. Introduction

In recent years, the miniaturization of the analytical procedures has been gaining importance due to its advantages over traditional methods. Millifluidic-based extraction systems use significantly lower sample consumption compared to traditional LLE and has been widely used in the last years. Miniaturized systems not only consume less sample volume, but also allow faster extraction, improve the portability, reduce the costs (low cost material) and allow the possibility of coupling to the analytical instrument for on-line analysis, among others. Traditional liquid phase microextraction (LPME) and electromembrane extraction (EME) are well-established techniques that have been applied in different fields, as environmental, pharmaceutical and food analysis,

among others [1,2]. Their theoretical principles have also been comprehensively described in different studies [3]. Firstly, millifluidic devices have been demonstrated in two-phase (inmiscible phases) [4] and three-phase configurations for the extraction of different compounds [5]. The first two-phase configuration in a millifluidic system based LPME was reported in 2000 [4] and this configuration was already compressively well described in recent reviews where the different operational schemes have been enumerated, either using a fluid interface in the form of a drop or film or using supported membranes [6,7]. Also, Alexovic et al, reviewed the microextraction techniques with [8] and without supported liquid membranes [9] for the extraction of compounds in immiscible phases and the different approaches. Since two phase LPME into a fluidic device has been already deeply reviewed, only LPME and EME in a three phase configuration will be discussed in this work.

The instability of the interface area in millifluidic systems (in two phases) together with the main disadvantage of the instability of the suspended drop in traditional systems (single drop microextraction, SDME), came up with the employment of supported liquid membranes (SLM). The use of SLM helped to overcome those stability problems, which has been widely used for the integration of LPME and EME into a fluidic device. The first millifluidic platform based LPME in a three phase configuration was reported by Audunsson et at in 1986 [5] where the author designed two different devices, consisting of two titanium or Teflon blocks, separated by a flat membrane which supports the organic solvent. Jönsson and coworkers also reported different studies and bibliography revisions on three phase SLM integrated in millifluidic platforms [10, 11]. In 2010, Petersen et al. presented for the first time a downscaled EME in a millifluidic device for sample preparation [12] using polymethylmethacrylate (PMMA). PMMA (a transparent, low-cost, light and reusable material) became the most suitable material for carrying out LPME and EME into a millifluidic device. Shortly after that, several developments associated with the miniaturization of LPME or EME were proposed. Initially, the proposed devices were limited to the determination of a single analyte [13,14] or analytes from a single family with similar properties [15-21]. Millifluidic devices offered very high extraction efficiencies by LPME [17] and EME [19] with very low enrichment factor under double-flow conditions. Later, fluidic devices with new geometries allowed higher enrichment factor with lower extraction efficiency under stopped-flow conditions, resulting in longer extraction times [19] compared to double-flow conditions. A comprehensive study between two different working modes was recently reported

by Ramos Payán in 2017 [22]. This study was applied to the simultaneous extraction of two different families of acidic drugs (non-steroidal antiinflamatories (NSAIDs) and parabens), resulting in high extraction efficiency and high enrichment factor under double-flow and stopped-flow, respectively. In double-flow, both solutions (acceptor phase and sample) are moving with a laminar flow in the same direction. Under stopped-flow, the acceptor solution is stationary, whereas the other phase (usually the donor phase) is always mobile. The method significantly decreased the sample consumption and the extraction time compared to previously described method for the extraction of NSAIDs and parabens. The reported procedure was successfully applied in biological (saliva and human urine) and environmental samples.

In the present, simultaneous extraction of acidic and basic drugs in a single step is a challenging task since the acceptor phase composition for each extraction is significantly different, according to their acidic or amino ionizable group (based on their pK_a). Several techniques were reported for simultaneous extraction of acidic and basic drugs using either the combination of LPME and EME [23] or double-EME in traditional set up [24,25] which required longer extraction time (over 15 min) and more sample volume (over 600 µL). Traditional set-up offered low extraction efficiencies and did not allow consecutive extractions. However, in 2016, the simultaneous extraction of basic and acidic drugs was addressed integrating EME by joining two individual millifluidic platforms using an external peak tube for one sample solution and two independent acceptor phases [26]. This procedure worked under stopped-flow conditions in order to pre-concentrate the sample, required 40 min extraction, did not allow more than one consecutive extraction and the final acceptor extract was diluted by half before a single injection into HPLC.

Recently, a new versatile millifluidic device was presented in order to overcome the limitations from previous devices. This new device allowed combining several or different techniques (for example LPME and EME) in a single fluidic platform without the necessity of an external tube, offering higher extraction efficiencies, shorter extraction times and lower sample consumption. It could combine either: (a) EME and LPME, (b) EME and EME, or (c) LPME and LPME using a common acceptor phase or a common sample solution. As the most recent design presented up to date, the proposed millifluidic device could be used for either (a) simultaneous extraction of drugs from different nature or (b) separation of drugs from a common sample solution [27]. Shortly after, a modification of this last geometry was addressed by Yamini for the simultaneous determination of acidic and basic drugs (using two model analytes) via EME [28].

