Development of Electromembrane Extraction Technologies for Future Bioanalysis of Pharmaceuticals and Peptides

Thesis for the degree Philosophiae Doctor

by

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CONTENT

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Oslo, October 2015
Chuixiu Huang

LIST OF PAPERS

This thesis is based on the following papers which are referred to by their roman numerals in the text:

I **C. Huang**, LEE. Eibak, A. Gjelstad, X. Shen, R. Trones, H. Jensen, S. Pedersen-Bjergaard, Development of a flat membrane based device for electromembrane extraction: a new approach for exhaustive extraction of basic drugs from human plasma, ***J. Chromatogr. A*** 1326 (**2014**) 7-12.

II **C. Huang**, A. Gjelstad, S. Pedersen-Bjergaard, Exhaustive extraction of peptides by electromembrane extraction, ***Anal. Chim. Acta*** 853 (**2015**) 328-334.

III **C. Huang**, A. Gjelstad, S. Pedersen-Bjergaard, Selective electromembrane extraction based on isoelectric point: Fundamental studies with angiotensin II antipeptide as model analyte, ***J. Membr. Sci.*** 481(**2015**) 115-123.

IV **C. Huang**, KF. Seip, A. Gjelstad, X. Shen, S. Pedersen-Bjergaard, Combination of Electromembrane Extraction and Liquid-Phase Microextraction in a Single Step: Simultaneous Group Separation of Acidic and Basic Drugs, ***Anal. Chem.*** 87 (**2015**) 6951-6957.

V **C. Huang,** A. Gjelstad, KF. Seip, H. Jensen, S. Pedersen-Bjergaard, Exhaustive and stable electromembrane extraction of acidic drugs from human plasma, ***J. Chromatogr. A*** 1425 (**2015**) 81-87.

Publications are not included in this thesis:

I **C. Huang**, KF. Seip, A. Gjelstad, S. Pedersen-Bjergaard, Electromembrane extraction for pharmaceutical and biomedical analysis - Quo vadis, ***J. Pharm. Biomed. Anal.*** 113 (**2015**) 97-107.

II **C. Huang**, H. Jensen, KF. Seip, A. Gjelstad, S. Pedersen-Bjergaard, Mass transfer in electromembrane extraction – The link between theory and experiments, ***J. Sep. Sci.*** 00 (**2015**) 1-10.

ABBREVIATIONS

AgNPs	Silver nanoparticles
AT1	Angiotensin I
AT2	Angiotensin II
AT2 AP	Angiotensin II antipeptide
BK	Bradykinin
CE	Capillary electrophoresis
CIT	Citalopram
CNTs	Carbon nanotubes
CTAB	Cetyltrimethylamonium bromide
DEHP	Di-(2-ethylhexyl) phosphate
DHE	Dihexylether
EE	Electro extraction
EME	Electromembrane extraction
ENB	1-Ethyl-2-nitrobenzene
FID	Flame ionization detection
FLU	Flurbiprofen
GC	Gas chromatography
GEM	Gemfibrozil
HF-LPME	Hollow fiber liquid-phase microextraction
HPLC	High-performance liquid chromatography
IBU	Ibuprofen
IL	Ionic liquid
IPNB	1-Isopropyl-4-nitrobenzene
ITIES	Interface between two immiscible electrolyte solutions

KEC	Ketorolac
KET	Ketoprofen
LC-MS	Liquid chromatography – mass spectrometry
LDS-USAEME	Low-density solvent based ultrasound-assisted emulsification microextraction
L-Enke	Leu-enkephalin
LPME	Liquid-phase microextraction
MWCNTs	Multi-walled carbon nanotubes
NPOE	2-Nitrophenyl octyl ether
NSAIDs	Non-steroid anti-inflammatory drugs
NT	Neurotensin
Pa-EME	Parallel electromembrane extraction
PEME	Pulsed electromembrane extraction
pI	Iso-electric point
PIM	Polymer inclusive membrane
PRO	Probenecid
QUI	Quinaldic acid
RSD	Relative standard deviation
SER	Sertraline
SLM	Supported liquid membrane
SPE	Solid-phase extraction
SPME	Solid-phase microextraction
TEHP	Tris-(2-ethylhexyl) phosphate
VS-PEME	Voltage-step pulsed electromembrane extraction

ABSTRACT

Electromembrane extraction (EME) was introduced in 2006 as a simple and miniaturized three-phase microextraction technique, where charged analytes are extracted under the influence of the electric field from an aqueous donor phase, across an organic phase (supported liquid membrane, SLM), and into an aqueous acceptor phase. EME has proved to be a fast and selective sample preparation method. EME can provide efficient sample clean-up, high recoveries, and substantial enrichment. Over 120 articles (Scifinder, October 2015) have been published with a focus on EME. The aim of this thesis was to develop an EME technical set-up using flat membrane instead of the hollow fiber membrane, and to study the effect of operational parameters on the EME efficiency and EME stability. Special attention has been paid to exhaustive EME, selective EME, the stability of the EME system, and the combination of EME with liquid-phase microextraction.

In **Paper I**, a single-well EME device was developed using a thin (100 μm in thickness) flat porous polypropylene membrane to support the SLM. One of the main features of this flat membrane-based EME device was the large acceptor phase volume. With this EME device, exhaustive extraction of some basic drugs from both acidified water samples and diluted human plasma (pH 7.4) has been demonstrated using quetiapine, citalopram, amitriptyline, methadone and sertraline as the model analytes. With 2-nitrophenyl octyl ether (NPOE) as SLM, exhaustive extraction was accomplished after careful optimization of sample matrix, volume of acceptor phase, extraction time, and extraction voltage. When the evaluation was carried out using liquid chromatography-mass spectrometry (LC-MS), this new EME device provided linearity in the range 10-1000 ng/mL with r^2 >0.990, repeatability at three concentration levels were < 10%, and the limits of quantification (LOQ, S/N = 10) were found to be in the range of 0.7-6.4 ng/ml.

In **Paper II**, exhaustive EME of peptides under low system-current conditions (< 50 μA) with a thin flat membrane-based EME device was demonstrated using bradykinin (BK), angiotensin II antipeptide (AT2 AP), angiotensin II (AT2), neurotensin (NT), angiotensin I (AT1), and leu-enkephalin (L-Enke) as the model peptides. Mass transfer of cationic peptides across the SLM was enhanced by complex formation with negatively charged di-(2-ethylhexyl)-phosphate (DEHP), and therefore the sample pH strongly affected the EME process. In addition, the stability and efficiency of the EME system was dependent on the

SLM volume. The EME recoveries of the peptides and the system-current during EME were affected strongly by the composition of the SLM and by the extraction voltage. The SLM of 1-nonanol diluted with 2-decanone (1:1 v/v) containing 15% (v/v) DEHP provided acceptable system-current and high recovery, when EME was carried out with a voltage of 15 V. The acceptor phase composition and extraction time were important factors for exhaustive extraction. After the optimization of the key parameters, recoveries of peptides were in the range of 77-94% (RSD <10%) with system-current < 50 μA.

In **Paper III**, selective EME of a target peptide based on iso-electric point (pI) was achieved for the first time using a two-step EME approach. AT2 AP (pI=5.13) was the target peptide, and AT2, NT, AT1 and L-Enke with pI > 5.13 were considered matrix peptides. In step #1, all peptides were extracted as cationic species from the sample (pH 3.50) and into an aqueous acceptor phase (pH 1.80) using the optimal EME conditions suggested in **Paper II**. Prior to step #2, pH of the acceptor phase collected after step #1 was adjusted to pH 5.25, and the target peptide was no longer net positively charged. In step #2, the matrix peptides were removed as cationic species, while the target peptide remained in the solution. The acceptor phase pH, the SLM composition, the extraction voltage, and the extraction time during the clean-up process (step #2) affected the selectivity of EME significantly. After optimization, 73% of AT2 AP (RSD 13%) and 48% of L-Enke (RSD 5%) were found in the solution after this two-step EME process, whereas the other three cationic peptides were not detected.

In **Paper IV**, EME and liquid-phase microextraction (LPME) were combined in a single step (EME/LPME) for simultaneous group separation of basic and acidic drugs. This simultaneous EME/LPME was carried out under optimum experimental conditions for both EME and LPME. NPOE and dihexyl ether (DHE) were used as the SLMs for EME and LPME, respectively. Longer extraction time (> 15 min) provided higher LPME recovery, but deteriorated the group separation. Simultaneous EME/LPME performance was not affected by coexisting drugs at high concentration, and the recovery from human plasma was improved by strong sample dilution and longer extraction time. The simultaneous EME/LPME approach was evaluated by LC-MS, 10-600 ng/mL and 1-60 μg/mL were the linearity range for the basic and acidic drugs, respectively, with R^2 >0.997. The repeatability at three levels was < 15%, and the limits of quantification (LOQ, S/N = 10) for basic and acidic drugs were 4.0-6.3 ng/mL and 0.6-0.9 μg/mL, respectively.

In **Paper V**, different types of organic solvents including different alcohols, ketones, and ethers as the SLMs for EME of acidic drugs were screened systematically. Seven acidic drugs with a broad log P range (1.01-4.39) were selected as model substances, representing a wide span in terms of physico-chemical properties. EME recovery and system-current across the SLM were studied, in relation to the organic solvent properties such as viscosity, and Kamlet and Taft solvatochromic parameters. Solvents with high hydrogen bonding acidity (α) and dipolarity-polarizability (π*) were found to be successful SLMs, and 1-heptanol was the most efficient one. Both hydrogen bonding and dipole-dipole interactions played an important role for efficient EME. EME efficiency normally increased with decreasing hydrocarbon chain length of the SLM due to the drop in viscosity and the rise in α and π* values. The system-current during EME depended on the sample matrices, the volume and the composition of the SLM. The stability of EME from human plasma was improved by using an SLM of 1-heptanol diluted with NPOE. Exhaustive EME from water samples and diluted human plasma was investigated. Satisfactory evaluation data for EME of acidic drugs from human plasma was obtained using HPLC-UV.

1 INTRODUCTION

Biological samples contain complex matrix, high concentration of ionic compounds, but low level of analytes interest. Therefore direct analysis of pharmaceuticals in biological samples is still a challenge [1,2]. As a consequence, proper sample preparation methods towards biological samples are on demand to minimize the effect of the sample matrices and to offer sufficient enrichment for the analytes of interest, which is significant to obtain reliable results and to maintain the life-time of the instruments [3-5]. In 1986, supported liquid membrane (SLM) extraction was presented to provide efficient sample clean-up and sufficient enrichment with very low organic solvent consumption [6]. Thus, sample preparation techniques with the utility of SLMs have obtained great interest especially for biological samples because of the challenges of the analysis of samples with complex matrices and the advantages of SLM extraction.

Liquid-phase microextraction (LPME) was introduced in the 1990s as a modified and more efficient format of liquid-liquid extraction using an SLM [7]. In LPME, the uncharged analytes in the sample migrate into the SLM by passive diffusion, further migrate into the acceptor phase, and are finally trapped in the acceptor phase because of ionization [8, 9]. Hollow fiber liquid-phase microextraction (HF-LPME) was one of the most popular formats of LPME [10, 11], and an illustration of HF-LPME is presenting in Figure 1-1.

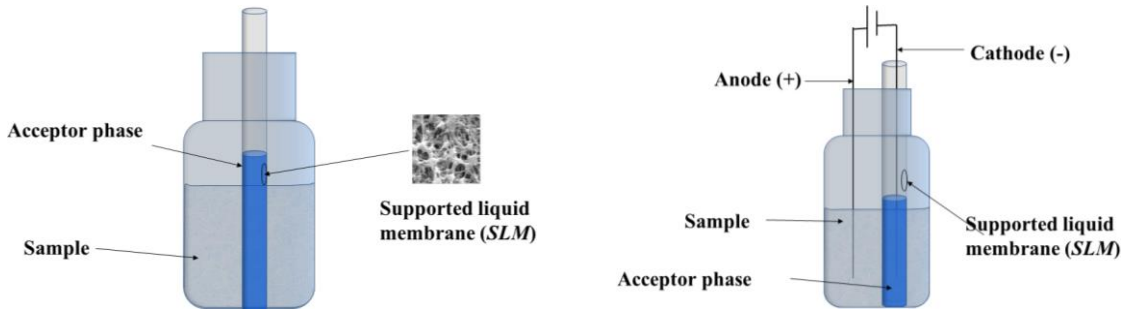


Figure 1-1. Schematic illustration of HF-LPME (left) and EME (right).

In HF-LPME, the organic solvent immobilized in the micro-pores of a hollow fiber membrane separates the sample and the acceptor phase, and migration of the analytes from the sample into the acceptor phase is governed by passive diffusion and a pH gradient. HF-LPME has been used for the determination of acidic drugs [12-15], basic drugs [16-18], peptides [19, 20], metal ions [21-23], organic pollutants [24-28] in biological fluids, food, and

environmental samples. HF-LPME can enrich trace level of analytes to meet the detection limit of the detector, and also can provide excellent clean-up because of the selectivity of the hydrophobic SLM. In addition, HF-LPME can be operated in both two-phase and three-phase mode. Normally, the acceptor phase in two-phase HF-LPME is an organic solvent, which is compatible to gas chromatography (GC) [29,30], while in three-phase HF-LPME, the acceptor phase is an aqueous solution,which is compatible with high performance liquid chromatography (HPLC) or capillary electrophoresis (CE) [31-34]. However, long extraction time is required because of the slow mass transfer, which is governed by passive diffusion in LPME [35, 36].

1.1 Electromembrane extraction principle and theory

In order to reduce the relatively long extraction times in LPME, an electrical field has been used to facilitate the extraction of charged species across organic-aqueous phase boundaries several times since 1994 [37-39]. In those papers, charged substances were extracted from ethyl acetate into an aqueous acceptor phase by electro extraction (EE), and the acceptor phases were subsequently analyzed by HPLC or CE. Afterward, Arrigan and coworkers proposed another approach named electrochemically modulated liquid–liquid extraction of ions, which was referred to the system of “interface between two immiscible electrolyte solutions” (ITIES) [40-42]. The analytes of interest were isolated from a flowing aqueous solution and into a pseudo-liquid acceptor phase (organo-gel phase).

Electro membrane extraction (EME) was developed by introducing an electric field across the SLM of HF-LPME system, and was introduced first by Pedersen-Bjergaard et al. in 2006 [43]. The traditional EME setup is shown in Figure 1-1 (right). A few microliters organic solvent immobilized in the pores in the wall of a hollow fiber polypropylene membrane is employed as the SLM acting as the barrier between the aqueous acceptor phase and donor phase. The acceptor phase is located inside the lumen of the hollow fiber membrane. Two electrodes are placed inside the acceptor phase and donor phase, respectively, and the direction of the electric field is dependent on the charge state of the analytes. If the analytes are positively charged in the donor phase such as basic drugs or peptides, the anode will be placed inside the donor phase, and the cathode will be placed inside the acceptor phase [43]. In addition, the acceptor and the donor phases should be under neutral or acidic conditions to make the analytes positively charged. For negatively charged analytes such as acidic drugs, the direction of the electric field is reversed, where the cathode should be placed inside the

donor phase and the anode should be in the acceptor phase, and the pH of the acceptor and the donor phases should be neutral or alkaline to make the analytes negatively charged [44]. Under influence of the electrical field, the ionized analytes in sample are migrating towards the oppositely charged electrode, which is placed inside the acceptor phase. The charged analytes migrate into the SLM first, and then migrate further into the acceptor phase to realize the enrichment, isolation and clean up [43,44]. As a consequence, the main component of mass transfer in EME is electrokinetic migration, while LPME is based on passive diffusion. Thus, in comparison with LPME, EME has been proved to be a faster sample preparation method because of the introduction of the electric field over the SLM [1].

Up to date, some research groups around the world have contributed to the development and application of EME since the introduction of EME in 2006. Recently, EME has been reviewed several times [1,9,45-56]. In addition, because of the promising advantages of EME, over 120 research articles can be found in SciFinder with a key word of "electromembrane extraction" in October, 2015. First of all, in comparison with other microextraction techniques such as LPME, extraction time is reduced because of the utility of a strong electric field, and 5 min or less is often used in EME [57]. Efficient sample clean-up can be achieved by EME which is due to the fact that the SLM is an efficient barrier for many sample matrices, which is another feature of EME [58]. In addition, the consumption of organic solvent for each sample is reduced to a few microliters, and EME is therefore a green sample preparation method. At last but not least, EME can be operated with high flexibility, which can offer good selectivity. As a consequence, the selectivity of EME can be controlled by the operational parameters, such as the direction or the strength of the electrical field [59,60], the composition of the SLM [61], or the composition of the acceptor phase [62]. These aspects will be discussed and exemplified later in Section 1.2.

The efficiency of EME can be evaluated by the enrichment factor (Ee) or the extraction recovery (Rec) of the analytes. Ee and Rec for each model analyte can be calculated by the following equations:

$$Ee = C_{Ai,t}/C_{Di,0} \quad (1)$$

$$Rec = (m_{Ai,t}/m_{Di,0})*100\% = (C_{Ai,t}*V_A/ (C_{Di,0}*V_D))*100\% = Ee* (V_A/V_D)*100\% \quad (2)$$

Here, $C_{Ai,t}$ and $m_{Ai,t}$ are the concentration and mass of the analyte at time t in the acceptor phase after extraction, respectively. $C_{Di,0}$ and $m_{Di,0}$ represent the concentration and amount of

the analyte at the initial stage in the donor phase, respectively. V_A and V_D represent the volume of the acceptor phase and donor phase, respectively.

