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Sensitivity enhancement in direct coupling of supported liquid membrane extractions to capillary electrophoresis by means of transient isotachophoresis and large electrokinetic injections

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ABSTRACT

Enhanced sensitivity for determination of basic drugs in body fluids was achieved by in-line coupling of extraction across supported liquid membrane (SLM) to large electrokinetic injection and transient isotachophoresis-capillary zone electrophoresis (tITP-CZE) in commercial CZE instrument. Twelve cm long tITP plug of 300 mM ammonium acetate was formed in the separation capillary just before the electrokinetic injection of acceptor solution containing nortriptyline, haloperidol and loperamide extracted across the SLM. The tITP plug ensured efficient stacking and preconcentration of the injected basic drugs due to the tITP action of ammonium and the drugs were then separated by CZE using 5.2 M acetic acid as background electrolyte. No interferences were observed from highly-abundant body fluid species (NaCl and human serum albumin) due to the excellent clean-up properties of SLMs and analytical sensitivity increased up to 340 times compared to SLM extractions coupled in-line to CZE with standard hydrodynamic injections. The SLM-tITP-CZE method was characterized by good repeatability (RSDs of peak areas below 7.8%), linearity over two orders of magnitude (r^2 better than 0.994) and limits of detection (defined as 3 × S/N) between 3 and 45 μg/L. Interfacing of SLM extractions to CZE instrumentation was achieved by low-cost, disposable micro-extraction devices, which can be routinely prepared in every analytical laboratory. These devices eliminated sample carry-over, minimized the need for manual sample handling and ensured fully automated determination (including extraction, injection, preconcentration and separation) of the three basic drugs in 20 μL of untreated body fluids.

1. Introduction

Analysis of biological samples is not a trivial task owing to the fact that complex samples, such as body fluids, contain large number of inorganic matrix components (salts), which are usually present in high concentrations, and interfere with determination of analytes of interest. In addition to inorganic salts, body fluids usually contain high-molecular mass matrix species (proteins, lipids, fatty acids, etc.), which result into analytical instrumentation fouling, and efficient removal of matrix components is therefore necessary prior to vast majority of analytical methods.

Traditionally, sample treatment is performed with large volumes of complex samples and reagents, is costly and time consuming [1,2]. During the last two decades, various micro-extraction techniques have been developed as an effective alternative to conventional sample treatment procedures, which have significantly reduced the requirements on sample/reagent volumes, costs and labor [3–11]. Solid phase micro-extraction (SPME) is the most widely used micro-extraction technique due to its commercial availability, solvent-less character and suitability for direct coupling to gas chromatography [4,9,12] and other separation methods [13,14]. Nevertheless, the SPME principle is based on adsorption of analytes onto porous fibers, which have limited durability, have to be replaced regularly and their costs are not negligible.

In liquid phase micro-extraction (LPME), analytes are separated from matrix components based on selective transfer of the former ones from aqueous samples into/across phase interfaces, which are formed from water immiscible organic solvents [10,15]. Matrix components are usually not compatible with the organic solvent and are thus retained by the established phase interface. LPMEs use low volumes of organic solvents and complex samples, extraction units are cheap and can be used as disposable devices, thereby eliminating problems with sample carry-over. Direct coupling of LPME to analytical method is therefore very attractive since it may enhance the method sensitivity, minimize the need for user

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intervention, eliminate possible user-induced sample contamination, and reduce overall costs and environmental impact. Nevertheless, coupling of LPME to analytical methods is not as straightforward as of SPME and LPMEs are usually performed in an off-line fashion [10,15–18].

Capillary zone electrophoresis (CZE) is a miniaturized analytical method that normally uses mL volumes of reagents, μL volumes of samples and nL–pL injection volumes. These characteristics make its direct combination with micro-extraction techniques, such as LPMEs, readily feasible. Indeed, recent review articles are available on direct coupling of LPME techniques based on extractions across supported liquid membranes (SLMs) [19] and single drop micro-extractions (SDME) [20] to CZE. They clearly demonstrate that direct coupling of LPMEs to CZE is desirable but rather unexplored, especially with respect to coupling of LPMEs to commercial CZE instrumentation [21–24].

In-line coupling of SLM extractions to CZE, formerly reported by our group, enabled efficient clean-up and direct CZE injections of samples with highly complex matrices from SLM surface, nevertheless, the method did not allow for analyte preconcentration [25,26]. In this contribution, the method was further examined with respect to increased sensitivity of the extraction/separation process. Major attributes of the two methods were therefore considered for the development of a preconcentration technique compatible with the hyphenated SLM-CZE system. (i) SLM extractions may yield acceptor solutions of low conductivity as most highly conductive matrix components are retained by the SLM and only analytes are transferred across SLMs [10,27]. (ii) Analytes in the low conductivity acceptor solutions can be injected more efficiently into CZE when electrokinetic injection and background electrolyte with sufficiently high conductivity are used; the large zone of injected analytes can be easily manipulated by on-capillary transient isotachophoresis (tITP), which ensures excellent analytes' stacking and separation efficiency [28–30].

