Journal of Chromatography A, 1326 (2014) 7–12

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Development of a flat membrane based device for electromembrane extraction: A new approach for exhaustive extraction of basic drugs from human plasma

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ARTICLE INFO

Article history:
Received 11 November 2013
Received in revised form 6 December 2013
Accepted 9 December 2013
Available online 16 December 2013

Keywords:
Electromembrane extraction (EME)
Basic drugs
Human plasma
Flat membrane
Exhaustive extraction

ABSTRACT

In this work, a single-well electromembrane extraction (EME) device was developed based on a thin (100 μm) and flat porous membrane of polypropylene supporting a liquid membrane. The new EME device was operated with a relatively large acceptor solution volume to promote a high recovery. Using this EME device, exhaustive extraction of the basic drugs quetiapine, citalopram, amitriptyline, methadone and sertraline was investigated from both acidified water samples and human plasma. The volume of acceptor solution, extraction time, and extraction voltage were found to be important factors for obtaining exhaustive extraction. 2-Nitrophenyl octyl ether was selected as the optimal organic solvent for the supported liquid membrane. From spiked acidified water samples (600 μl), EME was carried out with 600 μl of 20 mM HCOOH as acceptor solution for 15 min and with an extraction voltage of 250 V. Under these conditions, extraction recoveries were in the range 89–112%. From human plasma samples (600 μl), EME was carried out with 600 μl of 20 mM HCOOH as acceptor solution for 30 min and with an extraction voltage of 300 V. Under these conditions, extraction recoveries were in the range of 83–105%. When combined with LC–MS, the new EME device provided linearity in the range 10–1000 ng/ml for all analytes ($R^2 > 0.990$). The repeatability at low (10 ng/ml), medium (100 ng/ml), and high (1000 ng/ml) concentration level for all five analytes were less than 10% (RSD). The limits of quantification ($S/N = 10$) were found to be in the range 0.7–6.4 ng/ml.

1. Introduction

Sample preparation is a critical step in analytical chemistry, especially for the analysis of food samples, environmental samples, and biological fluids [1,2]. With the development of highly sensitive and selective detectors (e.g. mass spectrometry), miniaturized sample preparation techniques have attracted substantial attention in recent years [3,4]. Among several emerging and miniaturized sample preparation techniques, electromembrane extraction (EME), which was introduced [5], has been proved to be a rapid, green, simple, and cheap sample preparation technique [6,7].

In EME, ionized target analytes present in the sample first migrate into a supported liquid membrane (SLM), and subsequently migrate further into an acceptor solution under the influence of an electrical field. EME provides isolation of target analytes, sample clean-up and potential enrichment [8]. Because of these advantages, EME has been used to isolate acidic drugs [9], basic drugs [10,11], small peptides [12], metal ions [13] and organic pollutants [14] from biological fluids and water samples. In most cases, however, the extraction recovery of target analytes has been limited within the range of 20–70% with no possibilities for exhaustive extraction [15].

To the best of our knowledge, only a single research paper has reported exhaustive EME up to date [8], in which non-polar basic drugs were extracted from acidified water samples as well as from human plasma. Exhaustive EME was achieved by using three hollow fibers rather than one hollow fiber in each sample. With the three-fiber arrangement, the volume of acceptor solution was increased, and this contributed to extraction recoveries very close to 100%. However, the set-up with three hollow fibers is technically more complex than using a single hollow fiber. Also EME with the three-fiber approach is less amenable to automation.

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http://dx.doi.org/10.1016/j.chroma.2013.12.028

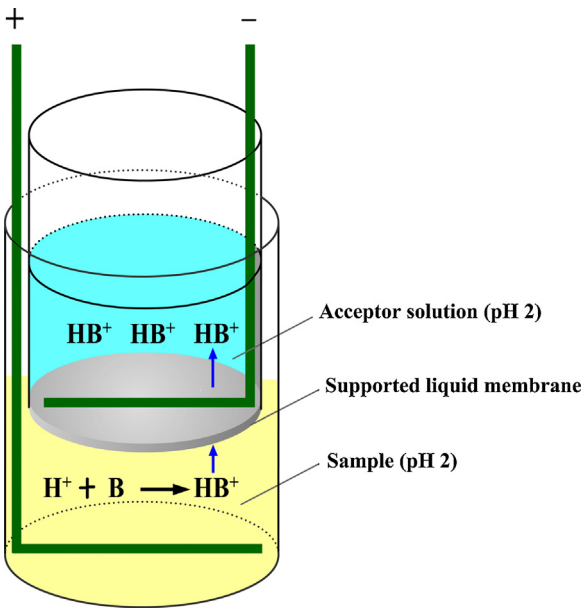

Fig. 1. Schematic illustration of the single-well EME set-up based on flat membrane.

For further progress and routine implementation of EME, simple and more user-friendly devices for exhaustive extraction have to be developed, and this was the objective of the present research article. Besides EME based on the hollow fibers [16,17], EME configurations based on a flat membrane have also been developed in the past five years. For example, EME based on a flat membrane bag/envelop was introduced by Lee et al. [14]. In their work, a membrane envelope was employed to replace the hollow fiber membrane with larger capacity of acceptor solution volumes, which can promote higher recoveries. Almost at the same time, another EME format termed drop-to-drop EME based on a flat membrane emerged [18]. Recently, the drop-to-drop EME has been further developed into a micro-fluidic chip EME using two polymethyl methacrylate plates with channels, which were isolated by an SLM and acted as the sample vial and the acceptor compartment, respectively [19].

