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Exhaustive extraction of peptides by electromembrane extraction

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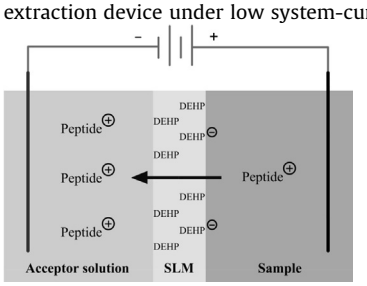
HIGHLIGHTS

- Exhaustive extraction of peptides achieved with a flat membrane-based EME device.
- The system-current was reduced and stabilized by increasing the SLM volume.
- The recoveries of peptides were increased with the increasing of the SLM volume.
- The average system-current was below 50 μA for a 25 min EME with a voltage of 15 V.

GRAPHICAL ABSTRACT

Exhaustive extraction of peptides was achieved using a flat membrane-based electromembrane extraction device under low system-current conditions.



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ABSTRACT

This fundamental work illustrates for the first time the possibility of exhaustive extraction of peptides using electromembrane extraction (EME) under low system-current conditions (<50 μA). Bradykinin acetate, angiotensin II antipeptide, angiotensin II acetate, neurotensin, angiotensin I trifluoroacetate, and leu-enkephalin were extracted from 600 μL of 25 mM phosphate buffer (pH 3.5), through a supported liquid membrane (SLM) containing di-(2-ethylhexyl)-phosphate (DEHP) dissolved in an organic solvent, and into 600 μL of an acidified aqueous acceptor solution using a thin flat membrane-based EME device. Mass transfer of peptides across the SLM was enhanced by complex formation with the negatively charged DEHP. The composition of the SLM and the extraction voltage were important factors influencing recoveries and current with the EME system. 1-nonanol diluted with 2-decanone (1:1 v/v) containing 15% (v/v) DEHP was selected as a suitable SLM for exhaustive extraction of peptides under low system-current conditions. Interestingly, increasing the SLM volume from 5 to 10 μL was found to be beneficial for stable and efficient EME. The pH of the sample strongly affected the EME process, and pH 3.5 was found to be optimal. The EME efficiency was also dependent on the acceptor solution composition, and the extraction time was found to be an important element for exhaustive extraction. When EME was carried out for 25 min with an extraction voltage of 15 V, the system-current across the SLM was less than 50 μA, and extraction recoveries for the model peptides were in the range of 77–94%, with RSD values less than 10%.

1. Introduction

Sample preparation has gained increasing attention in analytical chemistry, especially for the analysis of biological fluids [1,2]. Biological fluids without pretreatment are considered a too

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complex matrix for direct analysis, because the matrix might influence the quality of the results [3] and the life time of the analytical instrument [4]. Frequently, analytes are present in biological fluids at low concentrations, and pre-concentration of the analytes is necessary for a reliable quantitative and qualitative analysis [5]. Additionally, with the development of highly sensitive and selective detectors (e.g., mass spectrometry), miniaturized and green sample preparation techniques with a simple work flow have attracted substantial attention in recent years [6,7].

Microextraction techniques such as solid-phase microextraction (SPME) and liquid-phase microextraction (LPME) were introduced in the 1990s [8]. SPME is considered an open-bed and down-scaled format of solid-phase extraction (SPE) [9]. The idea of SPME is that a small amount of extractant coated on a solid support is exposed to the sample for a desired time, which is long enough for the extractant to adsorb the analytes and to establish an equilibrium [10]. Similarly, LPME is a down-scaled version of liquid–liquid extraction (LLE). Among the LPME formats, single-drop microextraction (SDME) was introduced first in 1996 [11]. Based on the distribution constants, the analytes are extracted from the sample into a single drop of the extraction phase [11], which can contain nanomaterial [12]. Subsequently, three-phase hollow fiber liquid phase microextraction (HF-LPME) was proposed as an alternative to SDME in 1999 [13]. In HF-LPME, a supported liquid membrane (SLM) containing a small amount of organic solvent in the micro-pores of a hollow fiber membrane is used as a barrier between the aqueous sample and the aqueous acceptor solution. Since the extraction is governed by passive diffusion, it requires a long extraction time to reach the equilibrium due to the slow diffusion across the SLM [14].