This tutorial is focused on the development of three phase membrane-based microextraction techniques (LPME and EME) in millifluidic platforms, including a discussion of its operation modes, types of geometries and configurations, established methodologies, reported applications and the future perspectives in this field based on the advantages and drawbacks. This tutorial is discussed with the idea to motivate the scientist community into this fascinating area.

2. Fluidic device designs and extraction principles

Millifluidic devices have been developed integrating two different techniques: LPME or EME. In LPME, the extraction mechanism is based on the passive diffusion of the analyte from the donor to the acceptor phase due to a pH gradient between both phases as driving force and the analyte must be neutral in the sample solution and ionized in the acceptor phase. On the other hand, EME is based on electrokinetic migration when a potential difference is applied between two electrodes and the analyte is ionized in both phases. Three important considerations in membrane extraction are the extraction efficiency (EE%), the enrichment factor (EF) and the relative recovery (RR%). The extraction efficiency is defined as the percentage of the mole numbers of the analyte extracted into the acceptor phase respect to the moles number of the analyte originally present in the donor solution. The enrichment factor is defined as the ratio of the analyte concentration in the analyte-containing acceptor to the initial concentration of analytes in the donor solution. The final analyte concentration in the analyte-containing acceptor is determined according to a calibration graph obtained from the direct injection of the standard solutions of the analytes. The relative recovery is defined as the percentage of the amount of analyte recovered in the acceptor solution from spiked real samples.

2.1 A single channel millifluidic device design

Millifluidic devices have mostly been designed for single extractions via LPME or EME. Figures 1A and 1B summarizes a scheme of the mechanism for millifluidic LPME and millifluidic EME devices, respectively, in their most general forms for a single channel. A straight microchannel with a T junction is the most common. In these devices, the flat membrane is located between two plates (made with a material which does not adsorb the analytes, usually PMMA) which contain one channel each (one for the sample solution and another for the acceptor solution, both symmetrical). At both ends of the channels, small i.d. holes (for example 1.4 mm i.d.) are drilled

through the plate to serve as inlet and outlet for the sample and acceptor solution. The channels must be aligned and the whole assembly is carried out either by solvent-assisted bonding with ethanol and cured in a 70°C oven [13] or by using four or six screws which make it reusable [17]. Both solutions are introduced by syringe pumps. To ensure that the channels are completely full without any bubbles, several minutes must past for SLM stabilization before starting the first extraction. The acceptor phase (outlet) is either collected and injected into the instrumental analysis for off-line analysis or coupled to an analytical instrument for its on-line analysis. For EME, small platinum wires are inserted into the outlet of the sample and the inlet of the acceptor channels, or along both channels of each phase (at the bottom of each channel).

2.2 A multiple channel millifluidic device design

LPME and EME were simultaneously integrated into a single millifluidic device by using multiple channels [27]. As seen in figure 2, the device consisted of three symmetrical PMMA layers and four channels assembled as a sandwich device. Figure 2A and 2B shows a two dimensional and three dimensional mechanism profile, respectively. The second layer contain two channels (the common acceptor phase) and are faced to the corresponding sample solutions separated by one membrane each.

In the first step, the analytes are extracted from the first sample solution to the acceptor solution by EME. In the second step, the analytes are extracted from the second sample solution to the common acceptor solution by LPME. This gives way for combining the advantages of both EME and LPME for the simultaneous extraction of very different nature compounds [23, 27]. Another example reported is the combination of dual EMEs in a single device [28]. This millifluidic platform consists of three layers containing three symmetrical channels in the form of a serpentine and combines a common donor phase and two individual acceptors.

3. Operation modes and automation

3.1 Operation modes

Two different working modes are generally distinguished: double-flow (or dynamic conditions) and stopped-flow conditions (or semi-dynamic conditions). As previously reported, depending on

the operation mode, high extraction efficiencies or high enrichment factors can be achieved under double-flow and stopped-flow conditions, respectively [22].

For non-miniaturized LPME and EME set-ups, both the donor and acceptor phase are generally kept stationary, for high enrichment factor to be achieved when there is a high-volume ratio between the phases. In millifluidic devices, where there is not a high-volume ratio between the phases, a high EF can be achieved by either (a) increasing the sample flow rate respective to the acceptor phase, under double-flow conditions or (b) increasing the sample flow rate while keeping the acceptor phase in stagnant conditions at different extraction times. Based on the reported results, it can be concluded that double-flow conditions lead to higher extraction efficiencies, while stopped-flow conditions achieve higher enrichment factors [12, 15-17,19, 22].

