

# **Electromembrane extraction - enhanced theoretical understanding and new applications**

*Thesis for the degree Philosophiae Doctor*

*by*

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Magnus Saed Restan

Oslo, August 2020

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If we knew what it was we were doing,  
it would not be called research, would it?

Albert Einstein

# Publications

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

- I. **Comprehensive study of buffer systems and local pH effects in electromembrane extraction**  
Magnus Saed Restan, Henrik Jensen, Xiantao Shen, Chuixiu Huang, Ørjan Grøttem Martinsen, Pavel Kubáň, Astrid Gjelstad, Stig Pedersen-Bjergaard.  
*Analytica Chimica Acta 2017, 984, 116-123*
  
- II. **Electromembrane extraction of unconjugated fluorescein isothiocyanate from solutions of labeled proteins prior to flow induced dispersion analysis**  
Magnus Saed Restan, Morten E. Pedersen, Henrik Jensen, Stig Pedersen-Bjergaard  
*Analytical Chemistry 2019, 91, 6702-6708*
  
- III. **Towards exhaustive electromembrane extraction under stagnant conditions**  
Magnus Saed Restan, Øystein Skjærvø, Ørjan Grøttem Martinsen, Stig Pedersen-Bjergaard  
*Analytica Chimica Acta 2020, 1104, 1-9*
  
- IV. **Electromembrane extraction of sodium dodecyl sulfate from highly concentrated solutions**  
Magnus Saed Restan, Frøydís Sved Skottvol, Henrik Jensen, Stig Pedersen-Bjergaard  
*Analyst 2020, 145, 4957-4963*
  
- V. **Influence of acid-base dissociation equilibria during electromembrane extraction**  
Magnus Saed Restan, Sindre Bergstrøm Ramsrud, Henrik Jensen, and Stig Pedersen-Bjergaard  
*Journal of Separation Science, published 03.06.2020*

Publication(s) not included in the dissertation:

- **Electromembrane extraction and mass spectrometry for liver organoid drug metabolism studies.**

Frøydis Sved Skottvoll, Frederik Hansen, Sean Harrison, Ida Sneis Boger, Ago Mrsa, Magnus Saed Restan, Matthias Stein, Elsa Lundanes, Stig Pedersen-Bjergaard, Aleksandra Aizenshtadt, Stefan Krauss, Gareth Sullivan, Inger Lise Bogen, Steven Ray Wilson  
Preprint of manuscript submitted to bioRxiv May 15 2020.



# Abbreviations

A336	<i>Aliquat 336</i>
CE	<i>Capillary electrophoresis</i>
DEHP	<i>Di-(ethylhexyl)phosphate</i>
DLLME	<i>Dispersive liquid liquid microextraction</i>
EE	<i>Electro extraction</i>
EME	<i>Electromembrane extraction</i>
ENB	<i>1-ethyl-2-nitrobenzene</i>
FA	<i>Formic acid</i>
FFE	<i>Free flow-electrophoresis</i>
FITC	<i>Fluorescein isothiocyanate</i>
GC	<i>Gas chromatography</i>
HL-LPME	<i>Hollow fiber liquid phase microextraction</i>
HPLC	<i>High performance liquid chromatography</i>
HSA	<i>Human serum albumin</i>
IEF	<i>Isoelectric focusing</i>
LC	<i>Liquid Chromatography</i>
LCMS	<i>Liquid Chromatography-Mass Spectrometry</i>
LLE	<i>Liquid liquid extraction</i>
LOD	<i>Limit Of Detection</i>
LOQ	<i>Limit Of Quantification</i>
LPME	<i>Liquid phase microextraction</i>
MS	<i>Mass Spectrometry</i>
MS/MS	<i>Tandem Mass Spectrometry</i>
NPOE	<i>2-nitrophenyl octyl ether</i>
Pa-EME	<i>Parallel electromembrane extraction</i>
PVDF	<i>Polyvinylidene fluoride</i>
SDS	<i>Sodium dodecyl sulfate</i>
SDME	<i>Single drop microextraction</i>
SLM	<i>Supported liquid membrane</i>
S/D	<i>Signal-to-noise</i>
SPE	<i>Solid Phase Extraction</i>
SPME	<i>Solid phase microextraction</i>
TDA	<i>Taylor dispersion analysis</i>

