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In-line coupling of microextractions across polymer inclusion membranes to capillary zone electrophoresis for rapid determination of formate in blood samples

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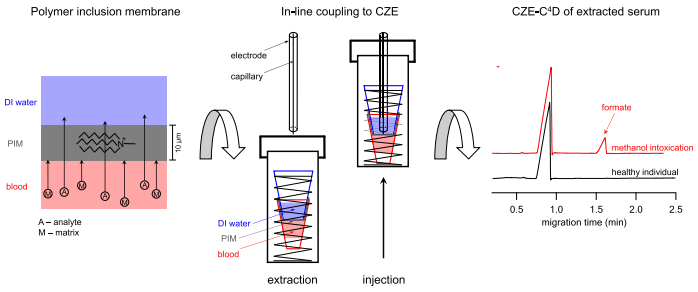
HIGHLIGHTS

- Microextractions across PIMs are in-line coupled to commercial CZE.
- Full automation of extraction, injection, separation and quantification is obtained.
- Formate is rapidly determined in clinical samples (undiluted whole blood and serum).
- PIMs exhibit excellent long-term stability for extractions of formate.
- Pre-assembled, disposable PIM microextraction devices might be of interest in clinical analyses.

GRAPHICAL ABSTRACT



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ABSTRACT

Polymer inclusion membranes (PIMs) have several important features, i.e., PIMs are dry and non-porous membranes, which can be prepared ahead of use and stored without noticeable deterioration in extraction performance. In this contribution, in-line coupling of microextractions across PIMs to a separation method for clinical purposes was demonstrated for the first time. Formate (the major metabolite in methanol poisoning) was determined in undiluted human serum and whole blood by capillary zone electrophoresis (CZE) with simultaneous capacitively coupled contactless conductivity detection (C^4D) and UV–Vis detection. A purpose-made microextraction device with PIM was coupled to a commercial CZE instrument in order to ensure complete automation of the entire analytical procedure, i.e., of formate extraction, injection, CZE separation and quantification. PIMs for formate extractions consisted of 60% (w/w) cellulose triacetate as base polymer and 40% (w/w) Aliquat™ 336 as anion carrier. The method was characterized by good repeatability of peak areas (≤7.0%) and migration times (≤0.8%) and by good linearity of calibration curves (r^2 = 0.993–0.999). Limits of detection in various matrices ranged from 15 to 54 μM for C^4D and from 200 to 635 μM for UV–Vis detection and were sufficiently low to clearly distinguish between endogenous and toxic levels of formate in healthy and methanol intoxicated individuals. In addition, PIMs proved that they may act as phase interfaces with excellent long-term stability since once prepared, they retained their extractions properties for, at least, two months of storage.

1. Introduction

Body fluids are complex samples, which contain high concentrations of bulk matrix components. The bulk components

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negatively influence performance of all major analytical methods and their efficient removal from sample matrices is therefore essential. A variety of microextraction techniques have been developed during the last two decades [1–14] and are currently receiving more and more attention since they efficiently eliminate the matrix effects and, in comparison to standard extraction techniques, they also significantly reduce the requirements on sample and reagent volumes, costs and labor.

Among the above mentioned microextraction techniques, supported liquid membrane (SLM) extractions have become very popular. Commercial availability of supporting materials in form of hollow fibers and planar sheets with various dimensions at negligible costs has significantly contributed to the wide acceptance of SLM extractions. Extractions across SLMs have been usually performed in an off-line arrangement [9,15]. Nevertheless, various instrumental set-ups for their in-line coupling to capillary zone electrophoresis (CZE) have been reported [16–18] and reviewed recently [19]. SLM extractions have been also in-line coupled to commercial CZE intruments, which has ensured full automation of the extraction and separation process and thus minimized the need for manual sample handling and user intervention [17,20–24]. In addition, sample contamination and analyte loss, two aspects usually associated with off-line sample treatment, have been minimized due to elimination of user induced errors.

Despite the excellent characteristics of extractions across SLMs, several drawbacks, which are associated with and limit the SLM extraction technique, have been recognized and reported recently [25,26]. The membrane instability in terms of long time performance leads to the reduction of solute flux and membrane selectivity and may be the consequence of solvent loss from the supporting material, either by its evaporation or dissolution/dispersion into the adjacent phases [25,26]. Organic solvents, which are used as liquid membranes for impregnation of the porous supports, are often volatile and may have toxic effects. Thus, increased volatility of the solvent increases its loss from supporting material and renders the SLM more unstable and toxicity caused by volatile solvents is not desirable for apparent reasons [26].