Under double-flow conditions, the extraction efficiency is expected to be lower as the sample flow rate increases due to the decrease in residence time of the sample. Under this operation mode, sample recirculation is not an attractive option since the extraction efficiencies are close to 80-100 % for a single extraction [17]. For this reason, very low acceptor and sample flow rate (about 1 μL/min) are desirable to enhance the efficiency. In addition, an increase of the sample flow rate may not significantly increase the enrichment factor [18].

On the other hand,stopped-flow mode is used when high EF is desirable. A decrease of the sample flow rate (which increase the contact area time) would involve a rise in the extraction time for a good preconcentration, and thus, instability in SLM. For this reason, the extraction time and the sample flow are two parameters that depend on each other and must be optimized together [19,22].

3.2 Automation

Millifluidic platforms have been demonstrated for off-line and on-line measurements. For off-line analysis, the analyte-containing acceptor phase is collected and subsequently injected into an analytical instrument, as for example, a high-pressure liquid chromatography (HPLC), capillary electrophoresis (CE) or Mass spectrometry (MS) [16,29]. However, these devices can be coupled to analytical instrument for on-line analysis. Figures 3A and 3B show the scheme of millifluidic extraction systems for on-line analysis coupled to an HPLC or MS, respectively. The donor phase introduction is usually carried out with syringes while the sample output is discarded [29]. Alternatively, it can be re-injected in the system to carry out several extractions of the same sample [1,2]. Nevertheless, it should be pointed out that the described off-line procedures usually can be

converted into an on-line procedure by the connection of their respective acceptor phase outlets to the analytical instrument. Although the procedure can be largely automated, the donor and acceptor solution syringes must be filled manually, meaning that full automation is not possible to date. Thus, an improvement in the automation process must be investigated in further research.

4. Operational parameters

The following operational parameters must be optimized for millifluidic systems based on LPME and EME: the geometry of the device, the donor and acceptor phase composition, the organic solvent (extractant), the sample and acceptor flow rates, the extraction time (under stopped flow systems) and the applied voltage (for EME). A stable miniaturized system for LPME and EME is important for reproducibility and robustness reasons, especially for EME since a low and stable current is required.

4.1 Supported liquid membrane (flat membrane)

Alternatives to conventional techniques have been developed using membrane-based techniques, resulting in an important advance in microextraction procedures. In LPME and EME, the organic solvent is immobilized into the pores of a membrane resulting in the supported liquid membrane (SLM). Membranes behave as selective barriers under certain conditions which makes it possible to determine analytes from complex matrices. As mentioned above, the use of membranes has improved the procedure robustness and reproducibility, and has also offered a more stable extractant phase.

There is a large variety of membranes with different structures, transport properties and separation mechanism and the most commonly used membranes in microextraction procedures are either organic or inorganic [2]. Two types of membranes can be distinguished according to the chemical nature and the type of polymer: hydrophobic or hydrophilic. Different flat membranes have been used in microextraction procedures, as for example polypropylene membranes of different porosity, as well as those composed of polydimethylsiloxane, carboxen/polydimentylsiloxane and polydimethylsiloxane/divinylbenzene, which have been applied as a clean-up and preconcentration procedure.

However, in millifluidic systems based LPME or EME, hydrophobic flat membranes (such as polypropylene) with a pore size of 0.2 µm, a porosity of 55% and a wall thickness between 25-200

μm have been the membrane most commonly used [22,26]. The selectivity and the separation process is significantly dependent on the size, shape and distribution of pores in a membrane. In millifluidic systems, a flat membrane is located between the sample and acceptor channel and the molecules (analytes) are extracted through the membrane from the sample to the acceptor phase by a process of diffusion (LPME) or by a gradient of electrical potential (EME). Recently, the importance of membrane thickness has been studied since it plays a key role in cross- SLM transfer of some model analytes [30]. The experimental results showed that thinner membranes offered faster and higher mass transfers through the membrane (25 μm thick membrane) and decreased the organic solvent consumption.

On the other hand, the extractant supported in the membrane (organic solvent) must offer high selectivity and good extraction efficiency for the compounds of interest. The most suitable organic solvent as supported liquid membrane (SLM) is directly dependent on the type of extraction principle used, either LPME or EME, the analytes properties and the material of the device in order to avoid any crack. Different solvents have been used as SLM, as for example: 1-octanol, diexylether, nitrophenil octyl ether (NPOE), 1-heptanol, 1-pentanol, among others. Unlike LPME, the organic solvent used for EME must meet certain requirements, i.e., organic phase must have a certain dipole moment or electrical conductivity to withstand a flow of relatively low current in the system, and it also needs certain chemical properties to allow the electrokinetic migration of the analytes. The stability of the SLM has been studied under double-flow conditions, observing that consecutive extractions can be carried out within a standard deviation of 2-4% [17,18].

