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Drop-to-drop microextraction across a supported liquid membrane by an electrical field under stagnant conditions

Nickolaj Jacob Petersen[a], Henrik Jensen[a], Steen Honoré Hansen[a], Knut Einar Rasmussen[b], Stig Pedersen-Bjergaard[a,b,*]

[a] Department of Pharmaceutics and Analytical Chemistry, Faculty of Pharmaceutical Sciences, University of Copenhagen, DK-2100 Copenhagen, Denmark
[b] School of Pharmacy, University of Oslo, P.O. Box 1068 Blindern, 0316 Oslo, Norway

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ABSTRACT

Electromembrane extraction (EME) of basic drugs from 10 μL sample volumes was performed through an organic solvent (2-nitrophenyl octyl ether) immobilized as a supported liquid membrane (SLM) in the pores of a flat polypropylene membrane (25 μm thickness), and into 10 μL 10 mM HCl as the acceptor solution. The driving force for the extractions was 3–20 V d.c. potential sustained over the SLM. The influence of the membrane thickness, extraction time, and voltage was investigated, and a theory for the extraction kinetics is proposed. Pethidine, nortriptyline, methadone, haloperidol, and loperamide were extracted from pure water samples with recoveries ranging between 33% and 47% after only 5 min of operation under totally stagnant conditions. The extraction system was compatible with human urine and plasma samples and provided very efficient sample pretreatment, as acidic, neutral, and polar substances with no distribution into the organic SLM were not extracted across the membrane. Evaluation was performed for human urine, providing linearity in the range 1–20 μg/mL, and repeatability (RSD) in average within 12%.

1. Introduction

For a long time, it has been known that liquid–liquid extractions can be improved by the application of an electrical potential difference over the two phases [1–4]. Primarily this principle has been utilized in chemical engineering and for industrial applications, whereas similar approaches in small scale for analytical applications are few. In pioneering work by Tjaden and co-workers [5–7], this type of electro extraction was accomplished in combination with capillary electrophoresis (CE) or high-performance liquid chromatography (HPLC). Target analytes present in extracts/solutions of ethyl acetate were electro-extracted into aqueous media by the application of several kV. In combination with CE [5,6], this phase-transfer from an organic extract and into aqueous solution was easily accomplished inside the CE instrument with the power supply of the latter, and required no additional equipment. The electro-extracted analytes were focused by isotachophoresis as a part of the extraction procedure, and were subsequently analyzed by CE. Electro extraction was also performed in combination with HPLC [7], and in this case the concept was realized by the development of a needle device coupled to a high voltage power supply. In spite of promising results, this approach was not further investigated.

Recently, Arrigan and co-workers reported another variant of electro extraction, namely electrochemically modulated liquid–liquid extraction of ions [8–10]. In this concept, ionic analytes were extracted from a flowing aqueous sample and into a stationary organo-gel phase by the application of an electrical field (d.c.). Whereas Tjaden et al. used several kV for their extractions the work of Arrigan et al. used low voltages (in the range −0.5 to +0.5 V). The concept of electrochemically modulated liquid–liquid extraction of ions was recently reported also in the micro-fluidic chip format as an interesting extension [10].

As another approach to electro extraction, we recently developed the concept of electromembrane extraction (EME) [11–19]. In this system, charged analytes were extracted from aqueous samples, through a very thin layer (200 μm) of an organic solvent immobilized in the porous wall of a hollow fiber membrane, and into an aqueous acceptor solution placed in the lumen of the hollow fiber by the application of an electrical field (d.c.). Subsequently, the acceptor solution was analyzed by CE or HPLC. The EME concept has been demonstrated for several basic [11,12,18] and acidic [13] drugs, and also peptides have been recently extracted by EME [19]. Typically, voltages in the range 10–300 V have been used to efficiently transfer the analytes into the acceptor solution. To further stimulate this process, strong agitation of the 300 μL samples was performed (1000–1200 rpm) as convection in the bulk sample

* Corresponding author at: School of Pharmacy, University of Oslo, P.O. Box 1068 Blindern, 0316 Oslo, Norway. Tel.: +47 22856576; fax: +47 22854402.
E-mail address: stigpe@farmasi.uio.no (S. Pedersen-Bjergaard).

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was found to be very important for the overall extraction kinetics [15]. Under optimized conditions, extraction recoveries in the range 40–75% have been reported after only 3–5 min of extraction, and excellent sample clean-up has been accomplished from biological samples. Thus, EME has shown promising aspects for future implementation in analytical chemistry.

With continuously improved detectability of analytical instrumentation (like in liquid chromatography–mass spectrometry), the recall for analyte enrichment has decreased for many applications, sample volumes have been reduced as in single drop microextraction (SDME) [20], and attention has been focused on miniaturization of the total analytical process in general. Thus, miniaturization of EME to handle low or sub μL volumes of sample and acceptor solutions may be an interesting direction in the future in order to implement this extraction technique to different micro-scale analytical approaches. The present work investigates for the first time miniaturized EME using flat membranes. The set-up used was very simple and required no special instrumentation except a low voltage power supply. With this simplified system, extractions could easily be performed from sample volumes in the order of 10 μL. The focus of the present paper was to demonstrate the proof-of-principle, and to characterize the system from a fundamental point of view. The influence of the membrane thickness, extraction time and voltage was investigated. Finally, we demonstrate a few applications of the system for human urine and plasma to illustrate the level of performance.