According to the above equations, the enrichment factor strongly depends on the ratio between the volume of the acceptor phase and the sample phase. Thus, a high enrichment factor can be achieved by using a large volume of the sample phase but a small volume of the acceptor phase in EME [63].

In an EME system, the SLM is the main component of electrical resistance, because the aqueous phases possess very high conductivities [43]. Thus the system-current in the EME system depends strongly on the properties of the SLM [64], and also on the applied voltage. As reported, the utility of potential in the EME system was supposed to improve the recoveries of the drugs from biological samples because of the reduction of the drug protein binding [65]. However, electrolysis on the two electrodes affects the stability of the EME system. With high system-current, the formation of bubbles at the two electrodes can be observed due to the formation of oxygen and hydrogen according to the following reactions at the anode and cathode, respectively. Electrolysis in the EME system also showed significantly effect on the pH of the donor and acceptor phases [66,67].

$$2\ H_2O \rightarrow 4\ H^+ + O_2\ \uparrow\ + 4\ e^- \quad (3)$$

$$4\ H^+ + 4\ e^- \rightarrow 2H_2\ \uparrow \quad (4)$$

1.2 Principle operational parameters in electromembrane extraction

As discussed above, the SLM and the applied voltage have to be optimized in order to develop stable and efficient EME system [43,68,69]. The extraction time, the pH of acceptor phase, agitation speed and other parameters also need to be optimized to provide high recovery and efficient sample clean-up. After the optimization of these parameters, an acceptable recovery and a substantial decrease in interfering compounds can be achieved by EME.

1.2.1 SLM

The composition of the SLM is one of the main operational parameters in EME, which determines the efficiency and the selectivity of EME. Thus, SLM located in the pores of the hollow fiber holding by capillary forces (Figure 1-1) is often optimized first in EME. The organic solvent used as SLM should fulfill a few requirements, such as very low water solubility, low volatility, and low viscosity, so that the SLM will not partly dissolve in the two

aqueous phases and not evaporate during EME. The analytes can then pass through the SLM with a relatively high diffusion coefficients to facilitate a rapid mass transfer across the SLM [55]. 2-Nitrophenyl octyl ether (NPOE) has become the most popular SLM for EME of non-polar basic drugs (log P > 2.5) [1,50-53]. However, for EME of polar basic analytes (log P < 2.5), NPOE is less efficient due to the poor transfer of analytes from the aqueous sample into the organic SLM, and an ion-pair carrier is required to improve the EME efficiency. Di(2-ethylhexyl) phosphate (DEHP) is an efficient and popular carrier because of the ion-pair formation between the positively charged analytes and the negatively charged DEHP at the donor/SLM interface to facilitate the mass transfer of the analytes from an aqueous donor phase into the SLM [24]. For acidic drugs, NPOE is inefficient, but higher alcohols such as 1-heptanol and 1-octanol have shown to be successful SLMs [44,68,70]. 1-Octanol containing DEHP is a successful SLM for EME of zwitterionic substances such as amino acids and small peptides [71,72]. From systematic studies, solvents with high Kamlet and Taft values for dipolarity-polarizability (π) and hydrogen-bond basicity (β) such as NPOE and IPNB were found to be the most efficient solvents for EME of non-polar basic drugs [64]. Before this thesis, similar information was missing for EME of acidic drugs, so this was addressed in one of this thesis work (**Paper V**). These studies indicated that both dipole-dipole interactions and hydrogen-bond interactions contributed for the mass transfer of charged species from the aqueous donor phase into the organic SLM.

1.2.2 Extraction voltage

The extraction voltage, which determines the strength of the electric field, is another operational key parameter in EME. The potential difference across the SLM serving as the driving force for the mass transfer in EME should be optimized as well. The common profile of EME recovery versus extraction voltage is presented in Figure 1-2 (left). Generally, EME efficiency is reduced with decreasing extraction voltage, as exemplified with 1-isopropyl-4-nitrobenzene (IPNB) as SLM in a recent publication [73]. However, when extraction is above a certain optimal voltage, the EME efficiency may also decrease. This might be due to the fact that the system-current across the SLM may be relatively high with higher voltage, and electrolysis may occur at the electrodes [55]. Excessive electrolysis will result in pH shift in the acceptor phase, and will provide an unstable EME system, which can potentially reduce the EME efficiency [66,67]. Generally, the system-current across the SLM should preferably be below 50 μA, and the control of the system-current should be performed during EME optimization. Another paper demonstrated that the extraction selectivity in EME can be

controlled by the voltage [60]. With NPOE as the SLM, five different basic drugs were extracted efficiently with a voltage of 50 V, whereas only the two drugs with lowest polar surface area were extracted when EME was performed with a voltage of 5 V.

1.2.3 Extraction time

The extraction time is another important operational parameter in EME, and it should also be optimized. The profile of EME recovery versus extraction time is presented in Figure 1-2 (right). Generally, the extraction recovery increases with increasing extraction time, until extraction recovery reaches a maximum at steady-state. The optimal extraction time also depends on the extraction voltage, and 5-10 min is a typical optimal extraction time in EME. However, longer than optimal extraction time often results in lower extraction recoveries, which is most probably due to partial back-extraction of the analytes into the SLM due to the occurrence of excessive electrolysis and pH shift in the acceptor phase [55].

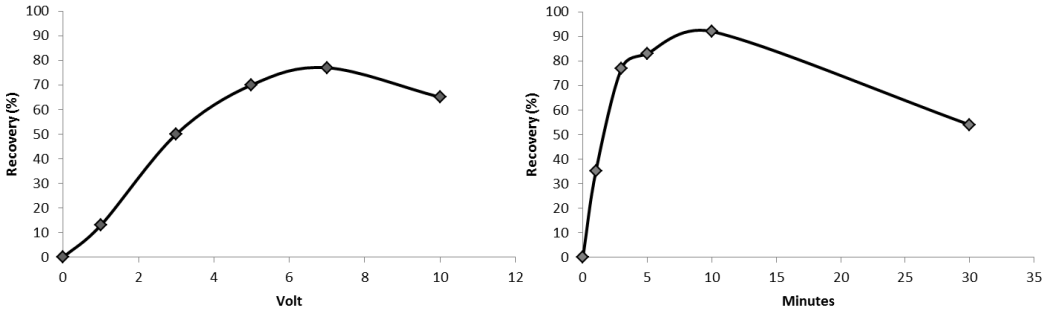


Figure 1-2. Extraction recovery versus extraction voltage (left) and extraction time (right) with an SLM of 1-isopropyl-4-nitrobenzene. Reprinted with permission from [73] © Elsevier B.V. (2007).

1.2.4 Acceptor pH

The pH in the acceptor phase is a key operational parameter. As mentioned above, the extraction recovery is very sensitive to the pH in the acceptor phase, and an unsuitable pH in the acceptor phase may cause trapping analytes in the SLM or back-extraction of analytes into the SLM [55]. Thus, the pH of the acceptor phase should ensure the ionization of the analytes efficiently, which is mandatory for efficient electrokinetic transfer. Normally, low pH in the acceptor phase is used for EME of basic drugs, amino acids or peptides [43,71,72], whereas high pH in the acceptor phase is used for EME of acidic compounds [44,68,70]. Hydrochloric acid (HCl) and formic acid are the most commonly used acids to maintain low pH in the acceptor phase, and sodium hydroxide (NaOH) is normally used to maintain high pH in the acceptor phase [55]. Due to electrolysis during EME, an increase of pH in the acceptor phase for EME of basic drugs and a decrease of pH in the acceptor phase for EME of acidic drugs

have been observed. In addition, the pH shift is magnified by using a small acceptor phase volume, and can be minimized by using strong buffers as the acceptor phase [66,67]. The pH in the donor phase plays less important role than that in the acceptor phase in an EME system [55].

1.2.5 Agitation and other parameters

Agitation during EME is an important factor for efficient EME [43,55]. For example, pethidine, nortriptyline, methadone, haloperidol, and loperamide were extracted with recoveries in the range of 8-10% without agitation, whereas the recoveries were improved to 70-79% using an agitation at 1200 rpm [43]. Agitation can provide sufficient convection and narrow the boundary layer between the SLM and the donor phase, which resulted in a more efficient mass transfer of analytes from the donor phase into the SLM [68,74]. In addition, the significance of agitation depends on the volume of the donor phase, and EME can be carried out as stagnant [75,57]. Typically, agitation speed between 500 and 1000 rpm are used for optimal performance. At lower than optimal agitation speed, analyte transfer from the bulk sample toward the SLM becomes less efficient, whereas at high agitation rates, air bubbles are formed at the donor/SLM interface, which may reduce the contact area between the sample and the SLM [55].

The ionic strength of the donor and acceptor phases and the extraction temperature can also play essential roles in EME. Higher concentration of ions in the acceptor phase than in the donor phase is often beneficial for the EME recovery [76]. As reported, extraction temperature affects not only the diffusion coefficient of an analyte across the SLM but also the electrokinetic driving force in the system, and higher temperature results in faster diffusion but smaller net flux of ions across the SLM according to the Nernst-Plank equation [77]. As a consequence, the effect of temperature is hard to be predicted, and most studies with focus on EME were accomplished in room temperature [55].

1.3 New configurations of electromembrane extraction

EME based on a hollow fiber membrane (Figure 1-1) has been proved to be an efficient sample preparation technique, and has become the most frequently used EME format since the introduction of EME [50,51]. Some other configurations of EME have also been introduced recently to fulfill the different requirements of the analysis.

1.3.1 Drop-to-drop EME

A drop-to-drop EME format using a porous polymeric polypropylene flat membrane was introduced in 2009 by Petersen and his coworkers [78]. The drop-to-drop EME configuration is illustrated in Figure 1-3, where the sample container is a well created on a piece of aluminum foil, which is connected to the positive outlet of the power supply and works as the anode. This configuration exploited the EME principle to extract basic drugs using NPOE as the SLM from a drop of sample (10 µl) into a drop of acceptor phase (10 µl of 10 mM HCl), which was the first time that EME was performed down to low microliter scale. In this work, Accurel PP 1E R/P with a thickness of 100 µm and a pore size of 0.1 µm, and Celgard 2500 with a porosity of 55% and with 0.21 µm×0.05 µm pores were investigated, and the EME recoveries of pethidine, nortriptyline, methadone, haloperidol, and loperamide from spiked acidified water samples were identical (in the range from 33-47%) with these two different flat membranes. The system-current generated in this drop-to-drop EME was in the range of 0.1-40 µA with an extraction voltage of 15 V. This EME format was simple and inexpensive. In addition, excellent clean-up of biological samples (urine and human plasma) were obtained, though the EME efficiency from human plasma was lower because of drug protein binding. Though this system was performed without agitation, rapid mass transfer of analytes from the sample and into the acceptor phase was observed because of the low microliter sample volume (short diffusion distance).

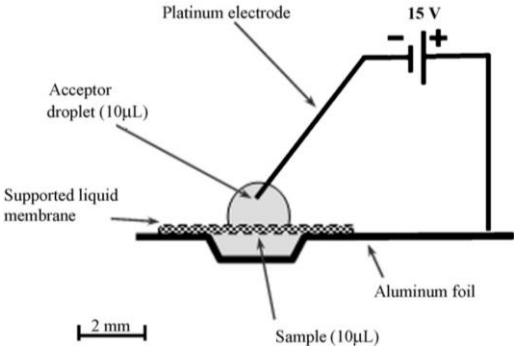


Figure 1-3. Schematic illustration of drop-to-drop EME. Reprinted with permission from [78] © Elsevier B.V. (2008).

1.3.2 On-chip EME

Recently, the drop-to-drop EME has been further developed into a micro-fluidic chip EME system using a flat membrane [79]. The on-chip EME was illustrated in Figure 1-4(left). One polymethyl methacrylate (PMMA) plate with channels was acted as sample channel, while another one was the acceptor compartment. The acceptor phase and the sample containing

five basic drugs were isolated by a piece of porous polypropylene membrane (25 μm in thickness) immobilized with an SLM (NPOE). Sample was pumped through the sample channels with a flow rate ranging from 1-20 μL/min, while the acceptor phase (7 μL of 10 mM HCl) was stagnant. This on-chip EME showed high efficiency, and 20-60% EME recoveries were obtained just after the sample (3 μL/min) contacted with the SLM for a short time (< 4 s).

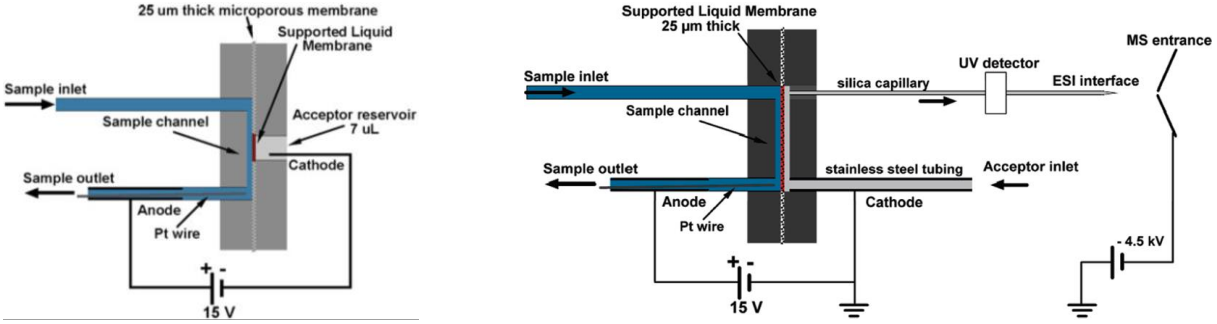


Figure 1-4. Schematic illustration of on-chip EME (left) and on-chip EME coupled with UV/MS (right). Reprinted with permissions from [79] © Springer-Verlag (2010) and [80] © American Chemical Society (2011), respectively.

Subsequently, on-chip EME was further developed and coupled with UV and mass spectrometric detectors for continuous monitoring using a dynamic acceptor phase (Figure 1-4 right) [80]. Two polymethyl methacrylate (PMMA) substrates with channels (50 μm in deepth facing the 25 μm thick porous polypropylene membrane) acted as sample and acceptor phase channels, respectively. The sample and the acceptor phase were separated by the membrane immobilized with an SLM of 0.2 μL NPOE. The dynamic sample and acceptor phase were pumped into the sample and acceptor channel with a defined flow rate, respectively. The EME recoveries for different analytes were in the range of 65-86%, when the dynamic on-chip EME was performed with a sample flow rate of 2 μL/min and an acceptor phase flow rate of 1 μL/min.

In another work, the dynamic on-chip EME was further applied for monitoring drug metabolism in real time by electrospray ionization mass spectrometry [81]. In this work, the acceptor phase outlet was directly coupled to the ESI-MS, and the sample inlet was connected to a metabolic reaction chamber containing rat liver microsomes in buffer to study the in vitro metabolism of amitriptyline in real time. The main advantage of this dynamic micro-fluidic chip EME is that the sample solution and acceptor phase can be delivered continuously into the chip which is beneficial for online analysis to realize the real time measurements and to

monitor drug metabolism. Fast kinetics was another advantage due to the short diffusion path, and this dynamic on-chip EME also demonstrated that a dynamic sample results in more efficient extraction than a stagnant sample [48]. Loss of the organic solvent in the pores of the membrane happened during the continuous operation.

1.3.3 Nano EME

After the development of the drop-to-drop EME and on-chip EME using flat porous polypropylene membrane, traditional EME was further downscaled to nano-EME using Plasmaphan P1LX polypropylene hollow fiber membrane with an internal diameter of 330 μm, a wall thickness of 150 μm and pore size of 0.4 μm, and with the assistance of fused silica capillary (50 μm internal diameter and 363 μm outside diameter) [82]. With this EME system (Figure 1-5), five basic drugs were extracted using an SLM of NPOE from 200 μL acidified sample into approximately 8 nL acceptor phase (phosphate buffer pH 2.7), which was located inside a fused silica capillary resulting in a direct analysis of the acceptor phase by capillary electrophoresis (CE). In comparison with traditional EME, nano-EME offered extremely high enrichment capacity (maximum 25000) because of the nL acceptor phase volume. The enrichment factor of loperamide by nano-EME in 5 min was > 500, although Nano-EME was a very soft technique. Thus, after nano-EME, over 99.2% of the analytes remained in the sample. However, carry-over was observed unless the SLM was carefully rinsed (with voltage applied) between each extraction.

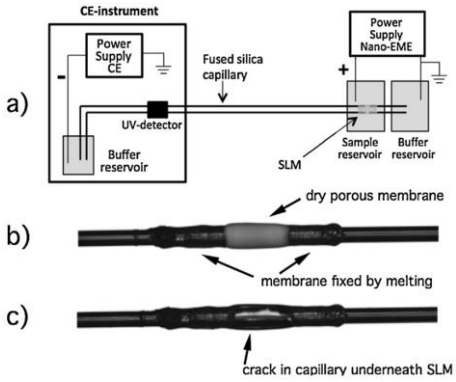


Figure 1-5. Schematic illustration of nano EME (a), before (b) and after (c) filling the SLM. Reprinted with permission from [82] © Elsevier B.V (2013).