4.2 Geometry

The geometry of the system (microchannel length, depth and width) plays an important role in the effectiveness of millifluidic devices, and consequently, in the extraction efficiency. Different geometries have been reported offering very different extraction efficiency and enrichment factor values. Some examples of different length (mm), width (mm) and depth (mm) sizes that have been tested are: 6x2x0.05 [12-14,16], 30x1x0.2 [19,33], 30x1x0.5 [20], 30x1x0.7 [21], 30x0.5x0.2 [26], 50x1x0.5 [28], 13x2x0.08 [17], 15x2x0.04 [18], 23x3x0.12 [22], 15x2x0.12 [27] and 23x3x0.06 [34].

In LPME, deeper sample channels lead to lower extraction efficiencies and hinder the passive diffusion of the analytes since the molecules are farther away from the supported membrane. In

EME, the increase in channel depth does not decrease the efficiency extraction in the same degree, as the extraction is carried out (and reinforced) by the electrical field generated by the two electrodes and not only by the passive diffusion of the analytes (a transport phenomenon that is always slower). In addition, the depth of the channels in EME depends on the diameter of the electrodes when they are placed along each channel. Regardless of whether it is EME or LPME, a very shallow channel could lead to the clogging of the donor phase, especially in the case of viscous samples. A compromise of the depth, length and width must be taken into account in order to keep a stable and regular laminar flow and to maintain the miniaturization benefits.

4.3 Sample and acceptor phase composition

The composition of both phases (acceptor phase and sample) will depend on the extraction technique (LPME or EME), as previously described. In LPME (passive diffusion mechanism), the analytes are neutral (uncharged) and charged (positively or negatively) in the sample solution and acceptor phase, respectively; whereas in EME, the analytes are charged in both phases. In EME, basic analytes are extracted across the SLM with a positive electrode placed in the sample and a negative electrode placed in the acceptor solution, and vice versa for acidic drugs extraction. The sample and acceptor composition and its pH depend on the pK_a value of the analytes of interest [31,32]. Additionally, sample and acceptor phase composition must be controlled to avoid pH shifts, especially when integrating EME where a negligible pH shift in both solutions and a low and stable extraction current is desirable. The employment of solutions with buffer capacities is recommended to maintain the pH of the acceptor and sample (donor) solution.

4.4 Sample and acceptor flow rate

Sample and acceptor flow rates are two effective parameters in the extraction efficiency. The enrichment factor decreases with the increase of the acceptor flow rate, whereas a low sample and acceptor flow rate increase the contact time and lead to an increase of the extraction efficiency.

However, the pressures of both phases (when introducing the sample and acceptor solution) must be carefully controlled to prevent one phase from penetrating the other or breaking the membrane. The major challenge is maintaining a stable and liquid stationary phase (under stopped-flow mode) when there is only pressure by the sample solution towards the membrane. In stopped-flow conditions, the maximum mass transfer that can be achieved only corresponds to the phase of

equilibrium after an extraction time. On the other hand, a no compensation flow rate between both phases can also break the membrane or alter the laminar flow under double-flow conditions (especially when the flow difference between both phases is over 20 µL/min) [18].

4.5 Voltage

For EME, voltage is also optimized. The selectivity of the millifluidic EME system depends significantly on the direction and magnitude of the electric field applied. A low and stable current (about nA or few µA) is required since a very high current is often associated with a decrease in the extraction efficiency due to bubbles on electrodes and pH shifts due to electrolysis.

5. Applications

Table 1 and table 2 summarize the applications for LPME and EME into millifluidic platforms, respectively, in a three phase configuration. As seen, the HPLC-UV technique has been the most applied instrumental technique [5,10,15,17,18-21,26,28,29]. Alternative detection systems include MS [13,14,16] and CE [12,16].

5.1. Millifluidic device integrating LPME

The procedures summarized in Table 1 follow the general scheme described in the Figure 1A. The first developed millifluidic system for LPME was applied to the determination of amperozide in human plasma [5,10] employing a modified dialysis system. The SLM was obtained by soaking a porous poly(tetrafluoroethene) (PTFE) sheet with dihexyl ether [5] or 5% tryoctil phosphine oxide in dihexyl ether [10]. These devices were coupled to an HPLC-UV system and the extraction was carried out using a stopped-flow procedure achieving extraction efficiencies in the order of 2 - 10%, which were improved by the recirculation of the donor phase for successive extractions of the same sample. Basic drugs (such as amitriptyline, methadone, haloperidol, loperamide and pethidine) were either analyzed off-line by CE for exact quantification, on-line by UV detection or electrospray ionization mass spectrometry (ESI-MS) for time profiling of concentrations [16]. Extraction efficiencies were in the range of 52-91% under double-flow conditions when analyzed off-line by CE. Also, the extraction was carried out online by coupling the acceptor outlet of the device to a UV detector. In that case, the device was tested under stopped-flow conditions using a sample flow rate of 3µL/min whereas the syringe pump (containing the acceptor phase) was turned

