Abstract
This paper discusses the recent development of hollow-fibre liquid-phase microextraction (HF-LPME). The principles of HF-LPME are presented with focus on both two- and three-phase extractions. Typical performance data are reported with major emphasis on recovery, enrichment, and sample clean-up, and the applicability of HF-LPME is discussed in relation to the performance data. Finally, several examples of new directions of HF-LPME are discussed to give a flavour of the future for the technique.

Keywords
Sample preparation; miniaturized liquid-liquid extraction; hollow-fibre liquid-phase microextraction
1 Principle and performance

Hollow fibre liquid-phase microextraction (HF-LPME) was presented for the first time in 1999[1], and was originally intended as a sample preparation technique for high-performance liquid chromatography (HPLC), capillary gas chromatography (GC), and capillary electrophoresis (CE). The general principle and a photo of a HF-LPME set-up are illustrated in Figure 1. A short piece of a porous hollow fibre is attached to a pipette tip in one end, and closed by mechanical pressure in the other end. Typically, this porous hollow fibre is made of polypropylene. Prior to HF-LPME, the hollow fibre is dipped for a few seconds into a water immiscible organic solvent, like 1-octanol. The organic solvent is immediately immobilized in the pores of the hollow fibre by capillary forces, forming a supported liquid membrane (SLM). The internal lumen of the hollow fibre is then filled with an acceptor solution, and the hollow fibre is placed in the sample. With strong agitation or stirring of the sample, target analytes are extracted from the sample, through the SLM, and into the acceptor solution. After a certain period of time, the acceptor solution is removed from the hollow fibre, and injected in HPLC, GC, or CE for the final analysis.

In one configuration, the acceptor solution can be the same organic solvent as used for the SLM. In this configuration, HF-LPME is performed as a two-phase extraction where target analytes are extracted from an aqueous sample and into the organic solvent based on classical partition. Since the acceptor solution is organic, it can normally be injected directly into GC. Therefore, two-phase HF-LPME is well suited for low and medium polar analytes which are to be analysed by GC. In another configuration, the acceptor solution can be an aqueous solution, providing a three-phase extraction system. For HF-LPME of basic substances, pH in the sample is made alkaline (to suppress ionization of target analytes), whereas the aqueous acceptor solution is acidified. Thus, target analytes are extracted from the sample and into the SLM in their neutral state, whereas protonation occurs when entering the acceptor solution. The protonation ensures a high solubility in the acceptor solution, and prevents back-extraction into the SLM. For extraction of acidic substances, the pH-gradient is reversed; the sample is acidified, whereas the

![Figure 1](principle_and_photo_of_LPME.png)
Acceptor solution is alkaline. Since the acceptor solutions in three-phase HF-LPME are aqueous, they can be injected directly into HPLC and CE. Three-phase HF-LPME is ideally suited for acidic or basic analytes which are to be analysed by HPLC or CE.

To further illustrate the principle of HF-LPME, a typical HF-LPME-extraction is summarized in Table 1. In this example, the acidic drugs ibuprofen, naproxen, and ketoprofen were extracted from human urine. Because the target analytes are acidic, and because the final analytical method was CE, three-phase HF-LPME was selected. The sample volume was 2.5 mL, and prior to extraction, the urine sample was acidified with HCl to suppress the ionization of the target analytes. Dihexylether was used as the SLM. The common HF-LPME solvents dihexylether, 1-octanol, and 2-octanone were tested during method optimization, and the highest extraction recoveries were obtained with dihexylether. When the hollow fibre was dipped into dihexylether for 5 seconds, approximately 15 µL was immobilized as SLM in the porous wall of the hollow fibre. The internal lumen of the hollow fibre was filled with acceptor solution, which in this case was 25 µL of 10 mM NaOH. The strongly alkaline conditions served to deprotonate the target analytes when entering the acceptor solution. HF-LPME was accomplished in 45 minutes by strong agitation (1200 rpm). Agitation was important to induce convection in the sample, for efficient transfer of target analytes from the bulk sample and to the surface of the SLM. After extraction, the acceptor solutions were collected by a micro-syringe or a pipette and transferred to the final analysis by CE.