TEHP	<i>Tris-(2-ethylhexyl) phosphate</i>
UV	<i>Ultraviolet (as detection principle)</i>

## Abstract

Electromembrane extraction (EME) is a sample preparation technique that utilizes an electrical field to extract charged compounds from a sample across a supported liquid membrane (SLM) and over to an acceptor solution. Over 300 scientific paper related to EME has been published since its introduction in 2006, demonstrating the applicability and versatility of the extraction principle. However, EME is still confined to academic settings, and further investigation on extraction mechanisms, extraction principles, and technical configurations are necessary to strengthen the fundamental understanding and move the concept towards routine laboratories and industrial settings. With this in mind, this thesis has been focused on these major objectives; fundamental investigation of buffers and pH during the extraction of small basic drug molecules (**paper I and V**), utilizing EME as a sample purification principle (**paper II and IV**) and improving EME under stagnant conditions (**paper III**).

In **paper I**, EME efficiency of five hydrophobic basic analytes using three different buffer systems in the pH range of 2.0-6.8, were investigated. Buffers are more resistant to pH changes caused by electrolysis than pure acids or bases, thus offering a more pH-stable EME system. A high degree of pH stability ( $\Delta\text{pH} \leq 0.1$ ) and low extraction current ( $\leq 6.0 \mu\text{A}$ ) was achieved for all tested buffer systems. Extraction recoveries for the model analytes were high and independent of buffer composition when system pH  $\leq 4.8$ , while recoveries declined when system pH  $\geq 5.8$ . This was interesting since pH 5.8 should be sufficient to achieve complete ionization of the model analytes. Using a pH-indicator in the acceptor solution during extraction revealed a layer of elevated pH in the acceptor/SLM interface compared to the rest of the bulk acceptor solution, causing deionization of the model analytes. This explained why analyte recoveries declined even when pH in the bulk solution was sufficient for complete ionization, and why extraction efficacy in EME is more sensitive to acceptor solution pH than donor phase pH.

In **paper II**, EME was operated for the first time in “matrix removal mode” for the selective removal of unconjugated fluorescein isothiocyanate (FITC) from fluorescently labeled human serum albumin (HSA) samples. Fluorescence labeling is a common way of enhancing the signal sensitivity and detectability of proteins and is performed with a large molar surplus of fluorescent reagent compared to protein. To avoid excessive fluorescent background signal in the subsequent analysis, removal of unconjugated fluorescent reagent is mandatory. This paper demonstrated the applicability of EME towards this purpose. With an SLM composition comprised of Aliquat 336 and 1-octanol (1:1000 w/w) operated at 50 V, selective removal of FITC (92 %) was achieved, while 79 % of the fluorescently labeled complex (HSA/FITC) remained in the sample. The conserved functionality of the HSA/FITC complex after EME was further assessed in binding affinity study in combination with flow induced dispersion analysis

(FIDA). Both the dissociation constant and hydrodynamic radius of the complex was in concordance with previously reported values, demonstrating conserved binding affinity.

In **paper III**, five hydrophobic bases were extracted from buffer solutions and whole blood samples using a simplified and portable EME device under stagnant conditions. Previously reported EME setups under stagnant conditions have been restricted to short extraction times (1-10 min), due to the open nature of the extraction device, resulting in limited recoveries. This was addressed in this work by making a fully closed device, capable of prolonged extraction times without potential evaporative losses of the acceptor solution. High analyte recovery was correlated with small sample volumes, and 50  $\mu$ L was found to be optimal. However, due to slow extraction kinetics, an extraction time of 60 min was necessary to achieve exhaustive recoveries from buffer solutions. Exhaustive recoveries from whole blood samples were not achieved, even after 60 minutes, due to protein binding and higher viscosity. Nevertheless, evaluation data of methadone from whole blood samples showed excellent clean-up properties, and limit of detection (LOD) and limit of quantification (LOQ) below the therapeutic window.