off during the extraction. After 120 min extraction, the concentrated sample (acceptor phase) was transferred to the UV detector, obtaining an enrichment factor of 500 with a sample volume consumption of 360 µL. A potential application of the device was applied for the study of the metabolism of amitriptyline by rat liver microsomes under stopped-flow conditions, obtaining an EF of 70. The system was coupled on-line to ESI-MS by replacing the standard ESI needle of a standard HPLC-MS system in the ES nebulizer assembly with a 14 cm long and 50 µm i.d. fused silica capillary, whereas the other end of the spray capillary was directly coupled to the acceptor outlet of the device using a Teflon tube. This demonstrated for the first time that LPME into a millifluidic platform had great potential for very efficient analyte enrichment from limited sample volumes (as biological fluidics). Later, in 2014, five alkaloids (morphine, codeine, thebaine, papavarine and noscapine) were extracted from water samples by using carriers, resulting in efficiencies between 17-45% [29]. In this miniaturized online LPME-HPLC system, the membrane liquid could be regenerated automatically between every third injections, allowing full automatization without any operator interaction.

Acidic drugs were extracted for the first time in 2005 by Wang et al. [15]. Haloacetic acids were extracted using a millifluidic device (2 cm x 2 cm size) via LPME-HPLC-UV. The extract could both be collected and injected off-line to the HPLC or it could be also collected in the sample loop of the HPLC injector for direct on-line analysis. Different modules with acceptor and donor channels of different depths were machined and the effects on the extraction performance were compared, observing higher enrichment factor using shallower channels. A study of different operational modes were carried out, obtaining EF between 10-90 under stagnant conditions and EE between 16-50% under double-flow conditions. The highest enrichment factor (between 60-70) was reached using an acceptor flow rate of 3 µL/min. In the last years, other acidic drugs have been extracted by LPME. Ramos et al. reported a method for the extraction of five non-steroidal antiinflamatories (salicylic acid, ketoprofen, naproxen, diclofenac and ibuprofen) and its subsequent offline analysis by HPLC [17].

After 5 min extraction, the efficiencies were over 87% (double-flow conditions) and 5 µL sample volume. After 25 min extraction and a sample consumption of 500 µL (operating under stopped-flow), a high EF were obtained (between 29-75). Successful application of the procedure was made in environmental water and biological samples (urine and saliva). However, it was necessary to collect acceptor extracts under stagnant conditions due to the low volume capacity of the acceptor

channel. Parabens were also extracted after 5 min, resulting in over 80% efficiencies and good EF (9-11) requiring only 50 µL of superficial water. Later, a new versatile millifluidic device for stopped-flow and double-flow was reported [22]. A geometric modification resulted in high extraction efficiencies and good enrichment factor for double flow and stopped-flow conditions, respectively. Under stopped-flow conditions (20 µL/min sample flow rate), the highest enrichment factors (between 16 and 47) were obtained after 20 min extraction; whereas for all compounds the extraction efficiencies were within the range of 27–81%. This device was tested under double flow conditions, obtaining good but lower enrichment factors (between 9 and 20) and higher extraction efficiencies (between 45 and 95) after 7 min extraction, consuming a volume sample of 140 µL.

5.2. Millifluidic device integrating EME

Millifluidic platforms based EME has been mainly applied for the extraction of basic analytes. In 2010, Petersen et al. miniaturized for the first time a EME method under stagnant conditions [12]. Compared to traditional EME set-ups, this proposed system resulted in faster mass transfer and high extraction efficiencies (between 20-60 %) in short time. The method was applied to the extraction of five basic drugs (pethidine, nortriptyline, methadone, haloperidol and loperamide). In this way, a double-flow mode was presented for its coupling directly with UV/MS detection, providing recoveries up to 86 % [13]. Later, in 2014, millifluidic devices based EME coupled with ESI-MS was used to monitor in vitro drug metabolism in real time under double-flow conditions [14]. Later, an introduction of two consecutive millifluidic devices were made to extract basic drugs with different properties using two identical devices connected by two external tubes [19] (as shown in figure 4A). This resulted in efficiencies over 95% under stopped-flow conditions (semi-dynamic mode), and a consumption of 1000 µL of sample after 33 min extraction. A further development was introduced by Yamini et al, in 2016, where two individual millifluidic devices integrating EME were only connected by an external tube (containing the common sample phase) [20] as shown in figure 4B. With this method, acidic and basic drugs (betaxolol, diclofenac, mefenamic acid) were simultaneously extracted from urine and human plasma for the first time, resulting in EF between 15 and 18 after 33 min extraction and a sample consumption of 1000 µL (under stopped-flow conditions). Most recent, pulsed EME into a millifluidic device followed by HPLC-UV was developed for the analysis of codeine, naloxone and naltrexone as model analytes in biological fluids [21]. In this work, a solution was proposed to apply a pulsed electrical voltage

as an electrical driving force for the migration of ionized analytes, which provided effective and reproducible extractions. This solution could successfully overcome the disadvantages of applying constant voltage.