Another type of membrane suitable for extraction purposes is a polymer inclusion membrane (PIM) [27]. PIMs generally consist of a base polymer, a plasticizer and a functional carrier. Polyvinyl chloride (PVC) and cellulose triacetate (CTA) are most often used as base polymers for the preparation of PIMs [28–30]. The base polymer provides the mechanical support, the plasticizer acts as the solvent and the carrier is the mediator to form ion complexes and initiates ion-exchange interactions with target ions. PIMs are usually prepared by dissolving the three above mentioned components in a volatile organic solvent; the mixture is then dispensed into a suitable vessel and a thin film of membrane is obtained after evaporation of the solvent. PIMs provide high extraction efficiency and selectivity, moreover, they offer better mechanical strength, stability and ease of use than SLMs [31].

Transport of ions across PIMs has, however, been investigated to a very small degree. Most PIM research has involved the transport of metal ions across PIMs and has been reviewed by Kolev and coworkers [28,30]. Scientific reports on extraction and transport of small organic compounds are limited. To date, transport mechanisms of small organic compounds are not well understood and are likely to be more complex than those observed with the transport of metal ions [28,29].

Recently, there have appeared a few publications concerning the transport of selected organic acids across PIMs [32–35]. Obviously, these works indicate that further research concerning extractions of small organic ions across PIMs might be of significant importance. Moreover, there are no publications on practical use of PIMs for microextractions in clinical analyses.

This manuscript brings an example of a practical use of tailor-made PIM microextraction devices in-line coupled to CZE for the analysis of formate in clinical samples. Formate is the major metabolite of methanol dehydrogenation in humans and is specifically responsible for methanol toxicity. Formate concentrations in blood of healthy individuals are low and a reference range of 0–0.4 mM has been reported recently [36,37]. On accidental methanol consumption, blood concentrations of formate may increase up to 40 mM and formate is thus an excellent marker of methanol intoxication [37]. The unique in-line coupling between PIM surface and injection end of CZE separation capillary, presented in this manuscript, ensures rapid extraction of formate from untreated blood samples, reproducible injections of nL volumes of the pretreated samples and rapid separation of formate from other anions, i.e., characteristics, which are not feasible with other standard analytical techniques. In addition, the presented technique uses commercial CZE instrumentation and allows for complete automation of the entire analytical protocol. The actual study evaluates important practical aspects of the technique, such as, robustness of the PIM–CZE coupling, extraction speed, sensitivity for methanol intoxication diagnostics and long term stability of PIM extraction performance.

2. Materials and methods

2.1. Microextraction devices and PIMs

Tailor-made microextraction devices compatible with an Agilent 7100 CZE instrument (Agilent Technologies, Waldbronn, Germany) were prepared according to the recently described procedure [24]. Donor and acceptor units made of two segments of plastic micropipette tip were mutually separated with PIMs to form the microextraction devices. 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 extraction. The bottom part of the donor unit was not closed; donor solution was held by surface tension in the unit. Analytes present in the donor solution were diffusively extracted across the PIM and the transferred analytes were injected directly from the PIM surface facing the acceptor solution. The microextraction device was accommodated in a polypropylene sample vial (1.0 mL, Part No. 5182-0567, Agilent) by using a tailor-made soft compression spring (Pružiny Čermák, Brno, Czech Republic); for more details, see Ref. [24]. Positioning of the microextraction device into the spring ensured pressure release during the contact between the separation capillary injection end and the PIM and thus eliminated possible perforation of the membrane. After extraction, the microextraction device was disposed of.

PIMs were based on CTA base polymer. CTA was mixed with Aliquat™ 336 as anion-exchange carrier and o-nitrophenyloctyl ether (NPOE) as plasticizer amounting to a total of 100 mg and dissolved in 5 mL of dichloromethane (DCM). Aliquat™ 336 enables extraction of negatively charged species across the PIM and consequently selective transfer of anions in the direction from donor into acceptor solution. CTA, NPOE and DCM were purchased from Fluka (Buchs, Switzerland), Aliquat™ 336 from Sigma (Steinheim, Germany). The solution was poured and spread evenly into a 9 cm diameter glass Petri-dish and was left overnight in a fume hood to completely evaporate the solvent. The resulting sheets were cut with a cork borer to achieve circular PIMs with a diameter of 11 mm, which were inserted and pressed between the acceptor and the donor unit of the microextraction device according to ref. [24]. Up to 40 PIMs could be cut out of one sheet. The thickness of PIM membranes prepared in our laboratory was measured by electron scanning microscopy (Mira 3, Tescan-Orsay,