1.3.4 Envelope-EME

Another EME format using a membrane-based envelope to replace the hollow fiber membrane was first introduced by Lee and his coworkers [83,76] and the illustration of

envelope-EME is presented in Figure 1-6. The membrane-based envelop was prepared by folding and sealing two ends of the folded polypropylene sheet membrane. With this envelope-EME format, four acidic compounds were extracted using 1-octanol as the SLM from 4 mL sample (pH 6.8) into 20 µL of water (pH 6.8) located inside the membrane-based envelope, which was suspended in the sample solution containing the negative electrode [83]. In another work, using this envelope-EME, four chlorophenols (PCs) were extracted using 1-octanol as the SLM from 3 mL sample (pH 12) into 100 µL acceptor phase (pH 12) [76]. In addition, a tailor-made three-layer-envelope fabricated by sealing four pieces of Accurel 2E HF (R/P) polypropylene membrane sheet with a thickness of 157 µm and pore size of 0.2 µm was used for simultaneous extraction of acidic and basic drugs from samples with neutral pH [84]. In this EME system, the cathode and anode were placed in the acidic and alkaline buffer solutions, respectively, for the extraction of basic and acidic drugs. In another work, 1 mL acceptor phase located in a large membrane-envelop was used for EME of six PCs [85]. The acceptor phase volume in this envelop-EME system is very flexible, which were reported in the range of 20 to 1000 µl [79,83-85]. The principle of EME with membrane-based envelop is the same as traditional EME, but the major advantages of envelop-EME over EME using hollow fiber are larger effective area of the SLM and larger acceptor phase volume capacity, which can promote faster mass transfer and higher recoveries [48].

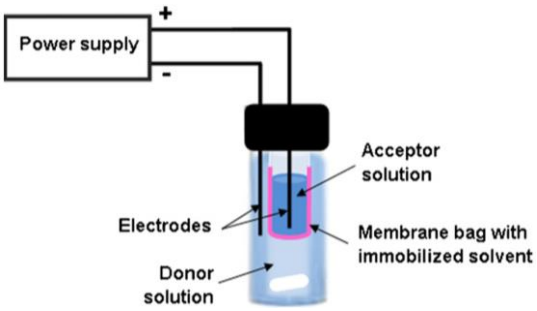


Figure 1-6. Schematic illustration of envelope-EME. Reprinted with permission from [83] © Elsevier B.V (2008).

1.3.5 Dual/Triple/Quadruple EME

Although EME with a flat membrane-based three-layer-envelope [84] and two hollow fibers [59] has been developed for simultaneous EME of basic and acidic drug, the concept of dual EME was brought up for the first time by Tabani and his coworkers [86]. The illustration of dual EME is presented in Figure 1-7. For the demonstration, ibuprofen and thebaine were selected as the model acidic and basic drugs. The anode and cathode were located in the

alkaline and acidic acceptor phase, respectively. Ibuprofen and thebaine were extracted using a voltage of 40 V from 4 mL aqueous neutral sample into 20 µL of alkaline (pH 12.5) and acidic (pH 2.0) acceptor phase, respectively. In another work, selective separation-preconcentration of Cr(VI) and Cr(III) from different environmental samples were achieved using dual EME, because Cr(VI) always forms anionic complex with the element of oxygen, while Cr(III) normally involved in the cationic complex with oxygen [87]. Subsequently, the dual EME with two hollow fibers was modified somehow as shown in Figure 1-7 (right) [88]. In this modified dual EME, the anode was located inside the sample, while two cathodes were located inside the two hollow fibers containing the acidic acceptor phase (pH 1). Dual EME either with flat membrane-based multilayer-envelope or two hollow fibers was developed for the simultaneous extraction of cations and anions [59,84,86,87], or of cations with different polarity [88]. The idea of using dual EME for simultaneous separation of cations and anions, or of cations with different polarity is very interesting. However, it is still a challenge to control the distribution of the electrical field across the sub-EME system. In addition, dual EME normally proved relatively low recoveries of some analytes, which might be due to the compromise experimental parameters (sample pH and extraction voltage) that were used, and neutral pH in sample was not sufficient to ionize the analytes [35,86,89].

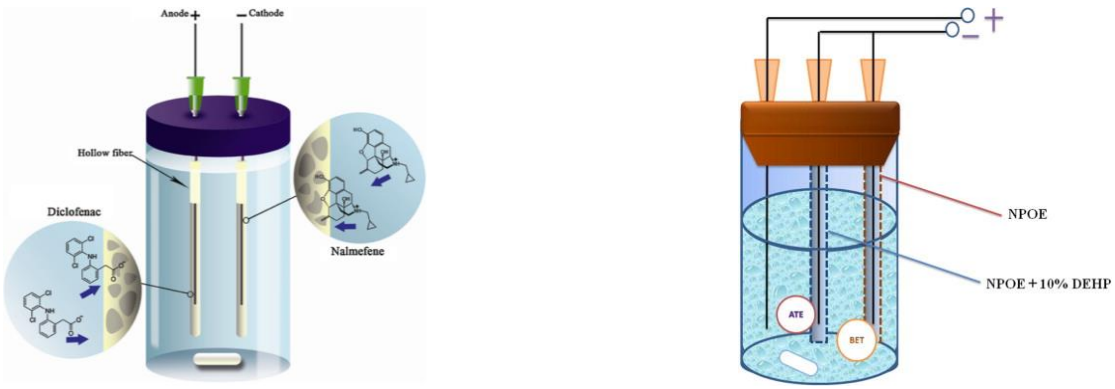


Figure 1-7. Schematic illustration of dual EME for simultaneous extraction of basic and acidic drugs (left), and for simultaneous extraction of basic drugs with different polarities (right). Reprinted with permissions from [86] © WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim (2012) and [88] © Elsevier B.V (2013), respectively.

Besides the simultaneous extraction purpose, another EME format using three-hollow fiber-arrangement shown in Figure 1-8 (left) was proposed for exhaustive extraction of some basic drugs from undiluted human plasma pH 7.4 [75]. In this arrangement, three cathodes were placed in each hollow fiber containing the acidic acceptor phase, and the anode was located in the sample. Six non-polar basic drugs were selected as the model substances to demonstrate exhaustive EME with this EME set-up. EME was performed with an SLM of NPOE and a

voltage of 200 V from 1000 µL sample into 3*30 µL acidic acceptor phases with or from 50 µL sample into 3*7 µL acidic acceptor phases without agitation. Exhaustive EME was obtained due to the increased contact surface area and acceptor phase volume by increasing the number of hollow fibers from one to three [75].

In another work, by combining the idea of group separation of cations and anions [84, 59,86,87], and the idea of group separation of cations with different polarities [86], an all-in-one EME set-up was proposed using four-hollow fiber shown in Figure 1-8 (right) [90]. The two anodes were located in the lumen of the hollow fibers each containing 10 µL alkaline acceptor phase (pH 12), while the two cathodes were located in the hollow fibers each containing 10 µL acidic acceptor phase (pH 1). Five basic drugs with logP ranging from 0.9 to 4.9, and three acidic drugs with logP ranging from 0.59 to 3.84 were selected as the model substances for the demonstration. The EME was performed with four different SLMs, which were 1-octanol, NPOE, 1-octanol containing 4% cetyltrimethylamonium bromide (CTAB), and NPOE+10% tris-(2-ethylhexyl) phosphate (TEHP)+10% DEHP for EME of hydrophobic acidic drugs, hydrophobic basic drugs, hydrophilic acidic drugs and hydrophilic basic drugs, respectively. The recoveries of those drugs from water sample (pH 6.5) were in the range of 33-66% with an extraction voltage of 50 V and an extraction time of 20 min. The quadruple EME configuration is very interesting and important for simultaneous EME of analytes with quite different properties such as acidity/alkaline and polarity. However, similar as dual EME, the experimental operation parameters was selected as a compromise [35,86,89].

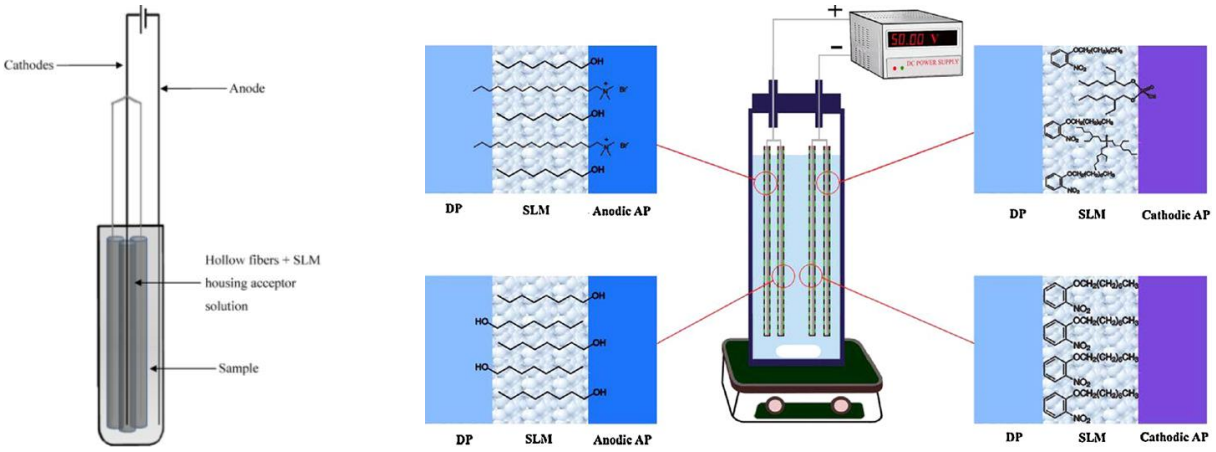


Figure 1-8. Schematic illustration of three-hollow fiber EME for exhaustive extraction (left), and four-hollow fiber EME for simultaneous extraction of basic and acidic drugs with different polarities (right). Reprinted with permissions from [75] © Elsevier B.V (2011) and [90] © Elsevier B.V (2014), respectively.

1.3.6 Parallel EME

Deriving from the aims of simultaneous extraction and exhaustive extraction, parallel EME was proposed to improve the throughput of the sample preparation. With the development of fast instrumental analysis, the sample preparation step normally takes 50-75% of the total analysis time [91]. Parallel EME (Pa-EME) was initially raised in 2010 by parallel collection of three individual traditional EME set-ups (Figure 1-9) [57]. This Pa-EME was performed using a 9 V battery and an SLM of NPOE for only one minute, and the performance of each individual EME were comparable with each other and also comparable with single EME. Afterwards, Pa-EME using porous polypropylene flat membrane, eight plastic vials, and a plate worked as the sample compartments was proposed and presented in a multi-well (eight-well) format [92]. In this work, four basic drugs were selected for the demonstration of Pa-EME using an SLM of NPOE with a voltage of 200 V, and Platemax™ aluminum foil was used as the electrodes. The optimal sample volume in each sample well was 240 μL, and the optimal acceptor phase volume in each acceptor well was 70 μL. Simultaneous and selective isolation, cleanup, and enrichment of the target from human plasma samples was achieved after Pa-EME for 8 min. Subsequently, this multiwell Pa-EME was further developed into a 96-well format [93]. In this work, the EME efficiency and the repeatability were studied in detail using three basic drugs as model analytes. It was demonstrated that the EME performance was affected neither by the collapse in part of the circuits nor by the processing samples from 1 to 96 in parallel in 10 min. Pa-EME showed high potential and may be an interesting high throughput sample preparation platform for the future [52,55,92,93].

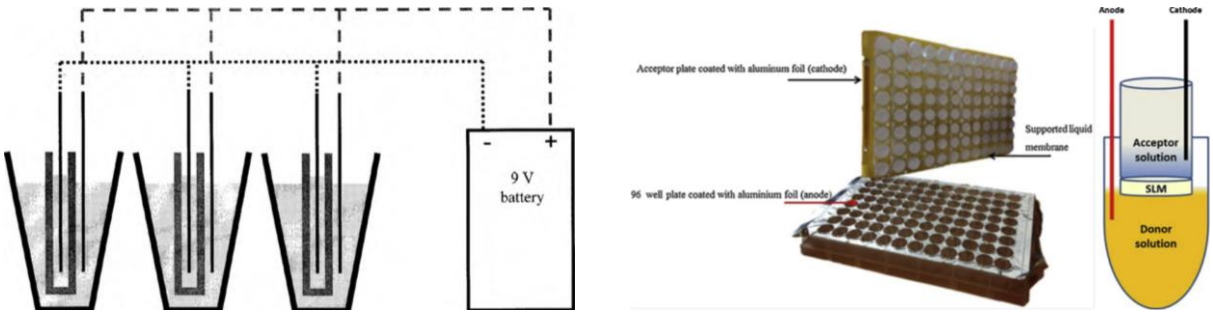


Figure 1-9. Schematic illustration of three hollow fiber EMEs for parallel extraction (left), and flat membrane – based EME for parallel extraction of basic drugs in 96-well format (right). Reprinted with permissions from [57] © Elsevier B.V (2010) and [93] © Elsevier B.V (2014), respectively.

1.4 Modified electromembrane extraction

1.4.1 EME with modified electric field

Originally, EME was proposed with a constant voltage across the SLM. In 2012, EME using stabilized constant d.c. electric current was introduced by Kuban and his coworkers [94]. In this work, the system-current was controlled with a resolution of 1 μA. This EME system provided better repeatability (RSD 2.8-8.9%) than EME at constant voltage (3.6-17.8%) for selected amino acids and basic drugs, because the total system-current was dependent on the contribution from analyte mass transfer [95]. However, the EME efficiency at constant current was not statistically better than that at constant voltage [94].

Subsequently, pulsed electromembrane extraction (PEME) was introduced by Yamini et al. using a pulsed voltage [96]. In comparison with traditional EME, PEME was supposed to improve the stability and extractability of EME because of the reduction of the thickness of the double layer at the interfaces and the elimination of this mass transfer barrier. In another work, PEME was applied for the extraction and quantification of histidine, phenylalanine and tryptophan from food and diluted biological samples (human plasma and saliva) using an SLM of NPOE containing 10% DEHP and 10% TEHP. The recoveries ranged from 7.6 to 21.6% with a repeatability < 4.5% (CV%) [71]. Recently, PEME was applied for the analysis of derived amino acids, and it was claimed that this approach can be used to differentiate the animal origins of gelatin samples used in food and pharmaceuticals [97]. In addition, the performance of PEME was evaluated in comparison with traditional EME using hydrophobic acidic drugs (diclofenac and mefenamic acid) as model analytes [70]. PEME offered slightly higher efficiency (65-69%) than EME (47-51%) from water samples with an SLM of 1-octanol, because a precise reversing of the polarity during the EME process was supposed to offer higher EME recovery [95]. When PEME was carried out from diluted plasma and urine, the recoveries were in the range of 23-33%.

Voltage-step PEME (VS-PEME) was introduced using a voltage programming [98]. VS-PEME was evaluated with three different groups of analytes including hydrophobic basic drugs, hydrophobic acidic drugs and hydrophilic basic drugs. It found that VS-PEME showed negligible influence on the hydrophobic basic drugs (NPOE as the SLM), but significant improvement on the recovery of acidic drugs (1-octanol as the SLM) and hydrophilic basic drugs (NPOE+10% DEHP+10% TEHP as the SLM) in comparison with EME at constant

voltage. VS-PEME started with a lower initial voltage and ended with a higher voltage. This was helpful in order to maintain the stability of the EME system with relatively low electrical resistance such as using alcohols as an SLM or SLM containing a carrier [99].

Recently, EME with a cylindrical cathode surrounding the hollow fiber membrane was used to extract diclofenac and mefenamic acid from biological fluids (plasma and urine) [100]. With this system, EME was carried out from 10 mL sample into 10 μL acceptor phase using 20 V for 15 min. The recovery ranged from 4.7-35.5 % from diluted plasma and diluted urine, which was higher than EME using traditional wire electrodes (1.8-17.3 %). Most recently, another work calculated the distribution of the electric field vector in EME with classical electrodes and cylindrical electrodes, and it was found that the electric field strength in classical EME depended on the position of the SLM while that in EME with cylindrical electrodes was angularly uniform [101]. However, in comparison with classical electrodes, EME with helical and cylindrical electrodes gave lower recoveries for the target analytes (naproxen and diclofenac), because the interaction between the inhomogeneous electric field and the uncharged analytes was involved in the mass transfer process.

1.4.2 EME with modified membrane support

Carbon nanotubes (CNT) were introduced into EME to improve the EME performance for basic drugs (buprenorphine as the model substance) by Fakhari and his coworkers [102]. In this case, prior to the immobilization of the SLM containing the nanotubes, the multi-walled carbon nanotubes (MWCNTs) were dispersed in the organic solvent after acid-treatment. Compared to traditional EME, CNTs-EME offered higher recovery in shorter time, because nanotubes possess large surface area and high adsorption capacity, and served as a solid-sorbent. In another work, functionalized nanotubes dispersed in 1-octanol was immobilized in the pores of the hollow fiber and used as the SLM to extract non-steroid anti-inflammatory drugs (NSAIDs) including Ibuprofen and naproxen from different sample matrices. The extraction recoveries from biological and environmental samples were reported in the range of 69-92% [103]. Recently, CNTs were used in two-phase EME for the determination of basic drugs (tramadol and methadone) in body fluids by GC, where CNTs dispersed in octanol was served as both the supported membrane and the acceptor phase [104]. CNTs-EME involved both EME and SPME (solid-phase microextraction), which resulted in an enhancement in extraction rate and recovery [102-104].