5.3. Millifluidic device integrating LPME/EME

LPME and EME have been simultaneously integrated into a millifluidic format for the first time [27] (as shown in figure 2). This device allowed to combine LPME and EME using two sample solutions and one common acceptor solution. Analytes with very different nature (fluoroquinolones and parabens) were extracted by LPME/EME, resulting in very high extraction efficiencies. The procedure significantly reduced the sample consumption and extraction time compared to other techniques for the simultaneous extraction of different classes of analytes. The versatile design proposed represented an important advance in millifluidic systems since it allowed any combination for LPME and EME either using two sample solutions and one acceptor solution, or vice versa.

5.4. Millifluidic device integrating EME/EME

Recently, a modification of the previous device was reported for simultaneous determination of acidic and basic drugs in a single millifluidic device [28]. The device allowed the extraction of Nalmefene and diclofenac by a double EME after 33 minutes extraction and a sample consumption of 1 mL. The procedure was successfully applied to urine samples obtaining enrichment factors of 17 and 19 and extraction efficiencies of 40 and 43 for diclofenac and nalmefene, respectively.

6. Future directions and perspectives

The current tutorial has discussed the miniaturization of different extraction principles into a millifluidic device. LPME methods present attractive sample preparation techniques by their simplicity of operation and a straightforward automation. The miniaturization into a millifluidic device has offered advantages such as the decrease of the extraction time and the sample volume consumption, portability, an excellent clean-up, low-cost, simple handling and increase the extraction efficiency under double-flow conditions. These millifluidic platforms can also be on-line coupled to analytical instrument for their automation, allowing their reusability and several consecutive extractions in most cases. However, its complete automation should be investigated

in future studies to avoid that the syringes have to be filled manually. In addition, the devices are environmentally friendly due to the minor amount (3-5 μL) of organic solvent employed. Potential future directions of development of these devices are related to their present drawbacks and the new challenges could be focused on: (1) the increase of enrichment factors, (2) the development of new applications and (3) automatization of the whole process. Automatization also gives the possibility to increase the sample throughput what brings LPME closer to routine analysis, especially for biological fluidics analysis (urine and plasma) when very few microliters of sample is available. The potential for acceptance and implementation in routine/research laboratories of microextraction in millifluidic devices is very high and there are automatic pumps available which allow automation of the whole process - from the first to the last step.

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TABLES AND FIGURES

Table 1. Millifluidic platforms for LPME approaches in a three phase configuration.

Table 2. Millifluidic platforms for EME approaches in a three phase configuration.

Figure 1. A single millifluidic device design: Acidic drugs extraction by (A) LPME, (B) EME and (C) real picture of a millifluidic platform for LPME.

Figure 2: A multiple millifluidic device design for LPME/EME: (A) Two dimensional and (B) three dimensional mechanism profile.

Figure 3. Automation: Millifluidic device integrating LPME coupled to (A) an HPLC instrument and (B) a MS instrument.

Figure 4. Miniaturization into a millifluidic device of EME/EME for (A) simultaneous extraction of basic drugs from different properties and (B) simultaneous extraction of acidic and basic drugs.

