Basic principles and applications of liquid phase microextraction techniques

Abstracts
The main liquid phase techniques for sample preparation have been explored. Among them, two phases devices such as single drop microextraction (SDME) and hollow fibre liquid phase microextraction (HFLPME), either in head space or total immersion modes, both static and dynamic ones; dispersive liquid phase microextraction (DLPME) and microextraction by demixture have been explained. Also three liquid phases using the donor-acceptor interactions, either spontaneous or electro-assisted using a membrane, were reviewed and explained. Several applications as example of each technique will illustrate the advantages and drawbacks of each technique.

Keywords: Microextraction, trace analysis, LPME, micro, devices.
1. Introduction

The demanding increase of sensitivity of analytical techniques and the complexity of real samples, either environmental, medical, biological or food samples, foster the development of new devices and techniques able to provide very low concentration values with a high accuracy. However, these requirements are not easy to achieve, as at so low levels many interactions occur, interferences appear and the confident on the final data diminishes. Sample treatment techniques are then extremely important to remove the interfering substances and to concentrate as much as possible the analytes to be measured. This way, the final analysis will provide the required accuracy and sensitivity without compromising the quantitation. For liquid samples the solid phase microextraction (SPE) has been established as a well known and standard technique. However, liquid phase microextraction, more recently explored, provides a set of techniques easy to handle and with a high potential for many applications. This paper shows the main liquid phase microextraction techniques, emphasizing the physic-chemical principle applied, advantages and disadvantages as well as some selected applications to illustrate the performance of each technique.

2. Single Drop microextraction (SDME)

This is a very simple microextraction technique that does not require special device or instrument. It consists of a hanging drop of solvent, of about 1 microliter, over the headspace in equilibrium with the sample or in total immersion in the solution containing the analytes. Figure 1 shows the scheme. As any other extraction technique the process is governed by the partition coefficient between the two phases, either between the liquid phase of the drop (extraction solvent) and the vapour (gas phase) in the headspace over the sample or the liquid phase containing the analyte. Of course the extraction solvent used will have very poor solubility in the liquid phase of the sample and very low volatility. The parameters affecting the SDME are the same as other solvent extractions, that is the solvent, temperature, agitation, presence of salt and the volume of solvent. Several scientific publications showed the advantages of this simple technique\textsuperscript{[1-6]} applied to very different areas of interest. Apparently easy to use, there are also some drawbacks. The first one to mention is the lost of the drop during the test. If the hanging drop falls off the sample is contaminated and the whole test have to be done again. It requires also some practice, to maintain the size of the drop for all the tests. To avoid lack of reproducibility and repetitivity it is recommended to use an internal standard dissolved in the extraction solvent, so that the quantitativity is not compromised. Another advantage of this technique is that derivatization reactions, which are always time consuming, can be carried out in the same step, by dissolving the derivatizing agent in the same extraction solvent. This is only possible in some cases, where the same solvent is used. This process has been successfully used for determination of explosives\textsuperscript{[7]}.

SDME is an equilibrium process and such qualitative and quantitative analysis can be carried out. In all cases the concentration factor is quite high, what represents an advantage versus other techniques. The availability at any laboratory and the general good performance make this technique attractive for trace analysis. As the drop is directly injected into the GC-MS, the technique does not required special injectors. However, it is a manual technique. The automation requires the development of microfluidics and a more complex system, but the basic technique is useful and well extended for many applications.
3. Hollow fiber liquid phase microextraction (HFLPME)

In contrast with other techniques, HFLPME is again quite simple, but it requires additional support and some automation to be successful. The extraction takes place between two non miscible liquid phases which are separated by a capillary semipermeable membrane of polypropylene (PP). The usual pore size of the membrane is 0.2 microns. The length of the capillary membrane used for the extraction process is selected by the analyst. With the control of size the total volume of extraction agent is controlled. Figure 2 shows the scheme of the basic technique. There are several modes of working with HFLPME. The first one is represented in Figure 3. The solution containing the analytes is placed in the 20 mL vial, where the membrane is inserted in total immersion mode. The capillary is filled with the extraction solvent. A magnetic stirrer shakes the solution to facilitate the extraction. After some minutes, depending on the analytes and conditions, the equilibrium is reached and the extract can be withdrawn with a syringe and injected in a microvial, which will be later analysed in the appropriate analytical instrument. The system has been successfully used for many applications in different modes of action.

One of the main drawbacks of this technique is the handling and accuracy of the technique when manually applied. Pezo et al in 2007 successfully developed an automatic instrument for simultaneous extraction of 6 samples.