Following the above work, EME using silver nanoparticles (AgNPs)-decorated hollow fiber as the liquid membrane support was proposed by Ramos-Payán et al. [105]. 1-octanol was used as the SLM. When AgNPs-EME of some NSAIDs was carried out from 10 mL water samples (pH 12) into 50 µL acceptor phase (pH 12) for 7 min using 10 V, the preconcentration of the NSAIDs was in the range of 51-86 times corresponding recoveries of 26-43%. It was claimed that in comparison to traditional EME, this new SLM resulted in a faster (30% reduction in extraction time) and more efficient EME system by a factor of 1.2-2.0.

1.4.3 EME without membrane support

Micro-EME across free liquid membrane was introduced by Kuban and Bocek (Figure 1-10) [106,107]. A segment of transparent perfluoroalkoxy tubing was used to house the acceptor phase (1.5 µL), free liquid membrane (1.5 µL) and sample, and this µ-EME principle was demonstrated visually using 1-pentanol as the free liquid membrane and an anionic and a cationic dye as the model analytes [106]. When µ-EME across a free liquid membrane (NPOE or 1-ethyl-2-nitrobenzene (ENB)) was carried out from 1.5 µL sample (water, urine and blood serum) and into 1.5 µL acceptor phase, the recoveries of three basic drugs were in the range of 19-52% [107]. Preconcentration in µ-EME across free liquid membrane (1 µL 1-pentanol) was demonstrated by using 10 µL sample and 1.0 µL acceptor phase [108]. The EME with free liquid membrane was further presented as a two-phase EME on a micro-chip for separation of amino acids [109].

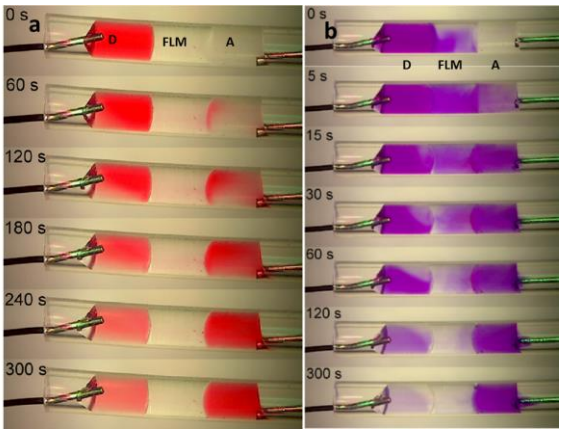


Figure 1-10. Schematic illustration of EME with free liquid membrane of anionic dye (red) and cationic dye (purple). Reprinted with permission from [106] © Elsevier B.V (2014), respectively.

1.4.4 EME with new membrane support

In addition to polypropylene membrane as the membrane support, EME has also been carried out using wheat stem to support the SLM, and 1-octanol containing 5% DEHP was

immobilized in the pores of the wheat stem to serve as an SLM for the extraction of thorium from aqueous samples [110]. Under the optimal conditions, an enrichment factor of 50 was obtained.

Most recently, a synthetic agarose film containing silver nanoparticles (20 μm in thickness, and containing 107.9 mg Ag/g film) has been reported as a new support for SLM in EME of NSAIDs, which provided better EME efficiency than the commonly used polypropylene support [111]. With this new EME support, the preconcentration of the five NSAIDs (logP $\geqslant$ 2.0) was in the range of 2.5-15.3 times (corresponding to 1.3-7.7% in recovery), when the EME was performed from 10 mL water samples (pH 12) into 50 μL acceptor phase (pH 12) with an SLM of dihexyl ether (DHE) and a voltage of 10 V for 10 min.

Furthermore, the polymer inclusion membrane (PIM) with a thickness of 20 μm containing 60% cellulose triacetate as base polymer, 20% NPOE as plasticizer and 20% Aliquat 336 (an anionic carrier) was synthesized and used for the extraction of glyphosate and aminomethylphosphonic acid from an aqueous sample (1 mL/min for 10 min) [112]. EME was carried out with a voltage of 1500 V. The enrichment for the two model analytes was in the range of 87-95 times. Inorganic anions extracted by EME with a PIM have been reported by the same group [113]. In this work, low lipophilicity anions required a membrane with high carrier content (60% cellulose triacetate and 40% Aliquat 336), while ions with high lipophilicity such as perchlorates were extracted well with a membrane without carrier (60% cellulose triacetate and 40% NPOE). In addition, lipophilic organic anions and cations were extracted across plasticized cellulose membrane containing Aliquat 336 and DEHP, respectively [114]. With a polymer inclusion membrane (consisting 75% cellulose triacetate, 12.5% of TEHP and 12.5% of Aliquat 336), chlorinated phenoxyacetic acid herbicides were determined by EME directly coupled to LC-MS [115].

1.4.5 EME with ionic liquid membrane

EME using an ionic liquid as the SLM was introduced by Chen et al. [99]. In this work, ionic liquid EME (IL-EME) was compared with traditional EME using ENB as the SLM with strychnine and brucine as the model substances. This work claimed that IL-EME operated at 1.5 V offered higher recovery than EME at 7.5 V with ENB as SLM. Most recently, by the same group, IL-EME was applied for the determination of four chlorophenoxy acid herbicides including 2,4-dichlorophenoxy acetic acid, 2-(2,4-dichlorophenox) propionic acid, 4-

chloromethylphenoxyacetic acid, and 2,4,5-trichlorophenoxyacetic acid in pig kidney tissue [116]. However, to our knowledge, the ionic liquid can partly leaks into the sample or acceptor phase because ionic liquids are charged, and significant higher system-current than with traditional EME may be observed. As a consequence, to reduce the system-current, very low voltage was used in IL-EME system [106, 116].

1.5 Electromembrane extraction coupled with other techniques

1.5.1 EME followed by ultrasound-assisted emulsification microextraction

Lee and Guo reported that trace level chlorophenols in environmental water samples was extracted using EME followed by low-density solvent based ultrasound-assisted emulsification microextraction (EME-LDS-USAEME), and analyzed by GC-MS after derivatization [85]. This was a two-step approach, where the analytes were extracted from the sample into an acceptor phase by EME with an SLM of 1-octanol in the first step, and then the analytes in the acceptor phase were extracted into a low density solvent in the second step of LDS-USAEME. This EME-LDS-USAEME provided high enrichment factors, and it was applied to determine chlorophenols in drainwater samples successfully.

1.5.2 EME coupled with dispersive liquid-liquid microextraction

EME was coupled with dispersive liquid-liquid microextraction (DLLME) to extract tricyclic antidepressants (TCAs) from untreated human plasma and urine [117]. TCAs were extracted by EME (14 min with a voltage of 240 V) following by DLLME and analyzed by GC-flame ionization detection (GC/FID). The preconcentration factors of TCAs in different sample matrices were in the range of 383-1065. In another work, 3-methylpyridine, 2,4-lutidine, quinoline and 4-dimethylaminopyridine (DMAP) in urine were determined by EME-DLLME [118]. In this approach, the pyridine derivatives were first extracted from the sample and into the acceptor phase by EME using NPOE containing 10% DEHP as the SLM, and then followed by DLLME. The reported enrichment factors were from 40 to 263, and it can be used as a simple and fast method for diagnosis of smokers.

1.5.3 EME-Solid phase microextraction/Solid phase extraction

The combination of EME and solid phase microextraction (EME-SPME) was first reported by Yamini and his coworkers in 2013 [119]. In this work, the positively charged analytes (amitriptyline and doxepin) in the sample were extracted first into the acceptor phase, and

then adsorbed on the carbonaceous cathode, which was a pencil lead fiber and acted as a solid sorbent as well. EME was performed from 24 mL sample (neutral pH) for 20 min with an SLM of 2-nitrophenyl octyl ether (NPOE) immobilized in the pores of a hollow fiber (HF), and a voltage of 120 V. Afterward, the pencil lead fiber was inserted into the GC injection port for thermal desorption and analysis. This approach provided high sample clean-up, and the extraction recoveries from different sample matrices were in the range of 3.1-11.5%. EME-SPME was further applied for the determination of acidic herbicides (2-methyl-4-chlorophenoxyacetic acid and 2,4-dichlorophenoxyacetic acid) in plant tissue by GC/FID [120]. In this case, EME was carried out for 20 min with a voltage of 50 V and an SLM of 1-octanol, and the anode located in the lumen of the hollow fiber was a pencil lead fiber. The recoveries of the analytes were from 0.6-4.8%. The same group reported on the use of a nano carbonaceous sorbent coated stainless steel as cathode for EME of amphetamine and methamphetamine in human urine and whole blood. The extraction efficiency was in the range of 9.0-18.8%, when EME was performed for 15 min with a voltage of 150 V and an SLM of NPOE containing 15% TEHP [121]. In addition, EME-SPME has been performed using reduced graphene oxide coated pencil lead and CTAB-doped graphene/polyaniline nanocomposite coated platinum wire as the electrodes and solid sorbent for EME-SPME of basic drugs [122] and NSAIDs [123], respectively.

Meanwhile, preconcentration of chlorophenoxy acid herbicides in environmental samples was proposed using SPE following by EME [124]. In this work, the samples were passing through an SPE column containing the synthetic graphene oxide, and the analytes of interest were eluted from the solid sorbents using methanol containing 8% acetic acide. After evaporation, the analytes were disolved into an alkaline aqueous solution (pH 9) as the sample for EME. After EME for 21 min with an SLM of 1-octanol and a voltage of 16V, high enrichment factor were achieved (1950-2000).

1.6 Electromembrane extraction applications

1.6.1 Basic drugs

EME of basic drugs have been reported most frequently among EME papers, and EME of basic drugs have been accomplished from human plasma, dried blood and saliva spots, serum, urine, saliva, and water samples [43,57,58,62,73,75,78,94,96,99,103,104,107,117,125-143], which supports that EME is compatible with biological fluids. In addition to the most

frequently used SLM (NPOE), ENB and isopropyl nitrobenzene (IPNB) have also been used in several papers [57,73,94,99,107,126,130]. All these solvents possess high Kamlet and Taft values in dipolarity-polarizability (π) and hydrogen-bond basicity (β). EME of non-polar basic drugs was often accomplished with the pure solvents as SLM [43,57,58,73,75,94,107,125,130]. When NPOE was used as the SLM, 100-300 V was used as the extraction voltage, whereas ENB and IPNB required relatively lower voltage [73]. Extraction times were typically in the range of 5-20 min. The acceptor phase pH is crucial in EME of basic drugs, and the pH often ranged from 1 to 2. In addition to HCl solutions, formic acid, acetic acid, and trifluoroacetic acid (TFA) have also been used as acceptor phase to make the acceptor phase compatible with direct injection into the instrument such as liquid chromatography–mass spectrometry (LC–MS). Crown ether (16-crown-6) in acetic acid has also been used as an acceptor phase to enhance the extraction of several salivary polyamines [62]. The extraction recoveries of target analytes were dependent on the properties of the target anlaytes and the EME conditions, and the recovery has been limited within the range of 20-70% [45]. Exhaustive extraction of basic drugs (97-115% in recovery) has been reported in a single publication using three hollow fibers [75]. Substantial interest for EME of basic drugs has been reported in literature, but a convenient and easy to operate EME set-up for exhaustive extraction is still missing.

1.6.2 Acidic drugs

EME of acidic drugs was first reported in 2007, where EME was carried out from 300 µl alkaline water samples and into an alkaline acceptor phase (30 µL). The extraction voltage was 50 V, and the SLM was 1-heptanol. The corresponding recoveries for eleven acidic drugs were in the range of 8-100% after 5 minutes of EME [68]. In another work, NSAIDs were extracted from 10 mL waste water (pH 12) and into an alkaline acceptor phase (50 µL). EME was carried out for 10 min using an SLM of 1-octanol and a voltage of 10 V, and the corresponding pre-concentration was in the range of 28-49 times [44]. AgNTs-EME has also been reported for EME of acidic drugs [105].

Recently, five NSAIDs were extracted from 10 mL water samples (pH 12) and into 50 µL acceptor phase (pH 12) with an SLM of DHE, which was immobilized in the pores of the agar films containing silver nanoparticles [111]. EME of acidic drugs has also been achieved by using PEME [70], voltage-step pulsed EME [98], EME with carbon nanotubes modified hollow fibers [103], and EME-SPME [123]. EME of acidic drugs from biological samples

have also been studied in a few cases [70,100,103]. Diclofenac (DIC) and mefenamic acid (MEF) were extracted by PEME from diluted human plasma and urine with an SLM of 1-octanol, and the recoveries were in the range of 23-33% [70]. In another paper, DIC and MEF have been extracted from 10 mL diluted human plasma and urine into 10 μL using 20 V and a cylindrical cathode, and recoveries in the range 4.7-35.5% was obtained after 15 minutes [100].

Several papers have been published related to EME of acidic drugs, but in most cases, EME was performed using 1-octanol or 1-heptanol as the SLM [44,68,70,98,100,103,105,123]. Other solvents such as DHE have also been tested but they were less efficient [55,111]. The solvent properties required for efficient mass transfer of deprotonated acidic analytes, and the interactions involved between the analyte molecules and the SLM, are still not fully clear. For future EME, however, this type of knowledge is necessary. Additionally, very little data has been reported on EME of acidic drugs from human plasma [70,100,103], and information on how to optimize recoveries from plasma, how to stabilize the SLM in contact with human plasma, and how to avoid excessive system-current is lacking. Thus, more research contribution in EME of acidic drugs is needed.

1.6.3 Group separation

Simultaneous EME of acidic and basic drugs were reported first by Lee et al. using a flat membrane-based three-layer-envelop EME device, where the cathode and anode were located in the acceptor phase for basic and acidic drugs, respectively [84]. EME was carried out with two different SLMs (NPOE and 1-octanol) and acceptor phases (acidic buffer and alkaline buffer) for basic and acidic drugs, respectively. Afterward, dual EME with two hollow fibers was also used to simultaneous extraction of basic and acidic drugs as discussed above [59, 86]. Selective extraction of Cr(VI) and Cr(III) from different samples matrices were also achieved using dual EME, because Cr(VI) was present in an anionic complex while Cr(III) was present in a cationic complex [87]. In addition, drugs with different properties were also extracted simultaneously by dual EME using different SLMs for each fiber [88]. In this case, the anode was located inside the sample, while two cathodes were located inside the two hollow fibers containing the acidic acceptor phase (pH 1). With this design, atenolol (ATE, logP 0.23) and betaxolol (BET logP 2.81) were extracted from water, urine, and plasma samples using PEME with a voltage of 100 V. BET was extracted with pure NPOE, while the ATE was extracted using an SLM of NPOE containing 10% DEHP because ATE was too

polar to be extracted without a carrier. Dual EME either with flat membrane-based multilayer-envelop or two hollow fibers was developed for the simultaneous extraction of cations and anions [59,84,86,87], or of cations with different properties [88]. In addition, basic and acidic drugs with different polarity were extracted simultaneously using four-fiber EME [90], which has been discussed in Section 1.4.5.

1.6.4 Amino acids and peptides

Definitely, EME of amino acids and peptides is an important direction in the future, and EME of amino acids and peptides have been reported in a few publications since the introduction of EME in 2006. EME of amino acids was reported first by Kuban and his coworkers, where 17 amino acids were extracted from acidified biological fluids with 2.5 M acetic acids across the SLM (ENB containing 15% DEHP) and into the acceptor phase (acetic acid, 2.5 M) with an extraction voltage of 50 V [145]. In another work presented by the same group, EME of amino acids was improved further by operating the EME system with constant current instead of constant voltage [94]. After the introduction of PEME (EME using a pulsed voltage) [96], PEME was applied for the determination of amino acids in food samples and in human plasma and saliva with an SLM of NPOE/DEHP/TEHP (80/10/10, v/v/v) [71]. Derivatized amino acids were also extracted by PEME from food and pharmaceutical samples to differentiate the animal origins of gelatin [97].

EME of peptides has been investigated in a series of research papers [61,72,145-149]. EME of peptides was first presented in 2008, where nine peptides containing three to 13 amino acids were extracted from acidified pure water samples (pH 3) across an SLM comprising1-octanol and 15% DEHP and further into the acceptor phase (100 mM HCl) [72]. In this work, when EME was performed for 5 min using a voltage of 50 V, the recoveries of the peptides were up to 53%. EME of peptides has been investigated from a fundamental point of view with focus on principal operational parameters [146], the components of the SLM [61], and the electrokinetic transfer of the positively charged peptides across SLM using 37 peptides with different physical–chemical properties [147]. Combined with LC-MS, EME of peptides from plasma has also been reported [145,148]. EME of peptides is still facing some challenges such as low recovery and high system-current because of the introduction of a carrier such as DEHP in the SLM [49]. Especially for peptides, development of predictable and highly selective separations in simple EME systems, which are compatible with biological fluids, may be a valuable tool for future applications.

1.6.5 Organic pollutants

Two-phase EME was used to extract organic pollutants (nitrobenzene, aniline and phenol) from water sample into n-octanol with a voltage of 60 V for 4 min, and the obtained recoveries were used to measure the partition coefficient values between n-octanol and water (log P) [150]. The measured values were in good agreement with the literature values. In another work, four nerve agent degradation products were extracted from different water samples with an SLM of 1-octanol and a voltage of 300 V [83]. EME of organic acidic pollutants has been achieved using an SLM of 1-octanol [89,151-153] or an SLM of toluene [154] from environmental or biological samples. Acidic herbicides in plant tissue were extracted by EME-SPME and analyzed by GC/FID [120]. In another work, after the environmental samples were passed through SPE, chlorophenoxy acid herbicides were extracted and preconcentrated by EME [124]. Chlorophenols have also been extracted by EME mainly from water samples [76,85,155,156]. In addition, organic basic pollutants have been extracted from food and biological samples by EME with an SLM of NPOE containing 10% DEHP [157] or an SLM of NPOE [158], respectively, depending on the properties of the analytes.