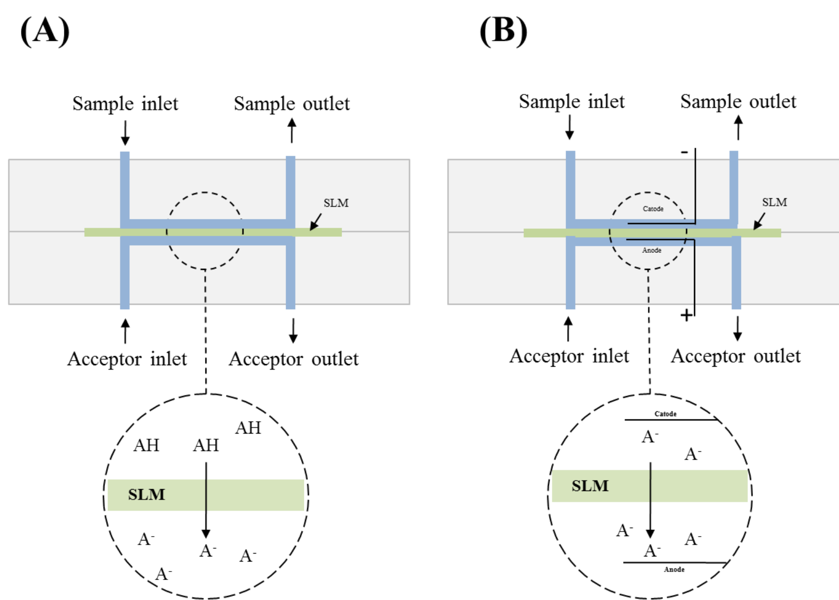
Analyte	Extraction time (min)	offline/ online	Analytical procedure	Matrix	Sample volume (µL)	Flow (µL/min) DF: double-flow SF: Stopped-flow Sample flow/acceptor flow	EF[a]	EE[b] (%)	Ref.
Amperozide	15 - 20	Online	HPLC-UV	Blood plasma	800	SF: 180/0	-	35	[10]
Haloacetic acids	30	Online	HPLC-UV	Water	62 – 228	SF: 126/0 DF: 126/4.4	10 - 65	16 - 50	[15]
Amitriptyline, Methadone, Haloperidol, Loperamide, Pethidine	7	Offline	CE	Standard solutions	10	DF: 1/1*	-	52 -91	[16]
Amitriptyline	10-120	Online	UV,MS	Rat liver microsomes	30-360	SF: 3/0*	42-500	-	[16]
Morphine, Codeine, Thebaine, Papaverine, Noscapine	5	Online	HPLC-UV	Urine	50	DF: 5/0.5*	-	17 – 45	[29]
Salicylic acid, Ketoprofen, Naproxen, Diclofenac, Ibuprofen	7	Offline	HPLC-UV	Urine, saliva and water samples	7	DF: 1/1*	-	87-100	[17]
Ethyl paraben, Propyl paraben, Isobutyl paraben, Butyl paraben	5	Offline	HPLC-UV	Natural waters	50	DF: 10/1*	9 - 11	84-100	[18]
Salicylic acid, Ketoprofen, Naproxen, Diclofenac, Ibuprofen, Ethyl paraben, Propyl paraben, Isobutyl paraben, Butyl paraben	20	Offline	HPLC-UV	Urine samples	400	SF: 20/0	21-47	27-81	[22]
	7	Offline	HPLC-UV	Urine samples	140	DF: 20/1*	9-19	44-94	[22]
Norfloxacin, Ciprofloxacin, Danofloxacin, Marbofloxacin	7	Offline	HPLC-UV	Urine	10	DF: 1/1*	-	35-62	[34]

*Allow consecutive extractions
[a]Enrichment Factor
[b]Extraction efficiency

Analyte	Extraction time (min)	offline/online	Analytical procedure	Matrix	Voltage (volts)	Sample volume (µL)	Flow rate (µL/min) DF: double-flow SF: Stopped-flow Sample flow/acceptor flow	EF[a]	EE[b] (%)	Ref.
Pethidine, Nortriptyline, Methadone, Haloperidol, Loperamide	10	offline	CE	Urine	15	25	SF: 2.5/0	2 - 15	20 – 60	[12]
Amitriptyline	12	online	UV,MS	Rat liver microsomes	15	108	DF: 9/3	>75	65-86	[13]
Amitriptyline	50	online	MS	Rat liver microsomes	15	1000	DF: 20/20	-	> 48	[14]
Amitriptyline, Nortriptyline	33[c]	offline	HPLC-UV	urine	40	1000	SF: 30/0	17-18	34-36	[33]
Betaxolol, Diclofenac, Mefenamic acid	33[c]	offline	HPLC-UV	Urine, plasma	40 /100	1000	SF: 30/0	15 - 18	-	[26]
Betaxolol, Naltrexone, Nalmefene	33[c]	offline	HPLC-UV	Urine, plasma	100	1000	SF: 30/0	15 - 19	-	[19]
Clonidine / Ephedrine	71	offline	HPLC-UV	Urine, plasma	74	2000	SF: 30/0	12-19	95-105	[20]
Codeine, Naltrexone, Naloxone	50	offline	HPLC-UV	Urine, plasma	110	2000	SF: 40/0	-	20 – 33	[21]
Norfloxacin, Ciprofloxacin, Danofloxacin, Flumequine, Ethyl paraben, Propyl paraben, Isobutyl paraben, Butyl paraben	8[d]	Offline µ-EME/LPME	HPLC-UV	Urine	30	<40	DF: 1/1*	-	78-100	[27]
Nalmefene, diclofenac	33[e]	Offline µ-EME/EME	HPLC-UV	Urine	60	1000	SF: 30/0	17-19	40-43	[28]

