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On-line coupling of a clean-up device with supported liquid membrane to capillary electrophoresis for direct injection and analysis of serum and plasma samples

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ABSTRACT

A simple sample clean-up device with planar supported liquid membrane (SLM) was developed and coupled on-line to capillary electrophoresis (CE) for direct injection of human body fluids. Donor and acceptor compartments of the device were filled with diluted body fluid and deionized water, respectively, and the two solutions were separated by a thin SLM. Analytes of interest were selectively transported from the donor solution through the SLM into the acceptor solution by diffusion whereas interfering matrix components were efficiently retained on the SLM. Equilibrium between the concentrations of analytes at the SLM was obtained typically in 5 min. Then a CE separation capillary was inserted into the acceptor compartment to firmly touch the SLM and the pretreated sample was hydrodynamically injected into the capillary. The analytical procedure was demonstrated by rapid pretreatment, on-line injection, and CE determination of selected amino acids in human serum and plasma samples. 1-Ethyl-2-nitrobenezene and bis(2-ethylhexyl) phosphate (15%, v/v) was used as the selective SLM for clean-up of the body fluids and 0.5 M acetic acid was used as a background electrolyte solution for CE analysis of the pretreated amino acids. Concentrations of amino acids on acceptor side of the SLM reached 40–58% of their original concentrations in donor solution after 5 min equilibration time and then remained constant proving that equilibrium was achieved at the SLM. Injection of the pretreated samples was highly repeatable with RSD values of peak areas 2.4–8.4% and 3.4–10.5% for standard solutions and real samples, respectively. Limits of detection between 0.75 and 2.5 μM were achieved, corresponding to 3.75–12.5 μM in 1:4 diluted real samples, which ensure sensitive determination of most amino acids in the body fluids. The developed method is fast, simple, efficient, cheap and selective and may be applied to determination of a wide range of analytes in various samples with complex matrices.

1. Introduction

Body fluids are complex biological samples and their analysis is a non trivial task. High molecular mass compounds in body fluids adhere to the inner surface of an analytical column (separation capillary in CE or separation column in HPLC and/or GC) and often result in irreversible poisoning of the column. This consequently evokes serious changes in separation efficiency and usually leads to peak broadening and detection interferences [1,2]. Qualitative and quantitative analyses of directly injected biological samples are therefore performed only scarcely [3,4] and various sample pretreatment procedures are normally applied prior to their injection in order to remove interfering matrix components.

Traditionally, pretreatment of biological samples was performed in an off-line fashion using stand-alone pretreatment techniques, such as liquid–liquid extraction [5], solid phase extraction [6] and protein precipitation with subsequent centrifugation [7]. More recently, alternative sample pretreatment techniques, such as ultrafiltration [8], electrodialysis [9], liquid phase microextraction (LPME) [10,11] and solid phase microextraction [12,13] were introduced. These pretreatment techniques exhibit minimum environmental impact due to their reduced use of organic solvents. They also speed-up the pretreatment procedures, require small volumes of biological samples and reduce the overall costs of an analysis. However, these techniques are usually performed off-line and their on-line and/or in-line coupling to separation techniques are reported less frequently [9,12,14–18].

One of the perspective pretreatment techniques is LPME using supported liquid membranes (SLMs), which was first described by Audunsson [19]. The method has gained significant attention in subsequent years and has become a basis for further development of various sample pretreatment methods (e.g. hollow fibre-liquid phase microextraction (HF-LPME) [11], and electromembrane extraction (EME) [20]). All these methods are based on the same

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fundamental principle, i.e. extractions of analytes across thin SLMs. A porous inert supporting material (usually polypropylene (PP) or polytetrafluoroethylene (PTFE)) is impregnated with water immiscible organic solvent to form the SLM and separates two compartments filled with donor and acceptor solutions. The donor solution is usually aqueous sample to be pretreated and the acceptor solution is either aqueous or organic solution. The SLM creates a selective barrier that enables transfer of analytes from donor to acceptor solution, whereas interfering matrix components are retained on the SLM. When the LPME is finished, the acceptor solution is injected into an analytical system. This simple experimental set-up can be used for simultaneous sample clean-up and preconcentration and has been widely used in many application areas [11,21]. LPME using SLMs is primarily attractive due to its negligible costs, low consumption of organic solvents, simple handling, and applicability to most analytical techniques including HPLC, GC, CE and MS [11]. Although LPMEs with SLMs are mostly performed off-line, several authors have demonstrated that on-line and/or in-line coupling of the pretreatment technique with various analytical methods is feasible.

Audunsson has developed an SLM-based pretreatment device coupled on-line to flow injection analysis (FIA) [19]. Basic set-up of this device was later used by Jönsson and coworkers who have demonstrated that, with few alterations, this instrumentation can be coupled on-line also to HPLC [22–24], GC [22,25], and atomic absorption spectrometry [26]. The system required additional external pumps to force the flows of the donor and the acceptor solutions through the device. Moreover, to replace the SLM, the extraction device had to be dismantled completely, which was quite complicated. To avoid the device dismantling, regeneration of the originally inserted SLM was used, which was time-consuming and could be a source of sample carry-over errors. Other authors have continued to use this approach in FIA [27–29], and extended the application field to microfluidic platforms [30–32] and to electroextraction assisted microfluidic platforms [33]. In the pretreatment devices, planar [25,31,34] as well as tubular [23,30,32,35] SLMs were used. Several reviews covering the major aspects of coupling SLM-based pretreatment devices to various flow-through analytical techniques and practical applications of the method are available [15–17,36].