1.6.6 Metal ions and inorganic anions

EME has also been employed for the extraction of metal ions from environmental and biological samples [63,159-162]. EME of metal ions was first reported by Lee and his coworkers in 2008, where the lead ions were extracted from lipstick, amniotic fluid, blood serum and urine samples, and detected by CE-UV [63]. In this work, EME was performed from 10 mL sample into the acceptor phase (phosphate buffer, pH 8.1) for 15 min with a voltage of 300 V and with an SLM of toluene. In another work, seven heavy metal ions were extracted by EME using an SLM of 1-octanol comprising DEHP and a voltage of 75 V [160]. Lithium in urine, blood serum, blood plasma, and whole blood has also been determined using EME with an SLM of 1-octanol [159]. Most recently, EME of heavy metal ions using a flat membrane instead of a hollow fiber membrane was reported, where the metal cations were extracted from the sample (pH 10) and into the acceptor phase (pH 1.2) using an SLM of 1-octanol containing 0.5% DEHP and 0.5% TEHP [163]. In addition, EME with a new membrane support of hollow cylindrical wheat stem has been applied for the extraction of thorium from water samples [110].

EME has been applied for the separation of inorganic anions from water samples, slightly water-soluble and water miscible organic solvents [164-166]. EME of inorganic anions was reported first by Zhu et al., where three inorganic anions including chloride, bromide, and sulfate were extracted from 8 mL ethyl acetate sample (a slightly water-soluble organic solvent) with a voltage of 600 V [164]. After 10 min of EME followed by analysis with ion chromatography (IC), the recoveries were in the range of 76-110%. EME of trace level perchlorate from water samples were also reported, and the recoveries of perchlorate from different water samples was in the range of 95.9-106.7%, when EME was carried out for 5 min with an SLM of 1-heptanol and a voltage of 25 V [165]. In another work, seven inorganic anions were extracted from water samples and water miscible organic solvent such as methanol, ACN and ethanol with an SLM of 1-heptanol and a voltage of 15 V [166]. As discussed above, separation of inorganic cations and anions simultaneously was achieved by dual EME [87].

EME has shown high potential for future bioanalysis of pharmaceuticals and peptides, though it is still facing some challenges. The work in this thesis is contribution in the direction with focus on both fundamental studies and applications to address some challenges in EME. Exhaustive extraction of drugs and peptides has been achieved by using the developed flat membrane-based EME device, which is easy in operation. The system-current during EME of peptides and acidic drugs were also controlled under 50 μA to minimize the possible excessive electrolysis during the extraction. In addition, selective extraction of peptides, and EME coupled with LPME have also been studied in this thesis. The details of the progress have been discussed in the corresponding publications (**Paper I-V**), and will be discussed in Section 3.

2 AIM OF THE STUDY

The current study was intended to develop the electromembrane extraction (EME) technologies using a piece of thin porous flat membrane. The attention was paid to study fundamental operational parameters of flat membrane-based EME, to apply this technology for the determination of pharmaceuticals in human plasma, and to propose stable EME system for future bioanalysis of peptides and pharmaceuticals. The latter set of experiments is mandatory for future EME applications. A focus in this thesis was to offer exhaustive extraction and selective extraction under stable and low system-current during the EME process. In addition, from a theoretical point of view, systematical study of some key EME parameters has also been performed. The following points have been addressed in this study:

- Development of a flat membrane-based EME device for exhaustive extraction of
    - Basic drugs **(Paper I)**
    - Acidic drugs **(Paper V)**
    - Peptides **(Paper II)**

- Proposal of an EME approach for selective EME of peptides based on the differences in the isoelectric points with focusing on efficient extraction and clean-up **(Paper III)**

- Development of stable EME system with system-current below 50 μA during EME of peptides **(Paper II & III)** and acidic drugs **(Paper V)**

- Investigation of the link between the properties of the organic solvent and the EME performance for acidic drugs **(Paper V)**

- Simultaneous extraction of basic and acidic drugs by intergradation of EME with liquid-phase microextraction (LPME) in a single step **(Paper IV)**

3 RESULTS AND DISCUSSION

In this section, only the main results were included. More detailed discussions are found in **Paper I-V**.

3.1 Electromembrane extraction set-up and model analytes

3.1.1 EME set-up

A new EME device using a flat piece of Accurel PP 1E (R/P) polypropylene membrane with a thickness of 100 µm was developed in **Paper I**. This flat membrane-based EME set-up is shown in Figure 3-1, and this EME device has been used in **Paper I, II, III and V**.

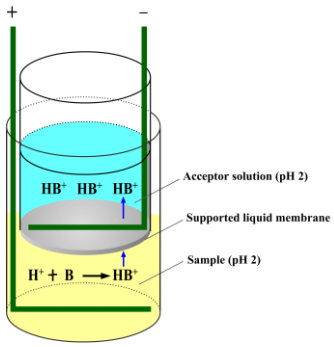


Figure 3-1. Illustration of flat membrane-based EME device. Reprinted with permission from **Paper I** © Elsevier B.V (2013).

A gap along the electrode in the sample enables excessive gas to escape from the sample. The acceptor compartment of the device consisted of a piece of flat membrane and a 10-1000 µL pipette tip, whose narrow end was cut off for easy operation. The flat membrane was sealed on the bottom of the pipette tip at 185 ^{o}C for 5 s using a Cotech Soldering iron station. An Eppendorf safe-lock PP tube (2.0 mL) was used as the sample compartment in this EME set-up, and the sample volume was 600 µL. Acceptor phase was filled into the acceptor compartment after the immobilization of the SLM on the porous membrane. The acceptor phase comprised 600 µL of 20 mM formic acid in **Paper I,** and 600 µL of 50 mM phosphoric acid in **Paper II and III,** whereas the acceptor phase comprised 100 µL of 10 or 100 mM NaOH in **Paper V.** The SLM was 5 µL NPOE in **Paper I**, 10 µL 1-nonanol diluted with 2-decanone (1:1 v/v) containing 15% DEHP (v/v) in **Paper II,** 10 µL 1-nonanol diluted with 2-decanone (1:1 v/v) containing 15% DEHP (v/v) and 10 µL 1-dodecanol containing 15%

DEHP (v/v) in **Paper III**, and 10 µL 1-heptanol containing 35% NPOE (v/v) in **Paper V**. The "L- shaped" cathode was inside the acceptor phase, and the anode was inside the donor phase in **Paper I, II** and during the first step of **Paper III**. The direction of the electric field was reversed in **Paper V**. After the acceptor compartment was inserted into the sample compartment with a gap of approximately 1 mm, the electrodes were connected to an ES 0300-0.45 power supply. The power supply was coupled to a home-made system-current monitor in **Paper II, III, and V**. The extraction was initialized by applying the power and starting the agitation. The extraction voltage was 250 V and 300 V in **Paper I**, 15 V in **Paper II**, 15 V and 10 V in **Paper III,** and 15 V in **Paper V.** After the desired extraction time (5-45 min), the extraction was terminated by turning off the agitation and the power supply. Immediately, the acceptor phase was collected and subsequently analyzed by micro-HPLC-UV or LC-MS in **Paper I**, or by HPLC-UV in **Paper II, III and V**.

In **Paper IV**, the acceptor compartment was modified slightly based on the set-up developed in **Paper I**, and the EME/LPME set-up is shown in Figure 3-2. A 10-200 µL pipette tip was used to fabricate the acceptor compartments for EME and LPME. The sample compartment was a disposable plastic pipette (SARSTEDT, Nümbrecht, Germany) with the tip cut off. The sample volume was 600 µL, and the volume of the acceptor phases for EME and LPME were 100 µL of 100 mM HCl and 100 mM NaOH, respectively. The SLM for EME and LPME were 3 µL NPOE and 3 µL DHE, respectively. The two acceptor compartments were inserted into the donor phase simultaneously, and the gap between the donor interface and the two SLMs was kept at about 1 mm. The cathode and anode was inside the EME acceptor and donor phases, respectively. The power supply was coupled to a home-made system-current monitor to measure system-current during EME, and 300 V was employed as the extraction voltage. The acceptor solutions after EME/LPME were analyzed by HPLC-UV or LC-MS.

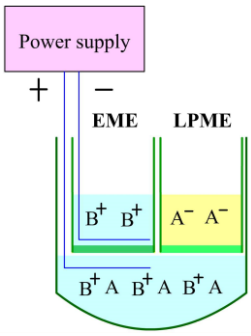


Figure 3-2. Illustration of flat membrane-based simultaneous EME/LPME device. Reprinted with permission from **Paper** IV © American Chemical Society (2015).

3.1.2 Model analytes

The selected model analytes represented a wide range in terms of physiochemical properties within the following categories: basic drugs **(Paper I** and **IV)**, acidic drugs **(Paper IV** and **V)**, and peptides **(Paper II** and **III)**. In order to evaluate the performance of the newly developed EME device properly, the selection of the model analytes were based on previous EME publications [43,44,57,60,61,64,65,68,69,72-75,77-81,84,92,93,125,126,130,145-149].

3.1.2.1 Basic drugs

Basic drugs used as model analytes in this thesis for the development of the thin flat membrane-based device for exhaustive EME in **Paper I**, and for the simultaneous EME/LPME in **Paper IV,** were selected based on previous EME publications [43,57,60,64,65,69,73-75,77-81,84,92,93,125,126,130] and are listed in Table 3-1. The selected basic drugs have log P-values in the range of 2.81-5.15, and are ideal candidates for EME. The pKa values of the model drugs ranged from 2.78 to 9.85, and all of them will be positively charged in acidified sample (10 or 100 mM HCl). All the model analytes were well separated by HPLC-UV except the internal standard, which was only used for the evaluation by LC-MS.

Table 3-1. Structure, protein binding, log P, and pKa values for all selected basic drugs in this thesis. Two pKa values are provided for drugs which can be doubly charged.[a]

Analytes	logP	pKa	Protein binding (%)	Used in
Methadone	5.01	9.12	85-90	**Paper I**
Quetiapine	2.81	2.78 7.06	83	**Paper I**
Citalopram	3.76	9.78	80	**Paper I** and **IV**
Sertraline	5.15	9.85	98	**Paper I** and **IV**
Amitriptyline	4.81	9.76	90	**Paper I**
Fluoxetine-d5 (Internal standard for basic drugs)	-	-	-	**Paper I** and **IV**

[a]*These data were adapted from* http://www.drugbank.ca/drugs.

3.1.2.2 Acidic drugs

The acidic model drugs were selected mainly based on previous EME experience [44,68]. The structure, protein binding, log P, and pKa values for all acidic drugs used in this thesis are listed in Table 3-2. The selected acidic drugs have log P-values in the range of 1.01 to 4.39,

representing a wide span in terms of the polarity. The pKa values of the model drugs ranged from 1.07 to 4.85, and all of them were negatively charged in alkaline sample at pH 8. All the model analytes were well separated by HPLC-UV.

Table 3-2. Protein binding, log P, and pKa values for all selected acidic drugs in this thesis.[a]

Analytes	pKa	logP	Protein binding	Used in
Quinaldic acid(QUI)	1.07	1.01	Not available	**Paper V**
Ketorolac(KEC)	3.84	2.28	99%	**Paper V**
Ketoprofen(KET)	3.88	3.29	99%	**Paper IV** and **V**
Probenecid(PRO)	3.53	1.52	75-95%	**Paper V**
Flurbiprofen(FLU)	4.42	3.57	>99%	**Paper V**
Ibuprofen(IBU)	4.85	3.84	90-99%	**Paper IV** and **V**
Ibuprofen-d3 (IS for acidic drugs)	-	-	-	**Paper IV** and **V**
Gemfibrozil(GEM)	4.42	4.39	95%	**Paper V**

[a] *http://www.drugbank.ca/drugs.*

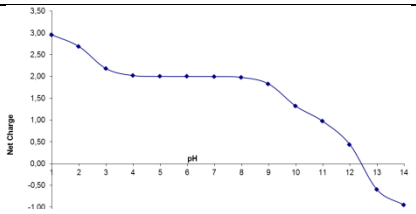
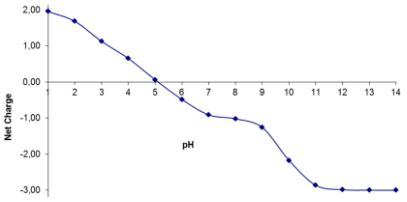
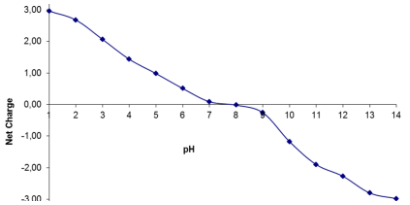
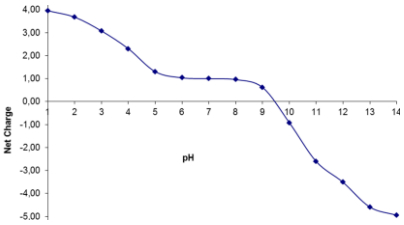
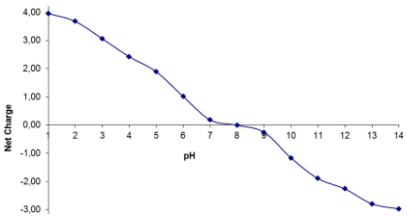
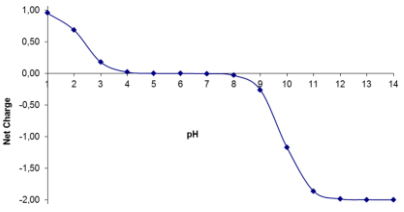
3.1.2.3 Peptides

The peptides were selected mainly based on previous EME experience [61,72,145-149] and commercial availability. The amino acid sequence, isoelectric point (pI), and the plot of net-charge versus pH for all the selected peptides are presented in Table 3-3. The model peptides are with five to thirteen amino acids, and the pI of the peptides ranged from 5.13 to 12.40. The model peptides show different net-charge profiles in the pH range 1-14.

3.2 Exhaustive EME

One of the main achievements in this work was that the developed EME device using thin flat membrane could provide exhaustive extraction for basic drugs, acidic drugs and peptides. Before this work, exhaustive EME of acidic drugs and peptides has not been reported, and only a single research work reported exhaustive EME of basic drugs using three hollow fibers simultaneously [75]. However, the three-fiber-arrangement was not very user-friendly. Exhaustive EME of basic drugs, acidic drugs and peptides could be achieved by tuning the operation parameters using a single well EME device, which is simple and easy in operation.

Table 3-3. Amino acid sequence, iso-electric point, and plot of net-charge versus pH for all selected peptides in this thesis.[a]

Peptides	Sequence	pI*	Net-charge vs. pH	Used in
BK	RPPGFSPFR	12.40		**Paper II**
AT2 AP	EGVYVHPV	5.13		**Paper II** and **III**
AT2	DRVYIHPF	7.76		**Paper II** and **III**
NT	ρELYENKPRRPYIL	9.52		**Paper II** and **III**
AT1	DRVYIHPFHL	7.91		**Paper II** and **III**
L-Enke	YGGFL	5.93		**Paper II** and **III**

[a] *Data were calculated using the Innovagen peptide property calculator at www.innovagen.se.*

3.2.1 Basic drugs

Exhaustive EME of basic drugs was studied in **Paper I** using a flat membrane-based EME device, which enabled larger capacity of the acceptor phase volume with the same amount of SLM. By increasing the acceptor phase volume from 100 and up to 600 µL, exhaustive EME

of almost all model basic drugs were achieved, and the results are shown in Figure 3-3. The results clearly suggest that EME recoveries increased with increasing the volume of acceptor phase, though the reported data deviated somewhat from the theory that recoveries were independent on the volume of the acceptor phase [149]. With 600 μL acceptor phase and 600 μL acidified water sample, EME provided no enrichment, but citalopram, methadone, amitriptyline and sertraline were extracted exhaustively. The EME recovery for quetiapine was slightly lower (80%) with 600 μL acceptor phase, which was due to the fact that most of the quetiapine molecules were carrying more than one positive charges at pH 2 [167], and recent work has demonstrated that multiple charged analytes were extracted less efficiently in EME with NPOE as the SLM [60]. The high efficiency with 600 μL acceptor phase suggested that mass transfer was not only by electro-kinetic migration. Most probably, the charged fraction of analytes was extracted across the SLM by electro-kinetic migration, whereas the neutral fraction was extracted across the SLM based on passive diffusion [168].

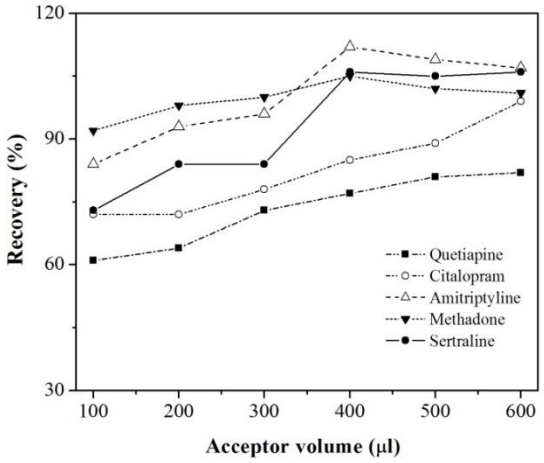


Figure 3-3. Effect of acceptor phase volume on the EME recoveries of the basic drugs from acidified water samples (n=3, all RSDs <10%). Reprinted with permission from **Paper I** © Elsevier B.V (2013).