In this contribution an analytical method that couples extraction across SLM in-line to large electrokinetic injection, tITP preconcentration and CZE separation of the extracted analytes is described for the first time. The device for SLM extraction is accommodated in autosampler carousel of a commercial CZE instrument and ensures that the extraction and analytical processes are performed fully automatically. A long plug of a low conductivity extract, resulting from the SLM extraction, is electrokinetically injected into separation capillary. Analytes in the injected extract are temporarily stacked and preconcentrated at the sharp tITP boundary and subsequently separated as narrow zones by CZE. The presented procedure significantly enhances sensitivity of the formerly reported hyphenated SLM-CZE method [25,26], while keeping its advantages, i.e. minimum sample/reagent consumption, low costs and fully automated determination of analytes in untreated body fluids. The presented method brings an important advantage in comparison to electrokinetic supercharging [28,29] where the clean-up step must be done prior to application of the supercharging method by an off-line procedure.

2. Materials and methods

2.1. Micro-extraction device and its function

Initially, a tailor-made micro-extraction device compatible with 7100 (Agilent Technologies, Waldbronn, Germany) CZE instrument was prepared. Dimensions of the device were optimized in order to fit the internal diameter of sample vials applied in the CZE instrument and to be fully compatible with its injection system. The device consisted of two plastic segments (donor and acceptor units), which were separated by a flat polypropylene (PP) membrane impregnated with an organic solvent to form the SLM, see Fig. 1, and pressed against each other. Ten μL of acceptor solution was pipetted into the acceptor unit and 20 μL of donor solution into the donor unit of the device before each analysis. Analytes present in the donor solution were diffusively transferred across the SLM based on their affinity for the organic solvent impregnated in the membrane pores and the extracted analytes were injected directly from the SLM surface in the acceptor unit of the device. The micro-extraction device was accommodated in the sample vial using a soft compression spring, which released the pressure when injection end of the separation capillary contacted the SLM.

Details on preparation of and injection from an SLM-based micro-extraction device compatible with the Agilent 7100 CZE instrument are depicted in Fig. 1. Donor and acceptor units were scalpel-cut from 200 μL PP micropipette tips (FL Medical, Torreglia, Italy, Part No. 28063) to fit the internal diameter (6 mm) of Agilent PP sample vials (Part No. 5182-0567). First, the top 7 mm of the micropipette tip was cut and discarded. Next, 11 mm long section of the tip was cut and was used as the donor unit. Finally, 10 mm long section of the tip was cut and used as the acceptor unit of the micro-extraction device. The remaining part of the tip was discarded. The three cuts are depicted with red vertical lines in Fig. 1A. PP membranes (Accurel PP 1E R/P, Membrana, Wuppertal, Germany, 100 μm thick, average pore size 0.1 μm) with a diameter of 11 mm were cut from the Accurel PP sheet with a cork borer. The micro-extraction devices were assembled according to the following procedure. (i) The PP membrane was impregnated with 5 μL of 1-ethyl-2-nitrobenzene (ENB), see Fig. 1B (left is before and right after impregnation with ENB). (ii) The membrane was placed on top of the donor unit and pressed against the bottom of the acceptor unit to assemble the micro-extraction device. (iii) The donor and acceptor unit was filled with 20 and 10 μL of donor and acceptor solution using standard Eppendorf micropipettes, respectively, and extraction time measurement was started. The bottom part of donor unit was not closed; donor solution was held by surface tension in the narrow (~3 mm ID) unit. (iv) Fig. 1C shows the assembled micro-extraction device and a tailor-made compressive spring (Pružiny Čermák, Brno, Czech Republic) before they were accommodated in a sample vial and closed with a plastic snap cap. A sketch of the micro-extraction device accommodated in the sample vial before injection is depicted in Fig. 1D. During sample injection, the sample vial was lifted up and the injection end of the separation capillary contacted the SLM. On further upward movement of the sample vial, the spring holding the micro-extraction device was compressed and injection was subsequently performed from the SLM surface, see Fig. 1E. The soft spring ensured direct contact between the capillary end and the SLM surface during injection and simultaneously eliminated possible perforation of the SLM. The micro-extraction device was disposed of after each extraction. The injection end of the separation capillary overlapped the rim of the tubular high voltage electrode by ca. 1.0 mm [26].

2.2. Capillary electrophoresis

The Agilent 7100 CZE system was operated at a potential of +15 kV applied at the injection side of the separation capillary for all runs. Detections were performed simultaneously using UV–Vis (diode array detector (DAD)) and capacitively coupled contactless conductivity detector (C^4D). Basic drugs were detected using DAD at 214 nm and tITP plugs were detected using C^4D. Separation capillary used was a fused silica capillary (75 μm ID/375 μm OD, 31 cm total length and 23/16.5 cm effective lengths for UV-Vis/C^4D, Polymicro Technologies). Prior to the first use, the bare capillary was rinsed for 15 min each with 1 M NaOH and deionized (DI) water. At the beginning of a working day, the capillary was rinsed with 15% phosphoric acid for 10 min, DI water for