Pretreatment devices using SLMs with the design originally introduced by Audunsson [19] were on-line coupled to CE [35,37]. The use of a complex pumping system and low flexibility for membrane replacement were the main limitations of these hyphenated systems. Moreover, a mismatch between the volume of pretreated sample and usual injection volumes in CE had to be overcome by a tedious and time consuming large-volume injection and double-stacking procedure [35,37]. Two alternatives for in-line coupling of a SLM directly to CE were presented by Valcárcel and coworkers [38–40]. Holes were cut out in an Eppendorf vial, which was then wrapped up in a planar PTFE membrane. The membrane was impregnated with organic solvent to form a SLM and the vial was filled with acceptor solution. Then the extraction assembly was placed in a conventional glass vial of a commercial CE instrument filled with a donor solution for extraction and injection [38,39]. Nozal et al. have also shown that efficient sample pretreatment can be achieved using a separation capillary of a commercial CE instrument coupled in-line to HF SLMs [40]. The HF was heat-sealed to the outer surface of two FS capillaries (64 and 0.5 cm long) and the distance between the two capillaries defined the extraction cell volume. Labor intensive procedure and low reproducibility of the manual fabrication of the extraction devices were mentioned as the main drawbacks of these arrangements [38–40] and the authors have preferred their repeated use and conditioning between two analytical runs. This, of course, increased the total pretreatment time and may be source of possible sample carry-over problems.

It has been shown, that coupling SLM to CE is a promising alternative for analysis of biological samples [35,37–40]. The on-line and/or in-line coupling to CE was, however, not fully solved until these days and many presented devices exhibit similar drawbacks, such as necessity of external instrumentation, low reproducibility, elaborateness, and possible sample carry-over. This work presents a simple pretreatment device with SLMs coupled on-line to CE for rapid pretreatment and analysis of human body fluids. Handling of the proposed device is simple and reproducible and was demonstrated by manual manipulation in this contribution. Planar SLM was screwed between two PTFE blocks with donor and acceptor solutions and was discarded after each use. Pretreated body fluids were injected hydrodynamically from the acceptor chamber by positioning the separation capillary directly onto the SLM surface. This ensured very short extraction times since equilibrium between the concentrations on both sides of the SLM was established rapidly. The method was applied to determination of amino acids in human serum and plasma and may be extended to a wide range of major and minor analytes since only 1:4 dilution of the original body fluids was necessary and the extraction selectivity could be fine-tuned by selection of a proper SLM.

2. Materials and methods

2.1. Instrumentation

2.1.1. Sample clean-up device

Fig. 1 shows the sample clean-up device and its connection to a CE system. It consists of two PTFE parts, screw and bolt type, with axial open hole channels of internal volume 50 μL (Link chambers, Harvard Apparatus, Holiston, MA, USA), separated by a polypropylene membrane (Accurel PP 1E R/P, Membrana, Wuppertal, Germany) with 100 μm thickness (porosity and pore size was not specified). The membrane was cut into 11 mm-radius circles, which exactly fitted into the inner part of the link chamber. Before sample treatment, the membrane was placed into one link chamber and was impregnated with 2.5 μL organic solvent to form a supported liquid membrane (SLM) in its centre. The organic solvent was let to soak into the membrane pores (this usually took ca. 5 s and the SLM became opaque) and the two link chambers were screwed together. Next, the donor chamber was filled with 50 μL of a body fluid using one micropipette and the acceptor chamber was filled with the same volume (50 μL) of an acceptor solution using a second micropipette. Measurement of the extraction time (typically 5 min) was initiated on filling the acceptor chamber with acceptor solution (see Fig. 1A). Simultaneously, the clean-up device was positioned into a holder, which was placed 30 cm (Δh) above the detection end of the separation capillary (C). The donor and acceptor solutions were held inside the chambers by capillary forces (the internal diameter of the chamber is approx. 3 mm) and no liquid losses were observed from internal chambers during up to 15 min. After the required equilibration time elapsed, sample injection was performed by inserting the separation capillary into the acceptor chamber to firmly touch the SLM. The capillary touches the SLM due to a slight tension created by the capillary bend (see Fig. 1B) and there is no gap between the capillary injection end and the SLM. After 60 s (injection time), the separation capillary was removed from the acceptor chamber, was placed into the vial with background electrolyte solution (E1) and high voltage power supply (HV) was switched on (see Fig. 1C). The clean-up device was then unscrewed, used SLM was discarded, internal channels of the link chambers were flushed with deionized (DI) water, dried out and immediately used for next sample clean-up. All clean-up experiments and sample injections were performed at ambient temperature of 25 ± 2 °C.