The SLM was optimized, and NPOE was superior among the tested five organic solvent including NPOE, 1-ethyl-2-nitrobenzene (ENB), 2,4-dimethyl-1-nitrobenzene (DMNB), isopropyl nitrobenzene (IPNB), and 2-undecanone. Different background electrolyte ions in the sample, including 10 mM HCl, 20 mM formic acid, 20 mM acetic acid, or 25 mM phosphate buffer (pH 2.5), did not show significant effect on the EME efficiency. However, the background electrolyte ions in the acceptor phase showed significant effect on the EME efficiency, and the results are presented in Table 3-4. The acceptor phase of 10 mM HCl and 20 mM formic acid provided high and comparable recoveries. The acceptor phase of 25 mM phosphate buffer (pH 2.5) provided lower recovery, and 20 mM acetic acid was relatively

inefficient as acceptor phase. A similar finding has been observed using peptides as model analytes [146].

Table 3-4. Effect of the acceptor solution composition on extraction recoveries (%) from acidified water samples, (250V, 5 minutes extraction time, NPOE as the SLM). RSD values presented in the parentheses (n=3).

Analyte	10 mM HCl	20 mM Formic acid	20 mM Acetic acid	25 mM Phosphate buffer (pH 2.5)
Quetiapine	65 (6)	45 (10)	0	2 (29)
Citalopram	78 (7)	77 (4)	9 (49)	20 (8)
Amitriptyline	80 (5)	79 (3)	32 (18)	62 (13)
Methadone	86 (6)	82 (3)	42 (8)	67 (6)
Sertraline	77 (7)	80 (4)	23 (40)	46 (15)

To clarify this fundamental observation, EME was performed for 5 and 15 min using 10 mM HCl and 20 mM acetic acid as the acceptor phases, respectively. The sample and the acceptor phase after EME were analyzed by HPLC-UV, and the mass balance data are summarized in Table 3-5.

Table 3-5. Mass balance data after 5 and 15 minutes of EME from acidified water samples (pH 2) with 10 mM HCl and 20 mM acedic acid as acceptor phase, respectively (250V, NPOE as the SLM).

	Distribution (%)		
	Acceptor solution	SLM	Sample
10 mM HCl for 5 minutes			
Quetiapine	65	15	20
Citalopram	78	22	0
Amitriptyline	80	15	5
Methadone	86	10	4
Sertraline	77	19	4
20 mM CH_3COOH for 5 minutes			
Quetiapine	0	0	100
Citalopram	9	15	76
Amitriptyline	32	26	42
Methadone	42	18	40
Sertraline	23	29	48
10 mM HCl for 15 minutes			
Quetiapine	91	9	0
Citalopram	92	8	0
Amitriptyline	101	0	0
Methadone	98	2	0
Sertraline	100	0	0
20 mM CH_3COOH for 15 minutes			
Quetiapine	3	40	57
Citalopram	42	32	26
Amitriptyline	98	0	2
Methadone	98	0	2
Sertraline	85	6	9

As seen, HCl as the acceptor phase provided a more efficient EME system than acetic acid. Interestingly, the acceptor phase of acetic acid strongly affected the mass transfer of the analytes from the sample into the SLM, which might be due to the fact that the acetic acid can partly diffuse into the SLM and modify the properties of the SLM. For EME of basic drugs, carboxylic acids have been reported to be inefficient SLMs due to their high proton donor properties [168]. Acetate ions might be extracted from the acceptor phase into the SLM, which might lead to complex formation between negatively charged acetate ions and positively charged basic drugs resulting in an inefficient EME. In addition, in electrochemical systems consisting of immiscible electrolyte solutions (such as water and NPOE), related observations have been reported, as compounds specifically interacting with the partitioning analyte in the organic solvent may affect the transfer of the analyte into (and out of) the organic solvent [169]. Similarly, the EME efficiency was reduced because of the penetration of acetic acid into the SLM. According to the data in Table 3-4, and due to the fact that formic acid is more compatible with HPLC or LC/MS than HCl, 20 mM formic acid was used as the acceptor solution.

Table 3-6. Effect of matrices, and the effect of extraction voltage and time on the recoveries (Rec, %) of model analytes from diluted human plasma (pH 7.4). RSD values presented in the parentheses, n=3.

Analyte	250 V, 15 min	300V, 30 min
Quetiapine	84 (5)	105 (5)
Citalopram	107 (7)	105 (5)
Amitriptyline	80 (8)	89 (5)
Methadone	96 (7)	103 (3)
Sertraline	64 (4)	83 (3)

As discussed above, when EME was carried out from 600 µL acidified sample into 600 µL acceptor phase for 15 minutes with 250 V and using NPOE as the SLM, exhaustive extraction of citalopram, methadone, amitriptyline, and sertraline was achieved. However, when EME was performed from spiked human plasma under the same conditions as used for acidified water samples, only citalopram and methadone were extracted exhaustively. The recoveries for quetiapine, amitriptyline, and sertraline from human plasma were improved by using higher extraction voltage (300 V) and longer extraction time (30 min), and the recoveries from human plasma together with those from water samples are presented in Table 3-6. As discussed above, at pH 2, most of the quetiapine molecules were doubly charged, which led to less efficient EME [60], so exhaustive EME of quetiapine from sample pH 2 was not

achieved. On the other hand, at pH 7.4, quetiapine was singly charged and partly uncharged [167], and exhaustive EME of quetiapine was achieved. In addition, the difficulties with exhaustive EME of sertraline and amitriptyline might be due to the strong protein binding [170].

3.2.2 Acidic drugs

EME of acidic drugs has been reported several times [44,68,70,100,98,103,105,123]. However, exhaustive EME of acidic drugs have not been reported. In this thesis however, using a flat membrane-based EME device and with a carefully optimized SLM (Table 3-7), exhaustive EME was obtained from alkaline water samples in **Paper V**. EME was carried out for 10 min from 600 µL of spiked phosphate buffer solution (pH 8) into 600 µL of 10 mM NaOH acceptor phase using a voltage of 50 V, and an SLM of 1-heptanol. Slightly lower recovery was obtained for quinaldic acid (QUI) due to the polar properties of this compound (log P 1.01).

Table 3-7. EME recoveries (Rec, %) of acidic drugs using different organic solvents as the SLM from water samples pH 8 (n=3, RSD values presented in the parentheses).[a]

Analytes	Alcohols					Ketones			Ethers	
	C7	C8	C9	C11	C12	2-C8	2-C10	2-C11	DHE	NPOE
QUI	94(3)	2(33)	10(31)	3(22)	0	0	0	0	0	0
KEC	102(3)	39(4)	51(25)	16(29)	0	6(16)	0	0	0	0
KET	104(1)	45(12)	64(26)	25(21)	2(18)	4(20)	0	0	0	0
PRO	100(2)	62(8)	81(21)	40(13)	6(57)	17(31)	17(6)	14(5)	0	0
FLU	99(2)	96(9)	106(6)	99(2)	33(15)	44(14)	24(5)	18(3)	5(33)	10(43)
IBU	110(2)	95(4)	73(15)	61(11)	3(80)	32(6)	10(16)	5(43)	0	0
GEM	96(2)	96(5)	109(5)	94(7)	21(14)	107(8)	93(6)	65(13)	39(8)	12(39)

[a] *C7, C8, C9, C11, C12, 2-C8, 2-C10, 2-C11, DHE and NPOE were 1-heptanol, 1-octanol, 1-nonanol, 1-undecanol, 1-dodecanol, 2-octanone, 2-decanone, 2-undecanone, dihexyl ether, and 2-nitrophenyl octyl ether, respectively.*

The introduction of NPOE in 1-heptanol as the SLM showed positive effect on the reduction and stabilization of the system-current, when EME was performed from six-fold diluted human plasma. However, the content of NPOE in 1-heptanol affected the EME efficiency from six-fold diluted human plasma as shown in Figure 3-4, where higher content of NPOE resulted in lower EME efficiency. Thus, the content of NPOE (35%) was selected as a compromise. When EME was performed for 10 min with a voltage of 10 V, the recoveries of

the analytes were limited to the range 25-55 %, except that of probenecid (95 %). Clearly, further optimization of the recoveries was requested.

In a following series of experiments, human plasma was diluted with different amounts of buffer solution, and the EME recoveries of all analytes are shown in Figure 3-4. Except probenecid, the EME recoveries of all analytes were improved significantly by higher dilution. The EME recoveries from 40-fold diluted human plasma were ⩾ 91 % except those of QUI (57 %) and FLU (63 %). The relatively low recovery of QUI was due to high polarity of this compound, and this indicated that hydrophobic interactions between the analytes and the SLM play a role for efficient EME. The low recovery for FLU was probably because of high protein binding (> 99%) (Table 3-2), which suggested that strong protein binding with the model drugs to some extent hindered exhaustive extraction as discussed before [170]. However, after 20 min EME, the recoveries of QUI and FLU were improved to 98% and 93%, respectively. Thus, exhaustive EME of acidic drugs from biological samples was facing more challenges than expected. Although the research in **Paper V** contributed with more knowledge on suitable SLMs for EME of acidic drugs and on how to reduce and stabilize the system-current during EME, more research in the area of EME of acidic drugs especially from biological samples is requested.

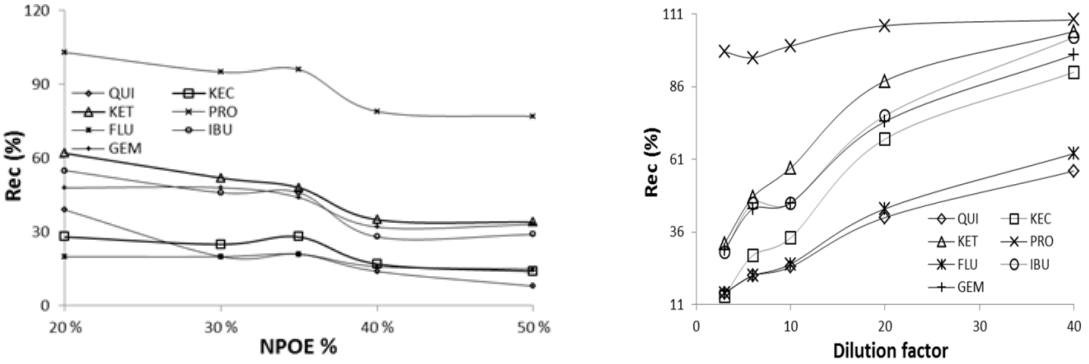


Figure 3-4. Effect of the NPOE content (left) and the dilution factor (right) on the recoveries of the acidic drugs from six-fold diluted human plasma. Reprinted from **Paper V** © Elsevier B.V. (2015).

3.2.3 Peptides

As mentioned above, EME of peptides has been reported in several publications [61,72,145-149]. However, the reported EME recoveries have been relatively low, and therefore exhaustive EME of peptides was investigated in **Paper II** using a flat membrane-based EME device and six peptides including BK, AT2 AP, AT2, NT, AT1 and L-Enke as the

model analytes. As discussed in **Paper II**, several operational parameters showed significant effect on the recoveries of the peptides.

The EME efficiency of peptides was dependent on the composition of the acceptor phase as shown in Table 3-8. The recoveries of all peptides obtained with trifluoroacetic acid (TFA), HCl, and phosphoric acid are high and comparable, but acetic acid and boronic acid provided much lower EME recoveries, which is consistent with previous findings [154]. The low recoveries obtained with acetic acid and boronic acid were due to excessive peptide trapped in the SLM.

Table 3-8. Effect of the acceptor solution composition on EME recovery (Rec) and the trapping of peptides in the SLM (Tra). RSD values presented in the parentheses, n=3.

Peptide	HCl (pKa -7)		Phosphoric acid(pKa 2.1)		Boronic acid(pKa 9)		Formic acid(pKa 3.75)		Acetic acid (pKa 4.75)		TFA(pKa -0.3)	
	Rec	Tra	Rec	Tra	Rec	Tra	Rec	Tra	Rec	Tra	Rec	Tra
BK	88(10)	12	87(6)	0	nd	93	88(8)	12	46(11)	54	91(4)	9
AT 2 AP	64(15)	23	77(4)	3	59(4)	20	78(2)	1	74(6)	3	73(4)	2
AT 2	79(13)	21	78(13)	22	nd	100	75(2)	25	30(8)	70	91(4)	5
NT	53(3)	47	72(5)	28	nd	100	75(10)	25	34(6)	66	81(5)	11
AT 1	75(12)	25	76(9)	24	nd	100	42(7)	58	7(19)	93	91(2)	4
L-Enke	64(14)	30	60(8)	31	15(16)	71	61(5)	31	37(9)	52	69(10)	23

nd: not detected.

Interestingly, the SLM volume (1-octanol containing 15% DEHP) was found to be an important factor for efficient EME of peptides as shown in Figure 3-5 (up, left). As shown, the extraction recovery was improved significantly by increasing the SLM volume up to 10 µL. Other organic solvents with lower water solubility including 1-nonanol, a mixture of decanol isomers, 1-undecanol, 2-decanone, and 2-undecanone were also tested. 1-nonanol was found to be as efficient as 1-octanol, whereas decanol isomers and 1-undecanol were less efficient. Interestingly, 2-decanone and 2-undecanone were as efficient as 1-octanol for almost all peptides except for AT2 AP and L-Enke. Besides the organic solvent, the EME efficiency was also dependent on the content of DEHP as shown in Figure 3-5 (up, right). By increasing the content of DEHP, the recoveries of AT2 AP and NT are improved, but the recoveries of other peptides are almost unchanged.

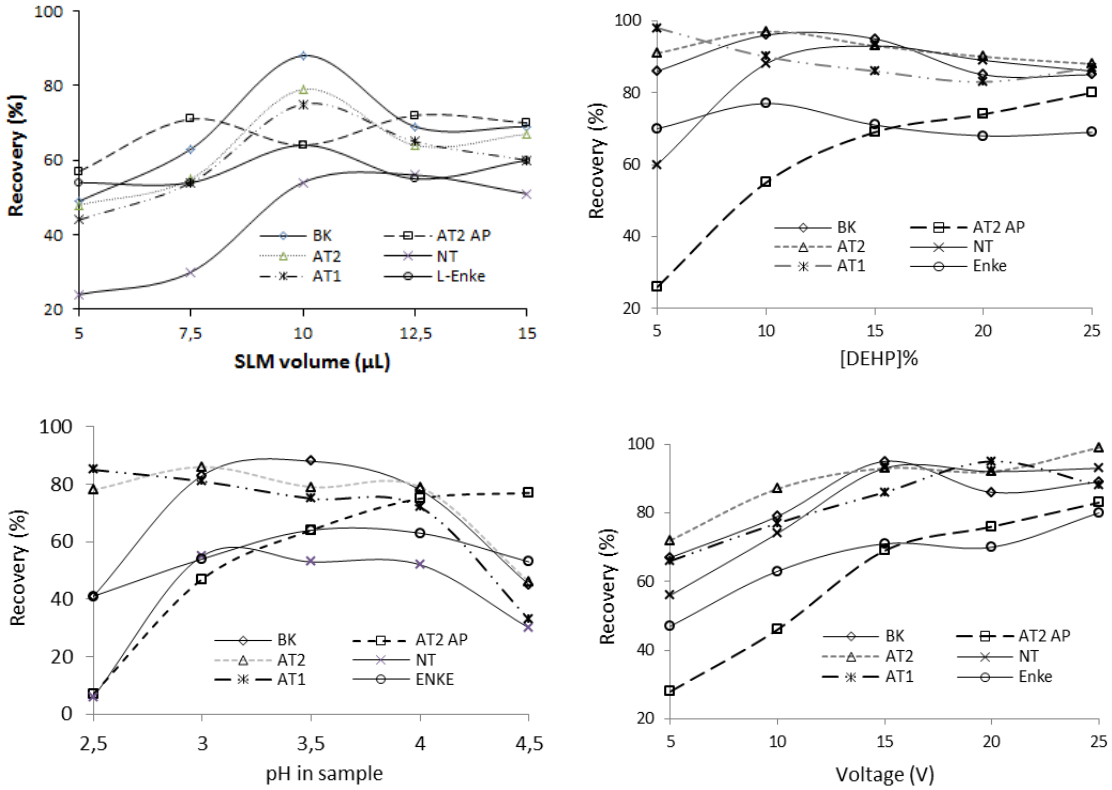


Figure 3-5. Effect of SLM volume (up left), DEHP content (up right), sample pH (lower, left), extraction voltage (lower, right) on the recoveries of peptides. Reprinted with permission from **Paper II** © Elsevier B.V. (2015).

As known, the sample pH affected the ionization of both the model peptides and DEHP at the SLM-donor interface, and therefore the effect of the sample pH from 2.5 to 4.5 on EME efficiency was studied. Since the pK_a value of DEHP is approximately 3.5 [171], DEHP was 10% ionized at pH 2.5 and 90 % at pH 4.5 [172]. Thus, sample pH showed a strong effect and complicated effect on the EME efficiency as shown in Figure 3-5 (lower, left). The recoveries of almost all peptides were increasing with increasing sample pH up to 3.5. Above pH 3.5 almost all peptides' recoveries decreased with increasing sample pH. This indicated that the complex formation between the negatively charged DEHP and the positively charged peptides at the SLM-donor interface played a significant role for the mass transfer. With increasing sample pH, the net-positive charge of some peptides decreased, but also the ionization of DEHP increased, which affected the complex formation mechanism and resulted in different amount of peptides trapped in the SLM and sample. Generally, the recoveries of all peptides can be improved by using higher extraction voltage as shown in Figure 3-5 (lower, right), but higher extraction voltage normally resulted in higher system-current during EME, which have been discussed in Paper II and will be discuss more in Section 3.4.