Based on the HF-LPME system, electromembrane extraction (EME) was developed in 2006 to overcome the limitation of the long extraction time in HF-LPME by introducing an electric field [15]. Among several miniaturized sample preparation techniques, EME is a rapid, green, simple and inexpensive sample preparation technique [16,17]. In EME of non-polar substances, ionized analytes in the sample cross the SLM containing a pure organic solvent, and subsequently migrate into the acceptor solution. As such, EME has been used to isolate acidic drugs [18], basic drugs [19,20], and organic pollutants [21] from biological fluids or water samples. However, EME of polar substances such as small peptides [22], metal ions [23], or amino acids [24], requires an ion-pair reagent in the SLM, and di-(2-ethylhexyl)-phosphate (DEHP) has been the most popular carrier [25].

Up to date, EME has been performed with porous hollow fibers to support the liquid membrane, providing a typical membrane thickness of 200 μm. With such systems, EME can provide fast isolation of target analytes, excellent sample clean-up, and high enrichment [26]. However, in some cases, the formation of gas bubbles occur due to excessive system-current (current running in the circuit) and electrolysis, and in most cases, exhaustive extraction is not possible [27]. Recently, a thin flat membrane-based EME device with a large capacity of acceptor volume was developed, and using this new EME configuration, exhaustive extraction of some basic drugs was achieved. The high extraction efficiency was due to the relatively large acceptor volume (600 μL) and the thin SLM (100 μm) [28].

To our knowledge, only a few articles have reported the analysis of peptides using EME [22,29–33]. These articles were all based on EME with porous hollow fibers as support for the SLM, and the extraction recoveries reported were typical in the range of 10–50%. In addition, due to the use of DEHP or related carriers in the SLM, the EME systems have to some extent been prone to excessive system-current [25]. EME of peptides is still in its infancy, and for future applications, higher extraction recoveries are mandatory. Therefore, the current fundamental work focused on exhaustive extraction of peptides by EME for the first time, using the thin flat membrane-based EME device developed recently [28]. With this device, the pH of the sample, the volume of SLM, the compositions of SLM and acceptor solution were optimized for exhaustive extraction, along with the extraction voltage and time. In addition to extraction recoveries, this work also focused on the level of system current. The latter is highly important in order to develop stable extraction systems in the future.

2. Experimental

2.1. Chemicals and materials

Bradykinin acetate (BK), angiotensin II antipeptide (AT2 AP), angiotensin II acetate (AT2), neurotensin (NT), angiotensin I trifluoroacetate (AT1), and Leu-enkephalin (L-Enke) were all supplied by Bachem (Bubendorf, Switzerland). Trifluoroacetic acid (TFA), 1-octanol, 1-nonanol, decanol isomers mixture, 1-undecanol, 2-decanone, 2-undecanone, and di-(2-ethylhexyl)-phosphate (DEHP) were all purchased from Sigma–Aldrich (St. Louis, MO, USA). A Milli-Q water purification system (Molsheim, France) was used to purify the water. Formic acid, acetic acid, boronic acid, phosphoric acid, hydrochloric acid, sodium dihydrogen phosphate monohydrate, and methanol were all obtained from Merck (Darmstadt, Germany).

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

2.2. Preparation of the sample solution

The individual stock solutions of the model peptides were prepared by dissolving the model peptide into deionized water with a concentration of 1–2 mg mL^{-1}. All these stock solutions were stored at −32 °C and protected from light. The sample solutions were obtained daily by diluting the stock solutions with 25 mM phosphate buffer with a designated pH before the extraction.

2.3. EME set-up and procedure

The schematic illustration and the real picture of the EME set-up are shown in Fig. 1, and the fabrication of this home-made flat membrane-based EME device has been described elsewhere [28]. Briefly, the acceptor compartment is a wide end-closed 10–1000 μL pipette tip with a piece of flat membrane, which was sealed on the pipette tip at 185 °C for 5 s using a Cotech Soldering iron station (Clas Ohlson AB, Insjon, Sweden). 600 μL of sample was filled into the donor compartment, which was a 2.0 mL Eppendorf PP tube. After the application of 5 μL of the SLM on the outside of the membrane, another 5 μL of the SLM was added to the inside of the membrane before filling the acceptor solution, which was 600 μL of 50 mM phosphoric acid. Two “L- shaped” electrodes (platinum wires with a diameter of 0.5 mm) were introduced into the sample and acceptor solution, respectively. Afterwards, the membrane based acceptor compartment was inserted into the 2.0 mL Eppendorf PP tube with a gap of about 1 mm between the interface of the sample and the SLM. The electrodes were connected with an ES 0300–0.45 power supply (Delta Elektronika BV, Zierikzee, Netherlands), which was coupled with a home-made current monitor. The current monitor was coupled to a computer to record the system-current using