Performance data for the extraction of ibuprofen, naproxen, and ketoprofen from urine are illustrated in Table 2. These data are representative for HF-LPME in general. Extraction recoveries ranged between 77 and 100 %. HF-LPME is a microextraction technique, and in most cases HF-LPME extractions are not exhaustive. Extraction recoveries are compound-dependent in HF-LPME, and are related to distribution coefficients. Generally, extraction recoveries decrease with the polarity of the compound, and highest recoveries can be expected for compounds with a log P-value (1-octanol-water partition coefficient) above 2. For quantification, calibration is performed based on HF-LPME from spiked (blind) samples to incorporate the exact extraction recovery into the calibration graph. Since the target analytes were extracted from 2.5 mL of urine and into 25 µL of acceptor solution, the HF-LPME system provided a 100 times analyte enrichment in case of exhaustive extraction. As illustrated in Table 2, this was

<table>
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<tr>
<th>Table 1</th>
<th>Typical conditions for LPME of ibuprofen, naproxen, and ketoprofen from urine.[2]</th>
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<tr>
<td>Sample</td>
<td>2500 µL urine + 250 µL 1 M HCl</td>
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<tr>
<td>SLM</td>
<td>≈ 15 µL dihexylether</td>
</tr>
<tr>
<td>Acceptor solution</td>
<td>25 µL 10 mM NaOH</td>
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<tr>
<td>Extraction time</td>
<td>45 min</td>
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<tr>
<td>Stirring</td>
<td>1200 rpm</td>
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<tr>
<td>Analyte</td>
<td>Extraction recovery (%)</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>101</td>
</tr>
<tr>
<td>Naproxen</td>
<td>84</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>77</td>
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accomplished for ibuprofen, whereas enrichment factors for naproxen and ketoprofen were somewhat lower (84 and 77 times, respectively) due to lower recoveries. Because the acceptor solution and the sample were separated by a non-polar and organic liquid membrane (SLM), most matrix components remained in the sample and were unable to pass the SLM. Thus, the HF-LPME process also cleaned up the samples very efficiently.

2 Applicability

Basically, HF-LPME has been developed for extraction of organic substances from aqueous samples. As discussed above, recoveries generally increase with decreasing polarity of the substances, and HF-LPME is most suited for substances with log P >1-2. For more polar substances, HF-LPME is less efficient due to unfavourable distribution coefficients. However, by addition of ion-pair reagents either to the sample or to the SLM, also polar analytes have been extracted successfully by HF-LPME\[3\]. If target analytes are neutral substances, two-phase HF-LPME has to be applied using an organic solvent as acceptor solution. The acceptor solution can be injected directly into GC after extraction, or can be evaporated and reconstituted in mobile phase for HPLC. If the target analytes are acidic or basic substances, three-phase HF-LPME can be used. In three-phase HF-LPME, the acceptor solution is directly injectable in HPLC or CE.

A major incentive for using HF-LPME is the high enrichment which can be obtained. The enrichment (Enrichment factor (E)) in HF-LPME is determined by the extraction recovery (R), and by the volume ratio between the sample (V_s) and the acceptor solution (V_a) as shown in Equation 1:

$$E = \frac{C_{a,final}}{C_{s,initial}} \cdot \frac{R \cdot V_s}{100\% \cdot V_a}$$

$$R = \frac{n_{a,final}}{n_{s,initial}} \cdot 100\%$$

C_{a,final} is the final concentration of target analyte in the acceptor solution, C_{s,initial} is the initial concentration of target analyte in the sample, n_{a,final} is the final amount of target analyte in the acceptor solution, and n_{s,initial} is the initial amount of target analyte in the sample. By increasing the volume of the sample while keeping the acceptor solution volume at a few µL, the enrichment can be increased significantly. Under extreme conditions, with a sample volume of 1.1 L and an acceptor solution volume of 20 µL, 27,000 time enrichment has been reported with HF-LPME\[3\]. Combined with highly sensitive MS-techniques, this has enabled drug substances to be detected down to the low pg/L level in sea water as an example\[3\]. Under more standard HF-LPME conditions, enrichment factors typically range between 50 and 500 times\[3\].

Another major incentive for using HF-LPME is sample clean-up. Especially in the three-phase mode, HF-LPME give a high degree of sample clean-up. The reason for this is that most water-soluble matrix components are not able to penetrate the SLM, and are excluded from the acceptor solution. Thus, salts, polar organic substances, and macromolecules (among others) all remain in the sample during HF-LPME. Finally, the consumption of organic solvent is extremely low by using HF-LPME. In a typical three-phase HF-LPME extraction, 10-20 µL organic solvent is used for the SLM, and this represent the total volume of organic solvent used per sample. In other words, HF-LPME is a green chemistry approach to liquid-liquid extraction.