Extraction time showed significant effect on EME recovery. The effect of the extraction time on the recovery of peptides in the acceptor solution and the depletion of peptides in the sample are presented in Figure 3-6. As seen in Figure 3-6b, multiple charged peptides BK, AT2, NT, and AT1 were depleted much faster from the sample than singly and non-charged peptides AT2 AP and L-Enke, and all peptides except AT2 AP and L-Enke were not found in the sample after 10 minutes of EME. L-Enke was almost non-charged at pH 3.5, so the depletion of L-Enke from the sample took 20 min due to passive diffusion kinetics. As shown in Figure 3-6a, the recoveries of all peptides increased with increasing extraction time up to 15 min except AT2 AP and L-Enke. After EME for 25 min, the obtained recoveries of BK, AT2 AP, AT2, NT, AT1 and L-Enke were 94%, 84%, 91%, 92%, 87% and 77%, respectively. Thus, BK, AT2, NT, and AT1 with recovery > 87% were extracted exhaustively. In addition, the recovery of AT2 AP increased to over 90% with the time up to 45 min where exhaustive extraction was accomplished, whereas exhaustive EME of L-Enke was not obtained even after 45 min EME. This was due to passive diffusion controlled mass transfer.

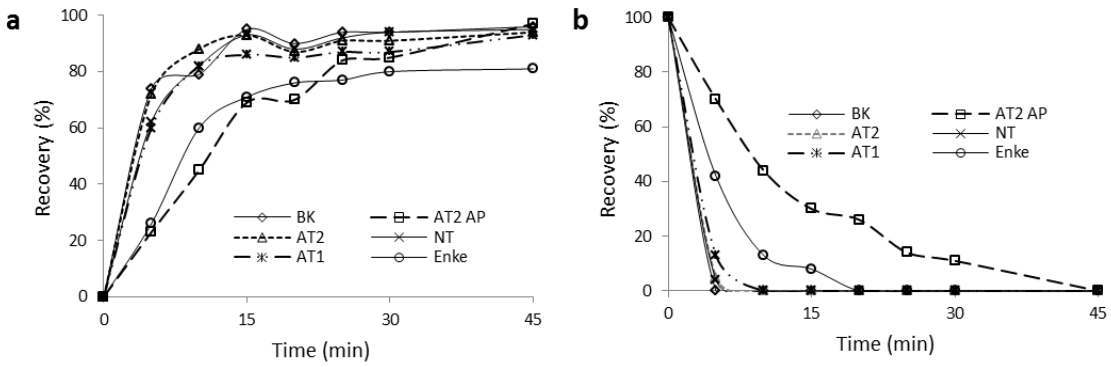


Figure 3-6. Effect of extraction time on the recovery of peptides in acceptor solution (a) and depletion of peptides in sample (b). Reprinted with permission from **Paper II** © Elsevier B.V. (2015).

3.3 Selective EME

Selective isolation of a target peptide based on the pI was investigated in **Paper III** using a two-step EME approach with a thin flat membrane-based EME device, and with AT2 AP as model peptide. AT2 AP has a pI of 5.13. The work flow of the two-step EME approach is illustrated in Figure 3-7.

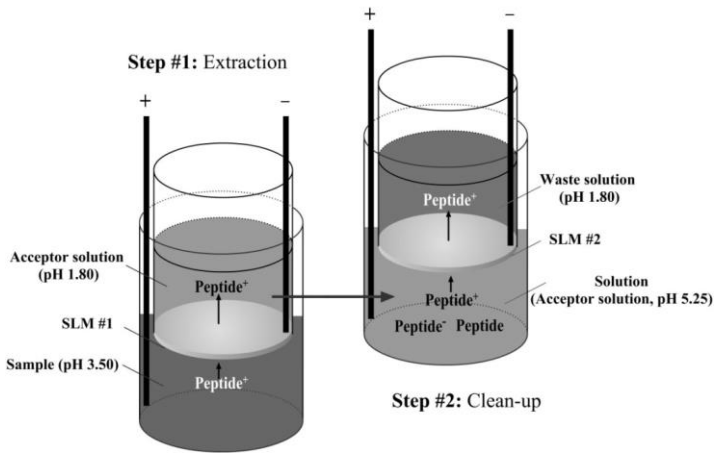


Figure 3-7. Illustration of the work flow of the two-step EME approach for selective extraction of AT2 AP. Reprinted with permission from **Paper III** © Elsevier B.V. (2015).

As shown in Figure 3-7, in the first step, the target peptide AT2 AP and the matrix peptides (pI > 5.13) including AT2, NT, AT1 and L-Enke were all extracted as cations from 600 µL sample pH 3.50 across an SLM, which comprised 1-nonanol diluted with 2-decanone (1:1 v/v) containing 15% (v/v) DEHP, and into 600 µL of 50 mM phosphoric acid solution. EME was carried out for 45 min with a voltage of 15 V, and the anode was in the sample. Prior to the second step, the acceptor phase collected after the first step of EME was adjusted to pH 5.25 and with the anode inside. To avoid back-extraction and diffusion into the SLM due to continuous contact between the SLM and the acceptor phase, another EME device was used for step #2. The second step was intended to remove the matrix peptides with pI > 5.13, which were net positively charged in the acceptor solution at pH 5.25. AT2 AP was not net positively charged in the acceptor solution at pH 5.25, which suppressed the complex formation with negatively charged DEHP, and AT2 AP remained in the acceptor solution at pH 5.25. To optimize the selectivity of EME, the stability of pH, acceptor solution pH, SLM, extraction voltage, and the extraction time in the second step were optimized.

According to the intention of step #2, a stable pH was mandatory to keep AT2 AP neutral or slightly negatively charged during step #2, so acetate buffer was selected to provide a stable pH during step #2. The selection of acetate buffer was mainly based on the pK_a (4.75), which is close to the target pH. In addition, the effect of the molarity of the acetate buffer on the stability of pH was investigated, and higher molarity resulted in more stable pH, which was consistent with buffer solution theory in general. Balancing both pH stability and the low system-current, acetate buffer with a concentration of 75 mM was used as a compromise to maintain the pH stability.

First of all, 15% DEHP in 1-nonanol, decanol mixture of isomers, n-decyl alcohol, 1-undecanol, 1-dodecanol, 2-octyl-dodecanol, 2-nonanone, 2-decanone, 2-undecanone, 2-nitrophenyl octyl ether, 2,4-dimethyl-1-nitrobenzene, and in 1-ethyl-2-nitrobenzene were tested as possible SLMs. After step #2 was carried out for 5 min, the amount of peptides remained in the acceptor phase pH 5.25 are presented in Table 3-9. As seen, 91% AT2 AP were found in the acceptor phase pH 5.25 when 1-dodecanol containing 15% DEHP served as the SLM during the clean-up process. Therefore, this SLM was selected for step #2, although it was not the most efficient one for removal of the other matrix peptides.

Table 3-9. The peptides found in the solution after the clean-up process (Rec) and the maximal system-current (Imax) during the EME with different SLMs. EME was performed from 75 mM acetate buffer (pH 5.25) with a voltage of 5 V for 5 min, n=3.

SLMs	I_{max} (μA)	Rec(RSD %)				
		AT2 AP	AT2	NT	AT1	L-Enke
1-Nonanol	108 ± 8	74 (11)	8 (8)	6 (72)	nd	30 (15)
Decanol mixture isomers	4 ± 1	63 (8)	nd	nd	4(20)	50 (7)
n-Decyl alcohol	67 ± 11	82 (8)	7 (43)	6 (110)	5 (31)	63 (3)
1-Undercanol	60 ± 10	64 (9)	7 (39)	5 (17)	8 (12)	52 (7)
1-Dodecanol	19 ± 4	91 (1)	10 (53)	5 (40)	6 (37)	73 (4)
1-Octyl-dodecanol	0	57 (11)	nd	nd	nd	54 (10)
2-Nonanone	53 ± 7	41 (5)	nd	nd	8(18)	31 (19)
2-Decanone	18 ± 4	42 (14)	nd	nd	nd	70 (10)
2-Undercanone	9 ± 2	48 (14)	nd	nd	nd	72 (5)
2-Nitrophenyl octyl ether	2 ± 1	37 (22)	nd	nd	nd	57 (2)
2,4-Dimethyl-1-nitrobenzene	13 ± 2	40 (5)	nd	nd	nd	17 (112)
1-Ethyl-2-nitrobenzene	11 ± 2	38 (6)	nd	nd	nd	41 (9)

The goal of step #2 was to separate AT2 AP from the matrix peptides based on the differences in the isoelectric point of the peptides.Thus the acceptor solution pH in step #2 was an important factor, which was studied ranged from 3.78 to 5.80 using 75 mM acetate buffers. After step #2, the amount of peptides remained in the acceptor solution versus the pH is plotted in Figure 3-8. The plot depicted that within the tested pH range, pH 5.25 was superior, because the amount of AT2 AP in the solution was highest, and the level of the three matrix peptides was lowest. However, L-Enke (pI=5.93) was poorly removed in step #2 because L-Enke was almost with zero net charge in a broad pH range (pH 4 to pH 8) as shown in Table 3-3. During step #2, the pH of the solution increased from 5.15 to 5.25, which was

slightly above the pI of AT2 AP. At pH above 5.13 (the pI of AT2 AP), AT2 AP will be neutral or slightly negatively charged.

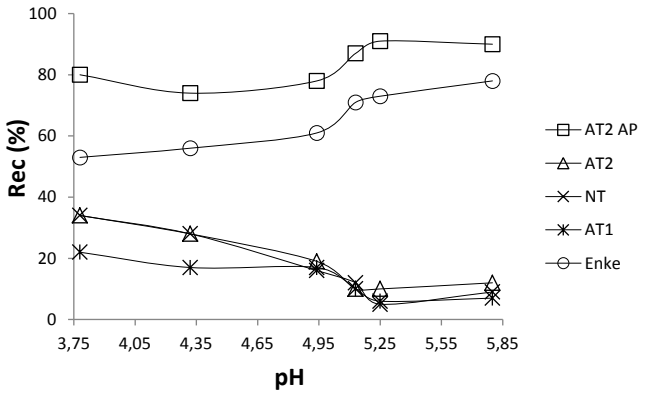


Figure 3-8. Effect of the solution pH on the amount of the remained peptides. EME was performed with 75 mM acetate buffer (pH varied from 3.78 to 5.80) with an SLM of 1-dodecanol containing 15% DEHP and a voltage of 5 V for 5 min, n=3. Reprinted with permission from **Paper III** © Elsevier B.V. (2015).

The content of DEHP in 1-dodecanol was investigated, and the results are illustrated in Figure 3-9a. All peptides remaining in the solution are decreasing with increasing DEHP content due to enhanced complex formation between the peptides and DEHP at the interface of SLM/acceptor solution pH 5.25, which was consistent with the observations in **Paper II**. In order to obtain high selectivity for AT2 AP and to minimize the system-current, 1-dodecanol containing 15% DEHP was selected as a compromise.

The effect of the extraction voltage during step #2 on the recovery of the peptides in the solution was depicted in Figure 3-9b. The voltage showed no effect on AT2 AP and L-Enke, because they were almost with zero net charge. The amount of the other three peptides was reduced significantly with increasing voltage up to 10 V (the insert in Figure 3-9b). Since removal of the charged peptides was comparable using 10 V or higher voltage, 10 V was used in step #2.

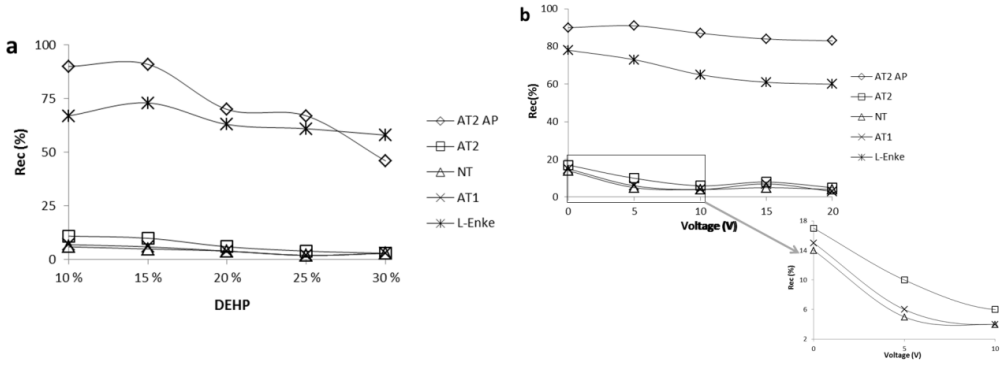


Figure 3-9. Effect of DEHP content (a) and voltage (b) on the remained peptides in the solution after step #2. Reprinted with permission from **Paper III** © Elsevier B.V. (2015).

The extraction time of step #2 was studied in a final experiment, and the results are presented in Table 3-10. When the extraction time increased up to 10 min, the amount of all peptides in the solution was reduced, and all matrix peptides with positive charges were removed completely after 10 min. In addition, further increasing the extraction time up to 20 min resulted in lower amount of L-Enke, but also lower recovery of the target AT2 AP. Thus, 10 min was selected.

Table 3-10. The amount of the peptides found in the solution after the clean-up process (Rec) with different time. EME was performed from 75 mM acetate buffer (pH 5.25) with an SLM of 1-dodecanol containing 15% DEHP and a voltage of 5 V for different time. RSD values presented in the parentheses, n=3.

Time (min)	AT2 AP	AT2	NT	AT1	L-Enke
5	87 (5)	6 (22)	4 (25)	4 (20)	65 (5)
10	83 (6)	nd	nd	nd	56 (6)
15	77 (5)	nd	nd	nd	42 (4)
20	74 (6)	nd	nd	nd	37 (16)

nd: not detected.

After the optimization, EME in step #2 was performed for 10 min with 10 V as the extraction voltage and 1-dodecanol containing 15% DEHP as the SLM from 75 mM acetate buffer pH 5.25 to the waste solution (50 mM phosphoric acid). The overall performance after step #1 and step #2 was tested, and the HPLC-UV chromatograms of the standard solution, and the acceptor solution after step #1 and after step #2 are presented in Figure 3-10.

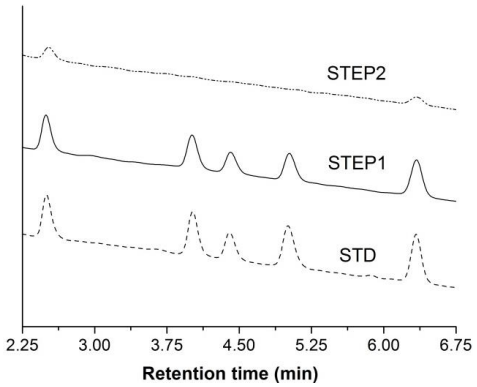


Figure 3-10. Chromatograms of the solution after the clean-up process (STEP2), the acceptor solution after the extraction process (STEP1) and the standard solution in 25 mM phosphate buffer with a pH of 3.50 (STD). The elution order is AT2 AP, AT2, NT, AT1 and L-Enke, respectively. Reprinted with permission from **Paper III** © Elsevier B.V. (2015).

The chromatograms demonstrate that selective EME of peptide based on differences in isoelectric point was possible. After this two-step EME, 27% AT2 AP was lost during the

process and 73% AT2 AP was found in the solution, which was considered reasonable. In comparison with isoelectric focusing using electromembrane process, very high separation factors (the ratio between the target peptide recovery and the matrix peptide recoveries) was obtained, because three cationic peptides (AT2, NT and AT1) were not found in the solution [173]. However, 48% of almost zero charged L-Enke was detected in the solution, which is in agreement with the separation of peptides or proteins by pI focusing, where controlled separation of peptides and proteins into acidic and basic fractions were achieved [174,175].

3.4 EME stability

The stability of the EME system for peptides has been considered in **Paper II** and **III,** and similar efforts for acidic drugs have been considered in **Paper V**.

3.4.1 SLM volume

In **Paper II**, when EME of six model peptides was initially carried out for 15 min from 600 µL of spiked phosphate buffer pH 3.5 using a voltage of 15 V and an SLM of 5 µL 1-octanol containing 15% (v/v) of DEHP, the recorded system-current increased from 100 to over 300 µA as shown in Figure 3-11.

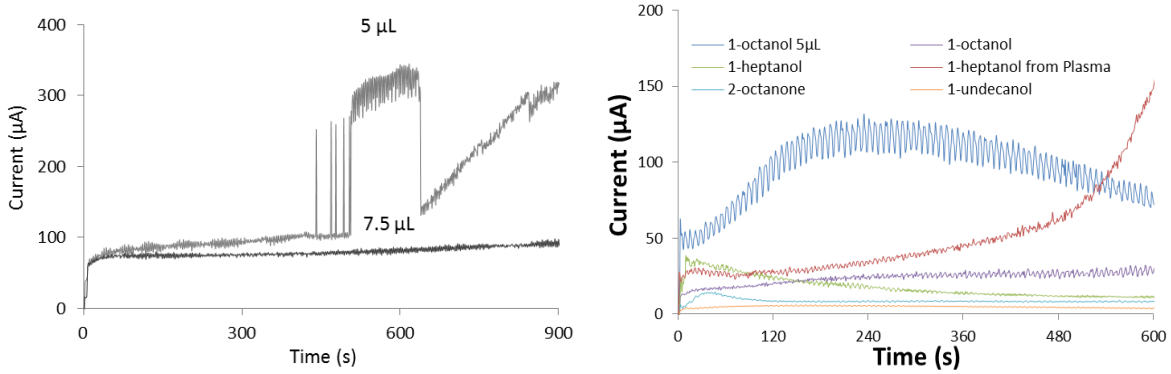


Figure 3-11. Effect of SLM volume on the system-current for EME of peptides (left) and acidic drugs (right). Reprinted with permissions from **Paper II** and **Paper V** © Elsevier B.V. (2015)**,** respectively.