HFLPME can be used in different modes in which several phases are involved. The simplest mode is that above described, where only two liquid phases participate separated by the semipermeable capillary membrane. However, other modes include three liquid phases, in which one is acting as donor phase, another one is the intermedia phase, usually the extraction solvent, and finally an acceptor phase, where the analytes are concentrated. The usual device consists of a concentric tubing system as shows Figure 4. For applying this three phases technique, the analytes should be susceptible of showing different physicochemical properties, for example, being affected by pH and then showing different chemical structures depending on pH. This way, the solubility of dissociated and molecular forms of the same molecule will be used for this specific and selective extraction.

A good example is the analysis of organic amines. In alkaline media the amines are in molecular state as neutral molecules. They are soluble in organic solvents and can be extracted by the solvent inserted in the pores of the semipermeable capillary fibre. The lumen of the fibre is filled in with an aqueous solution at acidic pH, where the amines will be dissociated and consequently transferred from the organic solvent inserted in the pores of the membrane. After reaching the equilibrium the final acceptor aqueous solution
containing the dissociated amines will be analysed by HPLC either with MS detector, UV, fluorescence or any other detector. The system has been successfully explored for a wide range of pharmaceutical drugs and pollutants. The only requirement is that the analytes work in the acceptor-donor role. One of the phenomena that can occur is the dimerization of some analytes in the organic solvent inserted in the pores, which would reduce the extraction recovery and affect the final analysis. This dimer formation depends on the analytes and has been registered in only a few publications\[19\]. Another advantage of this technique is the selectivity that can be achieved by selecting the appropriate extraction solvent, as was demonstrated by Rodriguez et al.[19].

Following the same principle above mentioned, the donor-acceptor procedure can be also enhanced either by electrical power when ionic substances are involved (Figure 5). Although attractive procedure, the requirements for achieving a feasible and reproducible extraction make this technique less useful and less extended in the analytical community.

4. Dispersive Liquid Phase Microextraction (DLPME)

The technique is again quite simple. It is based on ternary component solvent system, where an extracting solvent together with a disperser solvent are injected into
the sample, usually in an aqueous solution. The mixture is shaken and immediately a cloud dispersion is formed. Then the mixture is centrifuged and the dispersed phase, cloudy, remains at the bottom. Removing the supernatant liquid the DLPME containing all the organic analytes, is able to be analyzed. The recoveries are quite high, although the system needs careful optimization. But the technique is easy to handle and provides good enrichment factors and high recoveries for a series of analytes such as polyaromatic hydrocarbons, organochlorine and organophosphorous pesticides and benzene derivatives in environmental samples\textsuperscript{[20]}.

5. Microextraction by demixture

Although this technique has been less studied, interesting features and good performance were demonstrated for organochlorine pesticides in water and environmental samples. The principle is based on ternary diagrams of solubility, where the solubility of different mixtures is limited by the presence of salts and depends on their concentration. It responds to the salting-out principle. Initially the sample is in total dissolution. The extraction solvent and the salts to make the salting-out have to be carefully selected so that the solvent, initially completely soluble in the solution, can be demixed by the addition of salts. The analytes, usually organic compounds, are dissolved and concentrated in the low volume of organic solvent demixed. This way, this low volume of solvent can be directly injected into the GC. Only a few scientific publications are in the literature\textsuperscript{[20-22]}, although the recoveries, sensitivity and enrichment factors are very high. The main drawback of this technique is that the final extract is commonly saturated by salts and this fact can damage the detectors when many samples are analysed. However, for a few samples, the technique works very well, is easy to handle and very cheap, as no specific or expensive devices are required.

6. General comments

Without a doubt the microextraction techniques have been extensively explored and used. Nowadays they form part of any laboratory involved in trace analysis in a wide area of applications, from environmental issues to food, packaging materials, cosmetics, drugs and pharmaceutical products. The different options make the selection of the appropriate technique a difficult task. Exploring all the available techniques is not possible, as all of them require a careful optimization. There is not any technique that can be recommended for a specific task, as all of them have advantages and drawbacks. Thus, it is essential to consider first the analyte and the matrix in which the analysis has to be done. Later, the availability of the technique in the laboratory and of course the optimization requirements, the variables involved in each technique and the concentration level at which the analysis should be done, according to the legal limits for each analyte.

As other sections and tasks of the analytical work, the extraction step is still the bottleneck for the analysis. The small volumes and almost solvent free microextraction techniques contribute to a better performance of this step and enhance the environmental considerations of the laboratory work, as the volume of organic solvents are considerable reduced. But this is only one part of the whole analysis. The liquid phase microextraction techniques here described are only a part of the existing techniques. This is not an exhaustive list because this is an on-going development in which many laboratories are involved, looking for the ideal and clever technique, easy to apply, cheap and very efficient, applicable to every analyte. A dream that probably will be never achieved.
References


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