Enrichment, sample clean-up and low solvent consumption represent the major advantages of HF-LPME. As with all other sample
preparation techniques, there are also some disadvantages with HF-LPME. First, HF-LPME is a relatively slowly process, and typically extraction times from 15 to 45 minutes are required. The transfer of target analyte through the SLM is normally the rate limiting step in HF-LPME, and target analytes may partly be trapped in the SLM. Recently, a solution to speed up extraction kinetics was proposed by the introduction of Electromembrane Extraction (EME)\cite{3}. The set-up for EME is very close to HF-LPME, but in EME an external electrical potential is sustained over the SLM, and target analytes are extracted as charged species across the SLM. Due to this electrokinetic transfer, extraction times can typically be reduced to 5 to 10 minutes\cite{3}.

Another current disadvantage of HF-LPME is the lack of commercially available equipment. Thus, HF-LPME has up to date been performed with home-built equipment. The hollow fibres used are commercially available, but have to be cut to appropriate length and sealed as exemplified in Figure 1. This has limited the implementation of HF-LPME in routine laboratories and applications. However, as discussed in the next section, HF-LPME has recently been performed with flat membranes in a slightly modified commercially available 96-well plate, and this may open for more general acceptance and application of the technique\cite{4}.

Since 1999, more than 450 scientific papers have been published on HF-LPME (with hollow fibres). The field of HF-LPME and the applications have been reviewed several times recently\cite{5-12}. Most papers have been focused on environmental and bioanalytical applications of HF-LPME, but several other application fields like food and beverages have also been covered. It is not the purpose of the current perspective to review the HF-LPME applications, but information is available in several recent review articles\cite{5-12}.

3 Future directions

In addition to being developed towards routine applications, HF-LPME is currently also being developed in new and more specialized directions. To give a flavour of this progress, a few examples should be given from our own work. The first example is related to the development of HF-LPME into the 96-well format. This idea was presented recently, and termed Parallel Artificial Liquid Membrane Extraction (PALME)\cite{4}. The set-up for PALME is illustrated in Figure 2. Samples are filled into wells in a 96-well donor plate. The typical sample volume is 200-400 µL, and PALME is ideally suited for small volumes of biological fluids. The PALME set-up is not based on porous hollow fibres, but utilizes flat porous polypropylene membranes to support the SLM.
These flat membranes are located in the bottom of each well in a corresponding 96-well acceptor plate. Thus, after pipetting the samples into the donor plate (step #1), the SLMs are pipetted directly into the membranes in the bottom of the acceptor plate (step #2). In step #3, the acceptor solutions are pipetted above the SLMs in the acceptor plate. The donor and acceptor plates are clamped together, and the whole assembly is agitated for 15 to 30 minutes. During this period of time, target analytes are extracted from the sample in the donor plate, through the SLM and into the acceptor solutions located in the acceptor plate. Finally, the acceptor solutions are collected, and typically analysed by LC-MS.

Performance data for PALME are illustrated in Table 3, where the basic drugs pethidine, haloperidol, methadone, and nortriptyline were extracted from human plasma[4]. In this application, each sample was 200 µL, and prior to PALME, the samples were diluted with 200 µL of 20 mM NaOH. The SLM comprised 2 µL of dihexylylether, and the acceptor solutions were 50 µL of 20 mM HCOOH. The extraction time was 30 minutes, and the PALME plates were agitated at 900 rpm. As seen from Table 3, recoveries from plasma ranged between 34 and 74 %.

PALME can be performed with a very simple work-flow, and in 15-30 minutes, 96 samples can be extracted simultaneously. PALME provides a high degree of sample clean-up, similar to traditional HF-LPME, with a minimal consumption of organic solvent. Since PALME can be performed with commercially available 96-well plates, the technique will most probably be automated in the near future. The 96-well donor and acceptor plates we used for our experiments were commercially available, but were manufactured for filtration and membrane permeation assays. In the near future, we expect dedicated PALME plates to be commercially available, which are especially made and optimized for PALME.

Another example of a recent development is the implementation of LPME on a micro-chip[13]. The principle for this LPME-chip is illustrated in Figure 3. In this set-up, the SLM is located inside a micro-chip between a sample channel and an acceptor channel. The sample is pumped with a micro-syringe pump into the sample channel, and target analytes are extracted from the sample channel, across the SLM, and into the acceptor solution on the other side of the SLM. The acceptor solution is pumped with another micro-syringe pump, and the acceptor solution is either collected in a small vial for analysis by CE, or is pumped via an electro-spray ionization interface and directly into a mass spectrometer. In the latter case, the LPME process is followed and measured continuously with the mass spectrometer.