2. Experimental

2.1. Electromembrane extraction

The equipment used for the miniaturized EME is illustrated in Fig. 1. A platinum wire with a diameter of 0.5 mm (K. Rasmussen, Hamar, Norway) was connected to the negative outlet of the d.c. power supply and served as the electrode in the acceptor droplet. A small well with a diameter of 5 mm and a volume of approximately 15 μL was pressed into a 1 cm × 5 cm piece of aluminum foil with 15 μm thickness (Merck, Darmstadt, Germany), and this well was used as the sample compartment. The aluminum foil was connected to the positive outlet of the power supply and the whole foil served as the anode for the EME. Two porous polypropylene membranes with different thickness were tested; a 25-μm thick membrane (Celgard 2500 micro-porous membrane, Celgard, Charlotte, NC, USA) with a porosity of 55% and with 0.21 μm × 0.05 μm pores, and a 100-μm thick membrane (Accurel PP 1E R/P, Membrana, Wuppertal, Germany) where porosity and pore size was unavailable. EME was performed according to the following procedure; 10 μL acidified sample solution was filled into a well formed in the aluminum foil using a 20-μL Eppendorf Research pipette (Eppendorf, Hamburg, Germany), and the positive outlet of the power supply was connected to this foil. 1 μL of organic solvent NPOE (2-nitrophenyl octyl ether) was delivered by a pipette into a 1 cm × 1 cm piece of the porous polypropylene membrane for rapid immobilization. Excess of solvent was removed by a medical wipe. The membrane containing the SLM was placed on the top of the sample; the membrane squeezed the sample to fill the whole well and thus became in liquid contact with the sample. 10 μL of acceptor solution was applied as a droplet on top of the membrane, and was in direct liquid contact with the latter. The contact area with the SLM was 0.1 cm^2 on the acceptor side (assuming a hemispherical acceptor droplet) and less than 0.2 cm^2 on the donor side where the sample was sandwiched between the aluminum foil and the membrane. The negative electrode was inserted into the top of the acceptor droplet, and a constant voltage (typically 15 V) was applied for a certain period of time (typically 5 min) to accomplish the extraction. After the extraction, the acceptor droplet was immediately transferred by a pipette to a microinsert for analysis by capillary electrophoresis.

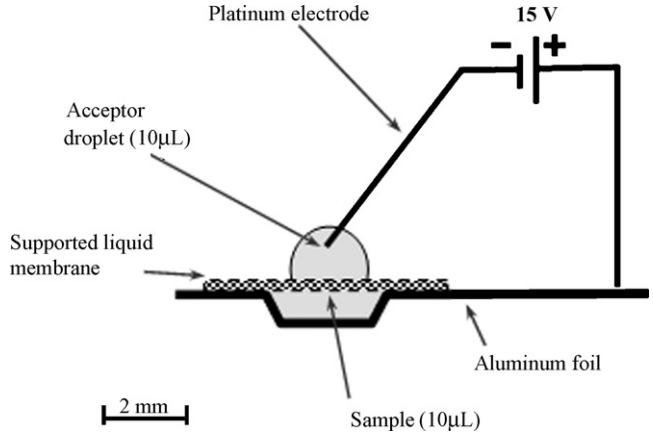

Fig. 1. Schematic illustration of the set-up for drop-to-drop EME.

2.2. Capillary electrophoresis

Capillary electrophoresis was performed with either a MDQ instrument (Beckman, Fullerton, CA, USA) or an Agilent Technologies HP^{3D} CE instrument (Agilent Technologies, Waldbronn, Germany) both equipped with a UV-detector operated at 200 nm. The running buffer was 15 mM phosphate adjusted to pH 2.7 with orthophosphoric acid. Separations were performed at 20 kV in 75 μm-I.D. fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) with an effective length of 20 or 24.5 cm respectively.

2.3. Chemicals

Pethidine hydrochloride, nortriptyline hydrochloride, methadone hydrochloride, haloperidol, loperamide hydrochloride, and 1-octanol were all obtained from Sigma–Aldrich (St. Louis, MO, USA). 2-Nitrophenyl octyl ether, 2-nitrophenyl pentyl ether, ethyl nitrobenzene, isopropyl nitrobenzene, and phenyltrimethylammonium chloride were obtained from Fluka (Buchs, Switzerland).

2.4. Solutions

A stock solution containing 1 mg/mL of each of pethidine, nortriptyline, methadone, haloperidol, and loperamide was prepared in ethanol and stored at −20 °C protected from light. Sample solutions were prepared by dilution of this stock solution either by 10 mM HCl (providing a pure water sample for fundamental studies and optimization), by human urine and 10 mM HCl (providing a spiked urine sample), or by human plasma and 200 mM ammonium format/formic acid pH 3.80 (providing a spiked plasma sample). 10 mM HCl containing 20 μg/mL phenyltrimethylammonium chloride was utilized as the acceptor droplet solution, where the latter component served as an internal standard to correct for possible evaporative loss of the acceptor droplet.

2.5. Calculation of extraction recovery and diffusion constants

Recovery (R_i) for the analyte, *i*, during the electromembrane extraction was calculated according to Eq. (1) for each analyte:

$$R_i = \frac{n_{a_i\ final}}{n_{s_i\ initial}} = \frac{V_a \cdot C_{a_i\ final}}{V_s \cdot C_{s_i\ initial}} \cdot 100\% \quad (1)$$

where $n_{a_i\ final}$ is the molar amount of analyte, *i*, transferred to the acceptor droplet and $n_{s_i\ initial}$ is the amount of analyte, *i*, originally