In **paper IV**, EME in “matrix removal mode” was investigated for the removal of sodium dodecyl sulfate (SDS) from highly concentrated solutions. SDS is an ionic detergent for the lysis of cell and solubilization of protein, but due to its detrimental effects on chromatographic columns and ion-suppression in mass spectrometry (MS), removal of SDS prior to analysis is necessary. To represent SDS concentrations used in real sample applications, EME was performed on highly concentrated SDS samples (0.1, 0.5, and 1.0 % (w/w)). Similarly to **paper II**, an SLM composition with A336 was demonstrated to be effective and complete SDS removal was achieved from 0.1 % samples. However, mass balance experiments revealed that most of the SDS was trapped in the SLM and only a small fraction was transferred to the waste solution. This limited the removal of SDS from 0.5 % and 1.0 % samples as the SLM capacity was exceeded and further mass transfer was impeded. Improved SDS removal could be achieved by extending the surface area of the SLM or by replenishing the SLM halfway through the extraction.

In **paper V**, a theoretical model to predict extraction recovery as a function of sample and acceptor pH was developed. Based on substances acid-base equilibria ( $pK_a$ ) and coupled equilibria in the partitioning of the neutral form to the SLM ( $\log P$ ), effective extraction was postulated if  $pH \ll pK_a - \log P$ . Twelve hydrophobic basic analytes with a wide  $\log P$  (2.19-4.89) and  $pK_a$  (6.32-10.38) range were extracted under different pH conditions with 2-nitrophenyl octyl ether (NPOE) as SLM and fitted to the theoretical model. Seven of the model analytes correlated with the prediction, while analytes with low hydrophobicity (i.e close to  $\log P = 2$ ) deviated from the general prediction. This was hypothesized to be caused by deprotonation and ion-pairing within the SLM.

# 1 Introduction

## 1.1 Sample pre-treatment

Analytical chemistry is a growing scientific field, for the separation, detection, and quantification of chemical substances [1]. Analytical chemistry is utilized in multiple areas, such as in pharmaceutical sciences, forensics, food chemistry, mining, environmental science, and biomedical sciences. Analytical determination of a target compound in a given sample usually involves a multi-step procedure, including sample preparation, separation, detection, identification, quantification, and data handling. Each step is equally important in order to get a reliable and accurate test result. In the field of bioanalysis, where scientists measure target compounds from complex biological samples such as whole blood, plasma, serum, saliva, and urine, sample pre-treatment is required to remove interfering matrix compounds such as phospholipids, salts, and proteins. Injection of matrix compounds on to chromatographic columns used in liquid chromatography (LC), can lead to series of detrimental effects, such as co-elution with target compounds, damage to the stationary phase, and shortened lifetime of the analytical column [2]. In cases of co-elution, chromatographic systems coupled to mass spectrometry (MS) may suffer from ion suppression or enhancement, leading to unreliable quantitative data [3]. Another challenge occurs when the concentration of target compounds in the sample is below the limit of quantification of the analytic instrument. In these instances, sample preparation involving enrichment of the target compounds is necessary.

The currently most used sample preparation techniques for aqueous samples are the well-established techniques liquid-liquid extraction (LLE) and solid-phase extraction (SPE) [4, 5]. Both techniques involve partitioning of compounds between two different phases. LLE is a two-phase extraction system consisting of two immiscible solvents in contact with each other, where the compounds are extracted based on their distribution coefficient ( $K_d$ ), as defined by the following equation:

$$K_d = \frac{[\text{Compound in organic solvent}]}{[\text{Compound in aqueous solvent}]} \quad (1)$$

Biological samples are normally aqueous, and one uses an organic solvent as extraction phase. The organic phase is either a pure organic solvent or a mixture, and one tunes this to extract the target compound while minimizing co-extraction of unwanted compounds. This is very applicable to biological samples, where most matrix compounds have low  $K_d$ -values. In the case of SPE, extraction relies on the partitioning of the target compound between a liquid mobile phase and a solid stationary

phase. The stationary phase comprises functional groups that retain target compounds based on their chemical properties. One performs SPE stepwise, including conditioning of the stationary phase, sample loading, wash, and elution of target analyte.