One interesting aspect of the LPME-chip is illustrated in Figure 4, where the enrichment of amitriptyline was studied versus extraction time. In this case, the acceptor solution flow was turned off during extraction, whereas the sample (alkaline) was pumped continuously into the LPME-chip with a flow rate of 3 µL/min. Under these conditions, the model analyte amitriptyline was extracted into 600 nL of acceptor solution.

| Table 3 Typical performance for PALME of pethidine, haloperidol, methadone, and nortriptyline from plasma[11]. |
|---------------------------------|-----------------|-----------------|-----------------|
| **Analyte** | **Extraction recovery (%)** | **% RSD (n=6)** | **Linearity** |
| Pethidine | 74 | 6 | 50-1000 ng/mL (R²=0.9994) |
| Haloperidol | 37 | 9 | 5-100 ng/mL (R²=0.9983) |
| Methadone | 70 | 5 | 50-750 ng/mL (R²=0.9955) |
| Nortriptyline | 34 | 12 | 10-250 ng/mL (R²=0.9984) |

1100 ng/mL assayed by LC-MS/MS.
(10 mM HCl) inside the acceptor channel. The enrichment factor increased linearly with the extraction time. After 10 minutes of LPME, the enrichment factor was 42, and at this time-point only 30 µL of sample has been pumped through the LPME-chip. After 120 minutes of operation, amitriptyline was enriched by a factor of 500 from 360 µL of sample solution. In other words, with the LPME-chip, very high enrichment factors can be obtained even from very small volumes of sample.

A final example of a recent development is illustrated in Figure 5, where LPME was combined with direct desorption electro-spray ionization mass spectrometry (DESI-MS)\textsuperscript{[14]}. In this set-up, samples are filled into extraction wells with a porous Teflon membrane in the bottom. Prior to extraction, 1.5 µL of hexadecane is pipetted into the Teflon membrane, and this serve as the SLM. After loading the SLM, the sample is filled into the well above the SLM. This initiate extraction, and target analytes are transferred into the SLM. Because the SLM is very thin (200 µm), the analytes rapidly distribute within the entire membrane volume. After extraction, the target analytes are measured by DESI-MS directly in the SLM. The analysis is performed on the back-side of the SLM, on the side which has not been in contact with the sample, to avoid ion-suppression from matrix components potentially fouling the membrane. Since the final analysis is perfor-
med within the SLM, this concept was termed “Thin Liquid Membrane Extraction” (TLME). The TLME step is performed in 5 to 10 minutes, which is followed by rapid analysis by DESI-MS. Thus, the time from initiating the extraction and completing the DESI-MS was typically 10 to 15 minutes.

Some results with TLME-DESI-MS are illustrated in Figure 6. Figure 6a) show a mass spectrum after TLME-DESI-MS of a human urine sample from a patient treated with 50 mg diphenhydramine. The signal at m/z 256 corresponded to diphenhydramine, whereas the signal at m/z 242 was from the metabolite nor-diphenhydramine. The signal observed at m/z 261 was due to a deuterated diphenhydramine, which was added to the sample for exact quantification. Figure 6b) shows a corresponding mass spectrum from a saliva sample from the same patient. As illustrated by Figure 6a) and b), and as confirmed by comprehensive reliability testing, it was concluded that TLME-DESI-MS can be used as a very rapid tool for identification. TLME-DESI-MS was also evaluated for quantitative purposes. For quantification, the DESI-spray was scanned across series of SLMs from multiple extractions, resulting in ion chronograms as illustrated in Figure 6c) and 6d). In both cases, the mass spectrometer was locked to the molecular ion of diphenhydramine at m/z 256. Each time the DESI-spray scanned across a SLM containing diphenhydramine from a biological sample, the signal increased as illustrated in Figure 6c) and 6d). The signals were integrated and used for quantification, measured relative to the deuterated internal standard. By using deuterated internal standards, calibration curves were linear (0.989-0.999), and accuracy was tested in a reliability study. In average, the deviation between the true concentration and the determined value was 17%. This demonstrated that TLME-DESI-MS potentially can be used for quantification in the future.

4 Conclusions

Since 1999, hollow fibre liquid-phase micro-extraction (HF-LPME) has been developed, evaluated, and published in more than 450 scientific research papers. This research has documented...
that HF-LPME is an attractive sample preparation technique, providing high enrichment of target analytes and excellent sample clean-up. In addition, the consumption of hazardous organic solvents is reduced to only 10-20 µL per sample, making HF-LPME a potential green chemistry approach for the future. The scientific literature contains more than 450 optimized applications of HF-LPME, especially related to environmental and biological samples, describing exact experimental conditions and performance. Commercially available equipment for LPME is hopefully available in the near future, making LPME available to routine laboratories. In parallel to this, LPME is also expected to be developed into more specialized directions, as exemplified in this perspective by the LPME-chip and TLME-DESI-MS applications.

References


Received: 10/17/2013
Accepted: 10/30/2013