The system-current profile might compromise the stability of the membrane [65]. The average system-current (≈ 160 µA) was comparable with previous current levels using EME with porous hollow fibers as support and a similar SLM [145]. However, the system-current was stabilized and reduced by increasing the SLM volume. As shown in Figure 3-11(left), when the SLM volume increased to ⩾ 7.5 µL, stable and relatively low system-current was recorded, and the average system-current was decreased from ≈ 160 µA with 5 µL of SLM to

$\approx$ 70 µA with $\geqslant$ 10 µL of SLM. When the partial loss of the SLM during EME was compensated for by using larger SLM volume, the EME system became more stable [66]. Similarly, when EME of acidic drugs was carried out using an SLM of 5 µL 1-octanol and a voltage of 50 V, the system-current exceeded 100 µA, and the system-current was reduced to < 50 µA with 10 µL 1-octanol as the SLM (Figure 3-11,right).

3.4.2 SLM composition

In **Paper II**, 1-nonanol diluted one to one (v/v) with 2-decanone was used instead of 1-octanol to reduce the system-current, because different organic solvents served as the SLM affected the stability of the EME system [64]. In addition, the level of system-current was also dependent on the content of DEHP, and higher DEHP content normally produced higher system-current as shown in Figure 3.12 (left). Similar observations on the effect of the SLM composition on the system-current have been observed in **Paper III** (Table 3-9).

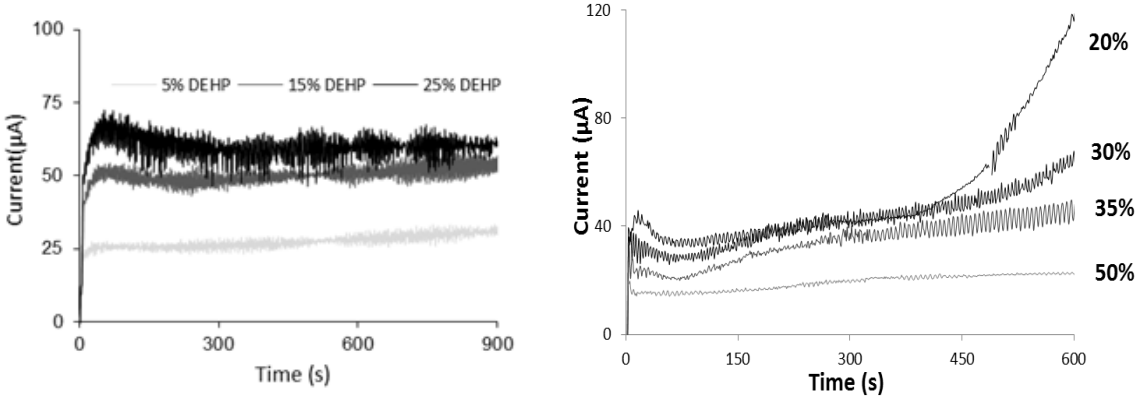


Figure 3-12. Effect of DEHP (left) and NPOE (right) on the system-current. Reprinted with permissions from **Paper II** and **Paper V** © Elsevier B.V. (2015), respectively.

As seen from Figure 3-11 (right), the system-current was found to be dependent on the type of the organic solvent. When EME was performed from water samples, 10 µL 1-octanol and 1-heptanol provided relatively higher system-current than the other tested solvents. When EME was carried out with a voltage of 5 V from human plasma using an SLM of 10 µL pure 1-heptanol, the system-current increased to 150 µA indicating an unstable EME system, which might be due to leakage of SLM into the human plasma sample. Thus, in order to stabilize the SLM, NPOE was used to dilute the 1-heptanol, and the influence of NPOE content on the system-current is illustrate in Figure 3-12 (right). As seen, higher NPOE content provided a more stable EME system, and NPOE content > 30% led to system-current lower than 50 µA. Finally, after the optimization, when EME was carried out for 20 min from

40-fold diluted human plasma with an SLM of 1-heptanol containing 35 % NPOE (v/v), a stable system-current < 50 µA was recorded (Figure 3-13).

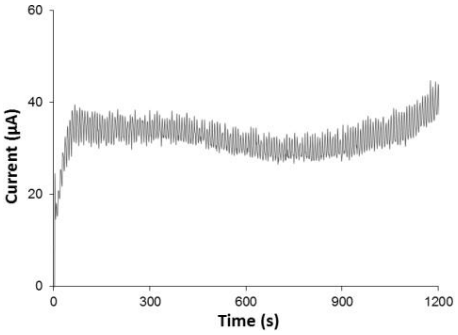


Figure 3-13. Recorded system-current profile during EME of acidic drugs from 40-fold diluted human plasma for 20 min using a voltage of 10 V and an SLM of 1-heptanol containing 35% NPOE. Reprinted with permission from **Paper V** © Elsevier B.V. (2015).

3.4.3 Background electrolyte ions

The background electrolyte ions in the acceptor phase gave different level of system-current, and TFA gave the highest system-current among all tested acceptor phases in **Paper II**. The system current exceeded 100 µA and was considered too high. In addition, the molarity of the acceptor phase also affected the system-current, and higher molarity led to higher system-current. In **Paper V**, system-current was found to increase with increasing the concentration of the background ions.

3.4.4 Voltage

As known, the applied voltage in the EME affects the system-current directly. Generally, EME with higher extraction voltage generates higher system-current as illustrated in Figure 3-14 (left). After the optimization of the extraction voltage, the recorded system-current for EME of peptides was < 50 µA and stable as shown in Figure 3-14 (right).

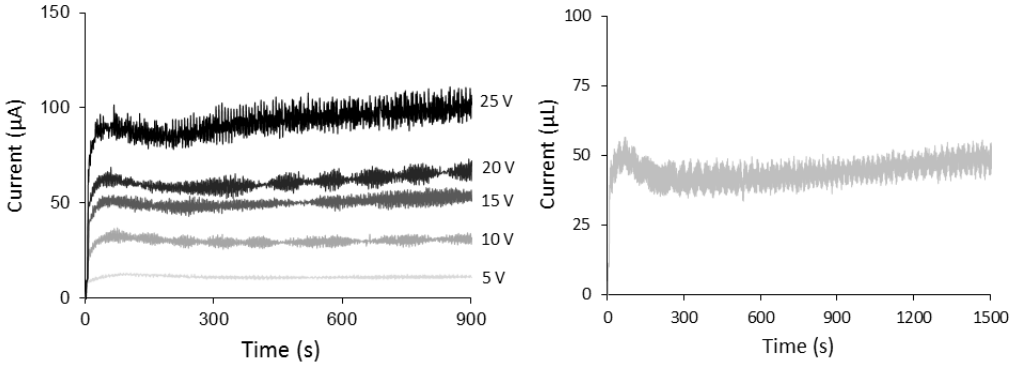


Figure 3-14. Effect of extraction voltage on system-current (a), and recorded system-current profile of a 25 min EME with a voltage of 15 V (b). Reprinted with permission from **Paper II** © Elsevier B.V. (2015).

3.5 Simultaneous EME and LPME

As discussed in Section 1.5, EME has been coupled with different techniques such as LDS-USAEME, DLLME, SPME/SPE for different applications. In addition, dual EME with compromise experimental conditions has been reported for simultaneous extraction of acidic and basic drugs in one step. In **Paper IV**, EME was combined with LPME for simultaneous group separation of basic and acidic drugs in a single step under optimal experimental conditions for both EME and LPME.

In simultaneous EME/LPME (Figure 3-2), EME and LPME were intended for the extraction of basic and acidic drugs from acidified sample into acidified and alkaline acceptor phases, respectively. When EME/LPME was carried out from six-fold diluted standard solution with 100 mM HCl, basic drugs were extracted exhaustively by EME, and acidic drugs were extracted by LPME with a recovery ranging from 76 to 86%. This was slightly lower than that obtained by single LPME (97-103%), because a small fraction of the acidic drugs were extracted into the EME SLM. The group separation performance of EME/LPME is illustrated with the chromatograms in Figure 3-15.

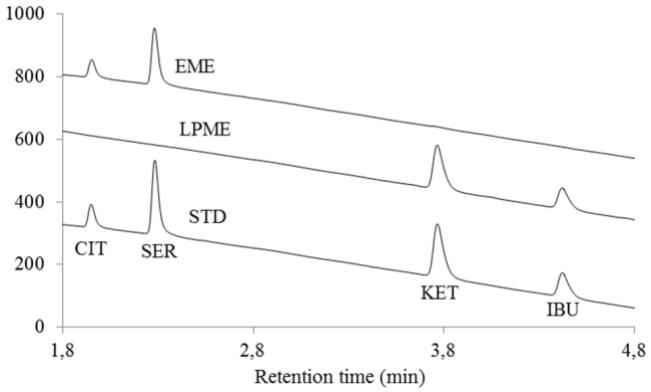


Figure 3-15. Group separation using simultaneous EME/LPME. Chromatograms of the acceptor solution after EME and LPME, and of the standard solution (STD, 10 µg/mL for each analyte). Reprinted with permission from **Paper IV** © American Chemical Society (2015).

The background electrolyte ion in the sample and in the acceptor phases was tested. The background of the sample was found to have some influence on the LPME recovery, and 100 mM HCl in the sample was optimal. This ensured neutral state of acidic drugs even with possible pH shift due to electrolysis induced by EME [59,86,122,176]. The LPME acceptor solution volume was also found to significantly affect the LPME recovery. As shown in Figure 3-16a, LPME recovery increased with increasing volume up to 100 µL. When

EME/LPME was carried out for 15 and up to 30 min, the EME recovery was unchanged, and the LPME recovery increased slightly with increasing time (Figure 3-16b). However, longer extraction time > 15 min caused a small fraction of the acidic drugs to be extracted by EME, due to the pH shift in the EME acceptor phase. [59,86,122,176]. Thus, 15 min was suggested to be the optimal extraction time for clear group separation and high efficiency, although higher LPME recovery required longer extraction time. The influence of the presence of acidic drugs on the EME of basic drugs was also studied, and vice versa. The observation was that the presence of acidic drugs showed no effect on EME of basic drugs, and vice versa. Those observations indicated that simultaneous EME/LPME has high potential to be used for real samples.

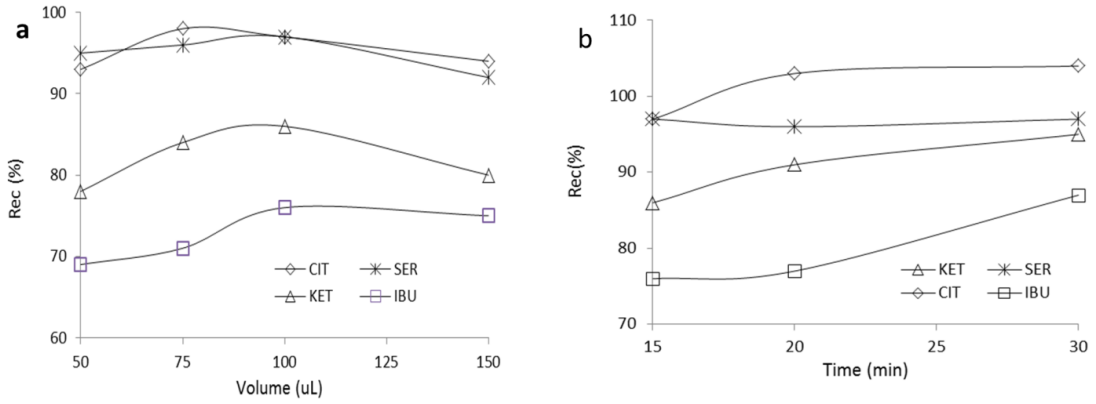


Figure 3-16. Effects of the LPME acceptor solution volume (a) and the extraction time (b) on extraction recovery. Reprinted with permission from **Paper IV** © American Chemical Society (2015).

Prior to the evaluation, simultaneous EME/LPME was carried out for 15 min from diluted human plasma with 100 mM HCl. The recoveries for CIT, SER, KET and IBU increased with increasing dilution times and extraction times as expected. The recovery of the analytes from both six-fold and 36-fold diluted plasma after 15 and 45 min EME are summarized in Table. 3-11. Finally, simultaneous EME/LPME of CIT, SER, KET and IBU from diluted human plasma was evaluated using LC-MS, and the evaluation results are presented in Table 3-12. The linearity range of the acidic and basic drugs was from 1 to 60 µg/mL and from 10 to 600 ng/mL, respectively, which covered the therapeutic ranges of the drugs [177]. In the investigated linearity range, the R^2 values were $\geqslant$ 0.997, and the repeatability was < 15% at 1, 6, and 30 µg/mL for acidic drugs, and at 10, 60, and 300 ng/mL for basic drugs (n = 6). The evaluation data of this approach were in accordance with the FDA requirements for bioanalytical methods validation [178]. Thus, EME/LPME showed high potential for the simultaneous quantification of basic and acidic drugs in real samples.

Table 3-11. Recoveries of the analytes from six-fold and 36-fold diluted human plasma using different extraction time (15 min and 45 min). Simultaneous EME/LPME was performed with NPOE and DHE as the SLM for EME and LPME, respectively. The voltage for EME was 300 V. RSD values presented in the parentheses, n=3.

Analyte	6-fold diluted human plasma		36-fold diluted human plasma	
	15 min	45 min	15 min	45 min
CIT	22 (15)	55 (4)	38 (13)	63 (14)
SER	38 (14)	65 (4)	65 (9)	95 (5)
KET	52 (7)	83 (7)	69 (6)	94 (5)
IBU	43 (8)	70 (10)	65 (6)	82 (5)

Table 3-12. Evaluation results with simultaneous EME/LPME-LC/MS from spiked acidified human plasma.

Analyte	R^2	Therapeutic level[a]	Linearity range	LOD (S/N=3)	LOQ (S/N=10)	RSD (n=6)		
						Low[b]	Medium[b]	High[b]
Bases			ng/mL				%	
CIT	0.997	20-200	10-600	1.2	4.0	14	10	10
SER	0.999	50-250		1.9	6.3	14	7	8
Acids			µg/mL				%	
KET	0.999	1-6	1-60	0.2	0.6	10	7	6
IBU	0.997	15-30		0.3	0.9	11	9	9

[a] *Data were adapted from Ref. [177].*
[b] *Low, medium and high were 10, 60 and 300 ng/mL for bases, but 1, 6 and 30 µg/mL for acids.*

4 CONCLUDING REMARKS

In the current thesis, a simple and easy to operate flat membrane-based electromembrane extraction (EME) device has been developed, and several fundamental aspects and applications of the device have been investigated. Theoretical considerations in EME such as the stability of the EME system and the relation between the SLM (supported liquid membrane) properties and the EME performance have been studied in this work. In addition, attention has also been paid to exhaustive EME of different basic drugs, acidic drugs, and peptides. The application of EME coupled with liquid-phase microextraction (LPME) has also been studied. All of those studies have been described in the five papers within this thesis, and some of the main observations within the thesis are listed below:

- A technical EME set-up with large capacity of acceptor phase volume using a flat porous polypropylene membrane instead of the hollow fiber was developed. Exhaustive extraction of several basic drugs was achieved from water samples and human plasma using a larger acceptor solution volume and longer extraction time, because the mass transfer of analytes across the SLM was caused mainly by electrokinetic migration of charged species and a minor degree by passive diffusion of uncharged species. In addition, a thinner membrane (100 μm in thickness) is also beneficial for EME recovery.

- System-current during EME of peptides using an SLM containing Di(2-ethylhexyl) phosphate (DEHP) was reduced and stabilized by increasing SLM volume from 5 to 10 μL. In addition, exhaustive EME of five model peptides with a thin flat polypropylene membrane based set-up was achieved with system-current below 50 μA.

- Selective extraction of peptide based on differences in isoelectric point using a two-step EME approach was achieved from an aqueous sample. The utility of two EME units in two steps provided high flexibility and enabled independent optimization of the two steps, individually.

- Simultaneous group separation of basic and acidic drugs has been achieved from both water samples and human plasma by combining EME with LPME, which was operated under optimum conditions for both EME and LPME.

- EME system-current and efficiency depends on the viscosity and Kamlet and Taft solvatochromic parameters of the solvents. Alcohols with high hydrogen bonding acidity (α) and dipolarity-polarizability (π*) could provide efficient EME of acidic drugs, and hydrogen bonding interactions, dipole-dipole and hydrophobic interactions were involved in EME of acidic analytes. With an SLM comprising 1-heptanol mixed with 2-nitrophenyl octyl ether (NPOE), exhaustive extraction was obtained from 40-fold diluted human plasma.

EME has shown high potential for future bioanalysis of pharmaceuticals and peptides, though it is facing some challenges. The work in this thesis is contribution in the direction with focus on both fundamental studies and applications to address some of the challenges in EME, which is beneficial for the improvement of EME performance for specific applications in the future.

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