PALME: A New Tool for Sample Preparation

Parallel Artificial Liquid Membrane Extraction (PALME)

Miniaturization of liquid-liquid extraction into liquid-phase microextraction (LPME) has gained significant interest in recent years, and represents an interesting green chemistry approach to efficient sample clean-up. However, up to date LPME has been performed with home-built equipment which has been difficult to automate for high-throughput applications. Here a new approach to miniaturized liquid-liquid extraction termed „Parallel Artificial Liquid Membrane Extraction“ (PALME) is presented, which enables extraction in commercially available 96-well plates using only 2-3 μl of organic solvent per sample.

Introduction

Prior to the determination of drug substances and metabolites in biological fluids by liquid chromatography/mass spectrometry (LC-MS) or related techniques, sample preparation is required. The main purposes of the sample preparation are to remove interfering matrix components and to ensure compatibility with the LC-MS system. Important sample preparation techniques in bioanalytical laboratories include protein precipitation, solid-phase extraction (SPE), and liquid-liquid extraction (LLE).

In recent years, substantial research has been devoted to the development of alternative sample preparation strategies. The major incentives for this have been to simplify the experimental work-flow, to improve sample clean-up, to improve analyte pre-concentration, to enable soft extraction, and to reduce the amount of hazardous organic solvents required (green chemistry). One important example has been the development of hollow-fiber liquid-phase microextraction (HF-LPME) [1]. In HF-LPME, target analytes are extracted from an aqueous sample, through a thin artificial liquid membrane (thin film of organic solvent) immobilized in the pores in the wall of a porous hollow fiber, and into an acceptor solution inside the lumen of the hollow fiber. Although more than 500 research papers have been published on HF-LPME, and although high pre-concentration, efficient sample clean-up, and low solvent consumption can be achieved by HF-LPME, no commercial equipment is
available and the technique has still not been automated. In order to achieve these goals, we recently transferred the principles of HF-LPME into slightly modified commercially available 96-well plates, which were originally intended for filtration, and termed this new microextraction technique “Parallel Artificial Liquid Membrane Extraction (PALME)” [2].

**Performance**
The technique may be characterized as a further development of HF-LPME but the concept of PALME is new.

It is highly efficient, and provides rapid extraction of up to 96 samples in parallel [2].

The principle is illustrated in Figure 1 and the experimental work-flow is shown in Figure 2. The set-up comprises (A) a 96-well donor plate and (B) a corresponding 96-well acceptor plate (with filters). In addition, a corresponding lid plate is required. In step #1, samples are loaded into individual wells in the 96-well donor plate (A). The sample is typically a biological fluid, and 96 different samples can be loaded into the donor plate simultaneously. Some type of buffer or acid / base is added to each sample to adjust pH. For PALME of basic analytes, each sample is made alkaline to suppress the ionization of the analytes. For the same purpose, each sample is made acidic in the case of PALME of acidic analytes. The total sample volume is up to 300 μl.

In step #2, 2-3 μl of organic solvent is pipetted into individual filters in the bottom of the 96-well acceptor plate (B), at locations corresponding to the samples in the donor plate. The organic solvent is immiscible with water. Each filter is comprised of a thin and porous polymeric membrane. When pipetting the organic solvent into the filters, the organic solvent is immediately dispersed in the entire porous structure of the filters, forming artificial liquid membranes. The artificial liquid
membranes are held in place by capillary forces. In step #3, the reservoir above each artificial liquid membrane is filled with acceptor solution. The volume of acceptor solution is typically 25-100 μl. For PALME of basic analytes, the acceptor solution is acidic, whereas a neutral or slightly alkaline acceptor solution is used for PALME of acidic analytes.

In step #4, the donor plate (A), and the acceptor plate (B) are clamped together, and the clamped device is covered by the lid plate and placed on a platform shaker. In step #5 the device is shaken for a predetermined time period, and extraction (PALME) takes place. After PALME, the acceptor solutions are collected for final analysis typically by LC-MS (step #6). The extraction time is typically 15-60 minutes for up to 96 samples.

**General aspects**

In PALME, the extraction across the artificial liquid membrane is promoted by passive diffusion. Basic analytes are extracted from alkaline samples, where the analytes are unionized, through the artificial liquid membrane, and into the acceptor solution. For basic analytes, the acceptor solution is acidic, and when entering the acceptor phase, the basic analytes become ionized. Thus, the basic analytes are prevented from reentering the artificial liquid membrane, and they are trapped efficiently in the acceptor solution. For acidic analytes, the pH gradient is reversed. Agitation of the device is important to promote mass transfer, and typical agitation rates are 500-1000 rpm.

The artificial liquid membrane serves as a very efficient barrier for substances of no interest (like salts, proteins, phospholipids), and high selectivity and sample clean-up can be obtained [2]. Typically 2-3 μl organic solvent is used to sustain the artificial liquid membrane for each sample, and the consumption of hazardous organic solvents can therefore be reduced to a minimum. It is directly compatible with modern analytical instruments like HPLC, LC-MS, and electrophoresis [1,2], and can be automated in modern laboratory robotics with a very simple work-flow.

As one example of the PALME performance, the basic drug substances pethidine, nortriptyline, methadone, and haloperidol were extracted from human plasma samples [2]. The plasma volume was 200 μl, 2 μl of dihexyl ether was used as artificial liquid membrane, 50 μl 20 mM HCOOH was used as acceptor solution, and the extraction time was 30 minutes. Extraction recoveries were up to 74 % from plasma, RSD values were below 12 %, and limit of quantification was in the range 0.01-0.35 ng/ml following LC-MS analysis. Calibration curves were linear in relevant concentration with r² values above 0.9955.
References

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