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Design and implementation of an automated liquid-phase microextraction-chip system coupled on-line with high performance liquid chromatography

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ABSTRACT

An automated liquid-phase microextraction (LPME) device in a chip format has been developed and coupled directly to high performance liquid chromatography (HPLC). A 10-port 2-position switching valve was used to hyphenate the LPME-chip with the HPLC autosampler, and to collect the extracted analytes, which then were delivered to the HPLC column. The LPME-chip-HPLC system was completely automated and controlled by the software of the HPLC instrument. The performance of this system was demonstrated with five alkaloids i.e. morphine, codeine, thebaine, papaverine, and noscapine as model analytes. The composition of the supported liquid membrane (SLM) and carrier was optimized in order to achieve reasonable extraction performance of all the five alkaloids. With 1-octanol as SLM solvent and with 25 mM sodium octanoate as anionic carrier, extraction recoveries for the different opium alkaloids ranged between 17% and 45%. The extraction provided high selectivity, and no interfering peaks in the chromatograms were observed when applied to human urine samples spiked with alkaloids. The detection limits using UV-detection were in the range of 1–21 ng/mL for the five opium alkaloids presented in water samples. The repeatability was within 5.0–10.8% (RSD). The membrane liquid in the LPME-chip was regenerated automatically between every third injection. With this procedure the liquid membrane in the LPME-chip was stable in 3–7 days depending on the complexity of sample solutions with continuous operation. With this LPME-chip-HPLC system, series of samples were automatically injected, extracted, separated, and detected without any operator interaction.

1. Introduction

The high complexity of biological samples and low concentrations of target analytes are the two of the main challenges for analytical detection and quantitation. Therefore, clean-up and enrichment procedures in order to resolve those analytical limitations are important, preferably in an automated way that is able to handle low sample volumes. For many years, liquid–liquid extraction (LLE), solid phase extraction (SPE), and solid-phase microextraction (SPME) have been the standard methods for sample preparation [1,2]. In recent years, substantial interest has also been devoted to extractions across supported liquid membranes (SLM) where an organic liquid is immobilized in the pores of a porous hydrophobic membrane. Analytes of interest can be selectively extracted across the SLM driven by either a pH gradient as used in the format of liquid-phase microextraction (LPME) [3–6] or a voltage gradient termed electromembrane extraction (EME) [7]. With LPME or EME, membrane microextraction has demonstrated a significant potential in pharmaceutical analysis [8], environmental [9–11] and food analysis [12].

Due to the high versatility of SLM based extraction techniques, they are readily incorporated into different platforms and coupled directly with high performance analytical instruments such as liquid chromatography (LC) [13], gas chromatography (GC) [14], capillary electrophoresis (CE) [15], or flame atomic absorption spectrometry (AAS) [16]. Chip-based SLM systems have been explored and coupled on-line with LC since the 1980s [17] due to their significant advantages in terms of miniaturization and automation [18]. Previous SLM-chip modules were made by packing a flat sheet membrane in between two grooved polymer holders, which were then clamped with bolts. The volume of the channels was generally in the range of 10–20 μL [19,20].

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The automated SLM-chip systems have been explored and applied for a wide range of biosamples, such as anesthetics (SLM-GC) [21], bambuterol in human plasma (SLM-CE) [22], and peptides in spiked plasma (SLM-HPLC) [19].

Recently, SLM extraction has been successfully downscaled to a microfluidic chip for sample enrichment and clean-up [23–26]. The advantages of such microchip membrane extraction include minimal organic solvent consumption, the ability to handle a wide range of sample volumes, ease of use, potentially high enrichment factors from small sample volumes, and the ability to provide selective extraction of analytes depending on their polarity and charge. The chemical binding of flat sheet membranes into polymethyl methacrylate (PMMA) blocks was developed in our group and high performance of this SLM-chip unit has been demonstrated by both EME [24,25,27] and LPME [23] work reported previously. In this microchip membrane extraction module, the sample solution was pumped into a 50 μm deep micro channel where the analytes were extracted through the SLM and into an acceptor channel located on the other side of the SLM. The driving force for the microchip membrane extraction was either a DC electrical potential [25], or a pH gradient [23]. With microchip membrane extraction, dynamic extraction was performed in which the samples were delivered continuously to the chip by a microsyringe pump. The enrichment factor (EF) was controlled by the ratio of the sample volume delivered to the device and the volume of the acceptor solution that could either be stationary (stopped flow) or delivered continuously [23,24]. In addition, in the microchip EME system, the EF was also controlled by the applied extraction voltage [24]. Both the microchip EME and LPME systems have been used for online and real-time measurement of *in vitro* metabolism of drug substances by rat liver microsomes [23,27].

The objective of this study was to integrate a microchip LPME system directly to a commercial high performance liquid chromatography (HPLC) system, and to fully automate the system. This report describes the design, construction, operation, and optimization of such a LPME-chip-HPLC system. The system was developed to automatically perform sample injection, LPME, SLM liquid regeneration, and fast HPLC separation. Different alkaloids were used as model analytes. The intention was not to develop an analytical method for the alkaloids, but rather to investigate fundamental aspects of the LPME-chip-HPLC system.