Inspired by the recent EME work with flat membranes, a single-well EME configuration based on a thin flat membrane was explored in the present paper in order to realize exhaustive EME (Fig. 1). The work investigated the development of the new EME device, and optimization of fundamental operational parameters was performed. Exhaustive extraction with the new device was discussed in relation to recent theoretical models for EME, and combined with LC–MS, the new EME device was validated with five non-polar basic drugs as model analytes.

2. Experimental

2.1. Chemicals and materials

Methadone hydrochloride and amitriptyline hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sertraline hydrochloride (50 mg tablet) was from Pfizer Italiana (Latina, Italy), citalopram hydrobromide (20 mg tablet) was produced by Lundbeck (Copenhagen, Denmark), and quetiapine (25 mg tablet) was provided by Astra Zeneca AS (Oslo, Norway). Fluoxetine-d5 solution in methanol was obtained from ISOTEC (Miamisburg, OH, USA) with a concentration of 1 mg/ml. 2-Nitrophenyl octyl ether (NPOE) was obtained from Fluka (Buchs, Switzerland). 1-Ethyl-2-nitrobenzene (ENB), 2,4-dimethyl-1-nitrobenzene (DMNB), and 2-undecanone (UD) were all purchased from Sigma-Aldrich (St. Louis, MO, USA). Isopropyl nitrobenzene (IPNB) was from Tokyo Chemical Industry (Tokyo, Japan). Water was purified with a Milli-Q water purification system (Molsheim, France). Formic acid, acetonitrile, acetic acid, hydrochloric acid, sodium dihydrogen phosphate monohydrate, and ortho-phosphoric acid were all obtained from Merck (Darmstadt, Germany).

Accurel PP 1E (R/P) flat membrane (polypropylene membrane, thickness of 100 μm) was purchased from Membrana (Wuppertal, Germany). The standard 10–1000 μl Biohit tips were from Sartorius Biohit Liquid Handling Oy (Helsinki, Finland). The Eppendorf safe-lock 2.0 ml PP tubes were obtained from Eppendorf AG (Hamburg, Germany). The platinum wires with a diameter of 0.5 mm were purchased from K. A. Rasmussen (Hamar, Norway).

2.2. Preparation of solutions

A single tablet of sertraline was grinded into fine powder. The powder was then dissolved in 10 mM HCl to obtain a stock solution with a theoretical concentration of 1 mg/ml. Stock solutions for methadone and quetiapine were also prepared from tablets in a similar way as for sertraline. The stock solutions of amitriptyline and citalopram at 1 mg/ml were obtained by dissolving pure amitriptyline and citalopram in ethanol. All these stock solutions were filtered through a 0.45 μm filter. The stock solutions were stored at 4 °C. The spiked acidified water samples were obtained by dilution of the stock solutions with 10 mM HCl. Drug-free human plasma, which was obtained from Oslo university hospital (Oslo, Norway) and stored at −32 °C, was diluted with Milli-Q water (1:5) prior to extraction.

2.3. EME set-up and procedure

The EME set-up based on a flat membrane is shown in Fig. 1. The acceptor compartment consisted of a piece of flat membrane connected to the wide end of a 10–1000 μl pipette tip. For easy operation, the narrow end of the pipette tip was cut off. The flat membrane was sealed on the wide end of the pipette tip using a cotech soldering iron station (Clas Ohlson AB, Insjon, Sweden) at 185 °C for 5 s. The sample compartment was a 2.0 ml Eppendorf safe-lock PP tube containing 600 μl of sample. After the immobilization of 5 μl of 2-nitrophenyl octylether (NPOE) on the porous membrane, 600 μl of 20 mM formic acid was filled into the acceptor compartment. The "L-shaped" anode and cathode (platinum wires) were placed oppositely into the sample and acceptor solution, respectively (see Fig. 1). The "L-shape" of the electrodes enabled the inter-electrode distance to be kept constant. The selection of platinum wires with a diameter of 0.5 mm as electrodes was based on previous experiences [8]. Thereafter, the acceptor compartment was inserted into the sample compartment with a gap of approximately 1 mm between the SLM and the interface of the sample. The electrodes were connected to an ES 0300-0.45 power supply (Delta Elektronika BV, Zierikzee, Netherlands), a voltage of 250 V was applied to initialize the extraction process, meanwhile the set-up was vibrated at a speed of 1200 rpm by a Vibramax 100 (Heidolph Instruments, Kelheim, Germany). After the desired extraction time, the extraction was terminated by turning off the power supply. Immediately, the acceptor solution was collected and subsequently analyzed by micro-HPLC or LC–MS.

2.4. Micro-HPLC

The Micro-HPLC consisted of a Cheminert injector with an injection volume of 50 nl (VICI AG, Schenkon, Switzerland), a MicroPro™ Pump (Eldex, Napa, CA, USA), a Spectra Flow-501 UV/VIS detector (Sunchrom, Friedrichsdorf, Germany) operated at 200 nm to maximize *S*/*N* ratios, and the software used for data acquisition was