## 1.2 Microextraction techniques

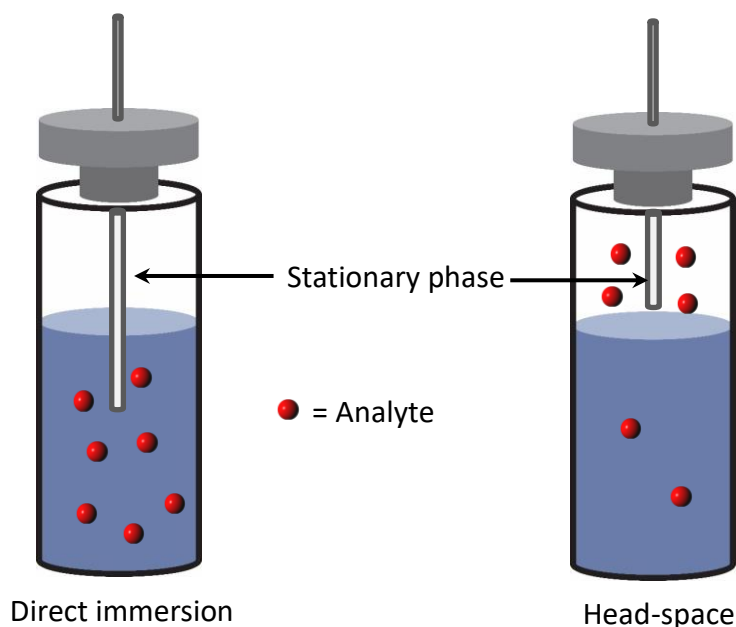
While conventional LLE and SPE are still widely used, there is a shift towards miniaturization [6]. Conventional extraction techniques require the use of relatively large amounts (mL) of potentially hazardous organic solvents, while only low  $\mu\text{L}$ -amounts are used in miniaturized versions. This is in concordance with the pursuit towards green chemistry in the analytical community, as well as reducing the cost per extracted sample. Furthermore, miniaturization offers other advantages such as reduced extraction time, easier automation, increased mobility, and reduced sample amount [7]. The latter is especially relevant in cases where the sample amount is limited (e.g cerebrospinal fluid, synovial fluid, natal blood).

### 1.2.1 Solid phase microextraction

Solid phase microextraction (SPME) was introduced by Arthur and Pawliszyn in 1990 [8]. In this work, the authors inserted a syringe containing a fused silica fiber coated with poly-dimethyl siloxane (PDMS) into an aqueous sample. The target analytes (trichloroethane, trichloroethene, and chloroethylene) partitioned into the PDMS coating (stationary phase) and equilibrium established within 1-2 min. The fiber was subsequently transferred to a gas chromatography (GC) inlet and the analytes were released by thermal desorption, thus eliminating the need for organic solvent as extraction medium. Following this pioneering work, numerous SPME geometries and principles have been developed, including coated tip, in-tube, in-needle, stir bar, and fiber-packed SPME [9-12].

The most common sampling techniques are direct immersion (DI-SPME) where the stationary phase is directly immersed in the aqueous sample and head-space (HS-SPME) where the stationary phase is placed above the sample (Figure 1) [13]. HS-SPME is applicable for the extraction of volatile and semi-volatile compounds from complex matrices such as urine, plasma, and whole blood. Since the stationary phase is not in contact with the sample, the extraction is less affected by matrix components resulting in highly pure and selective extracts, as well as an increased lifetime of the stationary phase [14]. DI-SPME is applicable to polar, semi- and non-volatile compounds. With direct immersion into the sample, more compounds are available for extraction compared to HS-SPME but at the cost of a higher degree of matrix interferences [15]. This has led to the development of membrane protected SPME, where a coating [16] or a membrane [17] prevents large matrix molecules from interacting with the stationary phase.