2. Experimental

2.1. Chemicals and sample solutions

Morphine (pK_a (base)=8.2, pK_a (acid)=9.7; log P=0.89) was obtained from Nycomed DAK (Copenhagen, Denmark), codeine (pK_a=8.2; log P=1.19) and noscapine (pK_a=6.3; log P=1.5) were obtained from Nordisk Droge and Kemikalie (Copenhagen, Denmark), thebaine (pK_a=8.4; log P=2.0) was obtained from Nomeco (Copenhagen, Denmark), and papaverine (pK_a=6.3; log P=3.0) was obtained from Mecobenzon (Copenhagen, Denmark). All these substances were hydrochlorides and with purities > 99%. LC–MS grade formic acid, acetonitrile, and sodium octanoate were purchased from Sigma-Aldrich (St. Louis, MO, USA). 1-Octanol and 2-nitrophenyl octyl ether (NPOE) were obtained from Fluka (Buchs, Switzerland). All water used was prepared with a Millipore Direct-Q3 UV system (Billerica, MA, USA).

Stock solutions containing 1 mg/mL of each model analyte were prepared in 10% (v/v) acetonitrile in 100 mM HCOOH and stored protected from light at 277 K (4 °C). Sample solutions of the compounds were prepared daily by adequate dilutions from the 1 mg/mL stock solutions by pure water or urine.

2.2. Instrumentation of the automated LPME-chip-HPLC

As shown in Fig. 1A, the integrated LPME-chip-HPLC consisted of three main parts: (1) an Agilent 1100 HPLC system (Agilent, Palo Alto, CA, USA) including an autosampler (model G1329A), a binary pump system (G1312A), and a UV detector (G1314A); (2) the home-built LPME-chip attached to a Valco Instrument (EHAM model, Houston, TX, USA) two position 10-port valve actuator control module; and (3) two microsyringe pumps (Kd Scientific, Holliston, MA). The HPLC software (Chemstation B.04.02) was applied for programming the sample injection, separation, and UV detection. The 10-port valve was used to synchronize the sample pretreatment, and to separate the low pressure of LPME-chip module from the high pressure of HPLC system by switching the positions between sample loading and injection. The automated operation of this 10-port valve was controlled by the remote control output (RS232 plug) on the HPLC system. The valve switching flow diagram for the LPME-chip-HPLC system is schematically illustrated in Fig. 1B. In this setup, the two switch positions A and B in the 10-port valve were alternatively changed for microchip LPME sample pretreatment and on-line HPLC analysis, respectively. The two microsyringe pumps were used to deliver the sample carrier buffer solution and the acceptor phase through the LPME-chip for the dynamic extraction, respectively.

The construction of the LPME-chip was published recently and only a short description is given here [23,27]. The porous polypropylene membrane (Celgard 2500 micro porous membrane; Celgard, Charlotte, NC, USA) with a 25 μm thickness (55% porosity, and 0.21 μm × 0.05 μm pores) used for the SLM was placed between two polymethyl methacrylate (PMMA) (53 mm × 53 mm × 2.1 mm) plates having 6 mm long channels with a depth of 50 μm and a width of 2.00 mm. The whole assembly was fixed by solvent-assisted bonding with ethanol and cured in a 343 K (70 °C) oven. At both ends of the channels, 1.6 mm I.D. holes served as inlet and outlet for the sample carrier liquid and the acceptor phase.

Prior to connection of the tubing to the chip, the supported liquid membrane was immobilized in the polypropylene membrane by filling approximately 0.2 μL of organic solvent (1-octanol or NPOE) into one end of the extraction channel using a micropipette. The solvent immediately immobilized into the polypropylene membrane by capillary forces, and this process was visually inspected as the appearance of the membrane changed from white to transparent during immobilization of membrane liquid. Subsequently, the tubings for the donor and acceptor flow were connected to the LPME-chip.

2.3. Procedure of carrier mediated LPME-chip-HPLC

Sample was loaded in the autosampler tray of the HPLC instrument in 2 mL LC vials (Microlab, Aarhus, Denmark), and extractions were normally carried out according to the following procedure; sample solution was prepared by the mixture of 500 μL analyte solution with 500 μL 50 mM sodium octanoate (ion-pair reagent) prepared in 25 mM pH 7.0 phosphate buffer. By means of the autosampler, 50 μL sample solution was draw into the injection needle and then directed back to the HPLC needle seat connected with the HPLC 6-port valve (all part of the Agilent® autosampler) (Fig. 1A). The microsyringe on the donor side was connected with the 6-port valve of the autosampler, and was filled with 25 mM phosphate buffer (pH 7.0) used as the sample carrier liquid. With a flow rate of 5 μL/min, the sample plug was transferred to the LPME-chip and the analytes were extracted through the SLM. The tubings used for connecting the LPME-chip to the autosampler had a small dead volume in the order of 1 μL, also on the accepter side there was a small dead