* Allow consecutive extractions
[a]Enrichment Factor
[b]Extraction efficiency
[c]Two individual millifluidic devices connected by external tube: EME/EME
[d]A single device integrating µ-EME/LPME
[e]A single device integrating µ-EME/EME

Figure 1



(C)

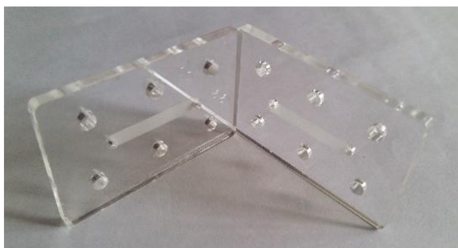


Figure 2

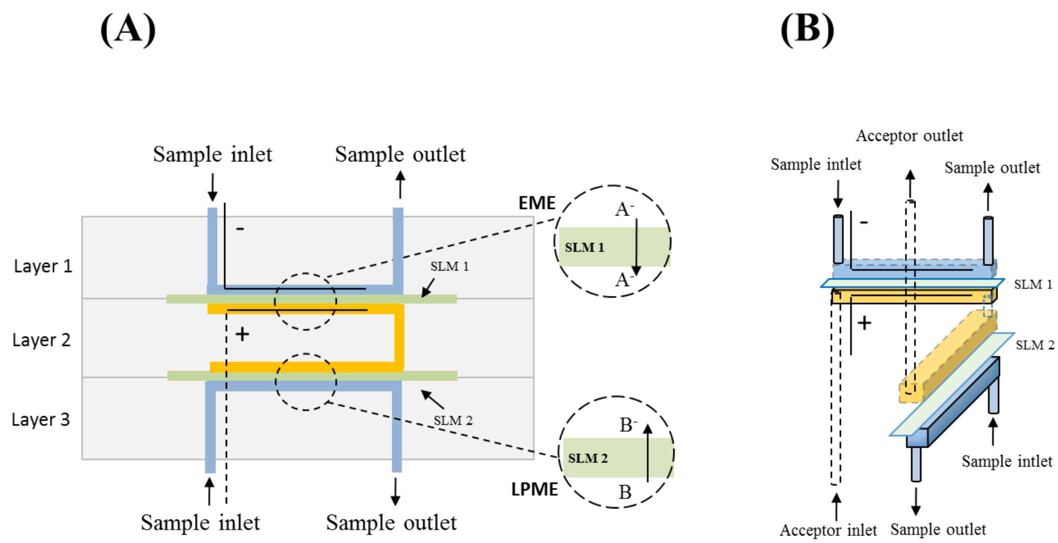


Figure 3

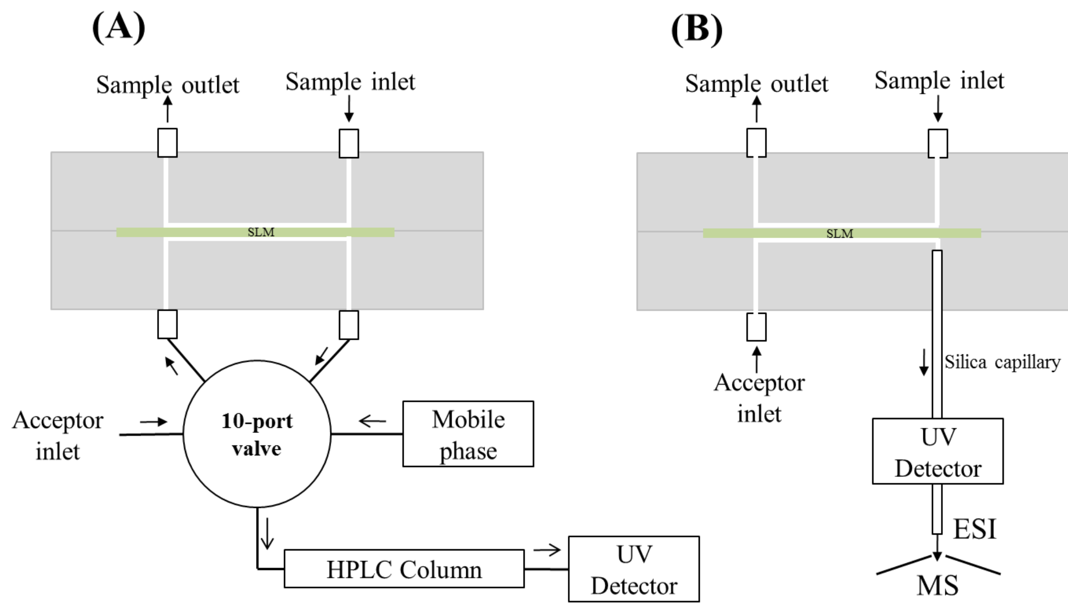


Figure 4