The simplicity and versatility of the SPME principle have led to its wide use in the analysis of pharmaceutical [18], environmental [19], food [20], and biological samples [21]. In most applications, SPME is coupled with GC, while coupling with LC is used in situations where target analytes are thermally unstable or non-volatile [22].



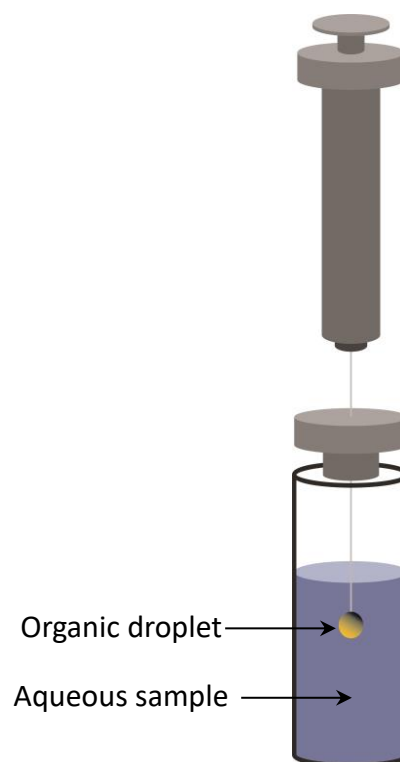
**Figure 1:** Extraction principle for direct immersion SPME and headspace SPME

### 1.2.2 Liquid-phase microextraction

Following the trend initiated by SPME, miniaturization of LLE emerged in the form of single drop microextraction (SDME) in 1996 [23, 24]. In this format, a micro-droplet of organic solvent is submerged into an aqueous sample solution and analytes are extracted into the droplet by passive diffusion (Figure 2). The organic droplet is introduced and held in place by a micro-syringe [25] or using a Teflon rod [24]. At equilibrium, the droplet is withdrawn and typically analyzed by GC. The SDME format is considered a simple, inexpensive, and fast extraction technique, with minimal usage of organic solvent [26]. The small volume of organic solvent (1-8  $\mu\text{L}$ ) enables high analyte enrichment, as there is a large volume ratio between the sample and the organic solvent [27, 28]. Extraction kinetics is also improved by the large surface-to-volume ratio [29]. However, the microscale size droplet imposes some drawbacks to the technique. The droplet is often dislodged or dissolute within the aqueous sample, especially during fast sample stirring and prolonged extraction times, leading to poor reproducibility and robustness [30]. Further modification of the SDME principle has led to the development of different extraction modes, which can be categorized as either two-phase or three-

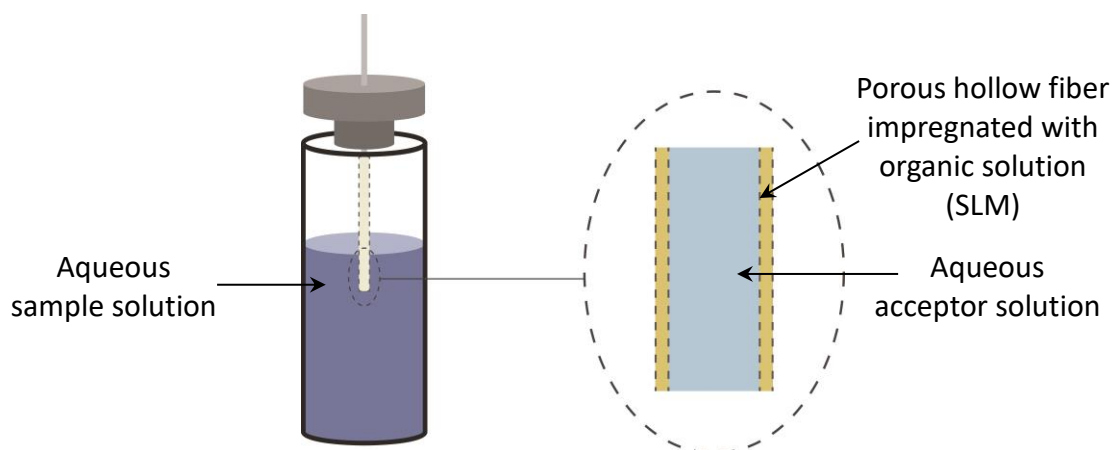
phase extraction systems [29]. The most important ones include dispersive liquid-liquid microextraction (DLLME), hollow-fiber liquid-phase microextraction (HF-LPME), 96-well liquid-phase microextraction, and solvent bar microextraction (SBME). DLLME is a very simple and efficient variant of liquid-phase microextraction, performed with standard analytical equipment in a conical test tube. The first article reporting on DLLME emerged in 2006 [31]. Using a micro-syringe, a water-immiscible extraction solvent and a water-miscible disperser solvent is injected into the sample. This results in a cloudy emulsion, where target analytes partition instantaneously into the dispersed extraction solvent. Subsequent phase separation is done by centrifugation and the extraction solvent is collected with a micro-syringe from the bottom of the test tube. Alternatively, phase separation can be achieved by cooling and the extraction solvent tends to solidify as a pellet. DLLME is currently a very active area of research, and applications are typically water samples (environmental analysis), food, beverages, and trace metal analysis. Bioanalytical applications of DLLME are relatively few, especially in combination with LC-MS, and mainly related to urine samples [30].

HF-LPME was introduced as an alternative to SDME in 1999 [32] (Figure 3). The development of HF-LPME offered a liquid-phase microextraction system with enhanced stability as compared to SDME. Extraction is performed from an aqueous sample, into a supported liquid membrane (SLM) and further into an extract solution (acceptor). The SLM comprises a thin film of water-immiscible organic solvent immobilized in the pores in the wall of a porous hollow fiber. The acceptor solution is placed in the lumen of the hollow fiber, protected by the SLM. While many scientists have developed HF-LPME with laboratory built equipment, routine laboratories request automated sample preparation with commercial 96-well plates. To address this, 96-well LPME was proposed in 2013 [33].



**Figure 2:** Schematic illustration of SDME principle. Analyte are extracted from the aqueous sample to the organic droplet via passiv diffusion.





**Figure 3:** Schematics illustration of HF-LPME in a three-phase format.

Solvent bar microextraction (SBME) is similar to HF-LPME and was proposed in 2004 [34]. The main idea of SBME was to enhance extraction speed as compared to HF-LPME. In SBME, a small piece of hollow fiber is tumbling freely in a stirred sample solution. Both ends of the hollow fiber are closed, and the lumen contains the acceptor. A water-immiscible organic solvent fills the pores in the wall of the hollow fiber, serving as SLM.

Extraction with HF-LPME (and even SBME) is relatively slow because the mass transfer is by passive diffusion. Thus, extraction times may be up to 45 minutes to reach equilibrium. In 2006, electromembrane extraction (EME) emerged to improve mass transfer [35]. In EME, electrodes are located in the sample and acceptor, and the electrodes are in connection with an external power supply. Forced by the electrical field, charged analytes migrate from the sample, across the SLM, and into the acceptor.

### 1.2.3 Electro-migration-based sample preparation

Electrical fields are utilized in separation science with capillary electrophoresis (CE) and gel electrophoresis [36] as well as in sample treatment techniques such as free-flow electrophoresis (FFE), isoelectric focusing (IEF), and electroextraction (EE) [37].

The following equation expresses the electrostatic force ( $F$ ) on charged analytes under a constant electrical field:

$$F = qE \quad (2)$$

where  $q$  is the charge of the analyte and  $E$  is the strength of the electrical field. The electrophoretic mobility ( $ue$ ) of a charged analyte is determined by  $E$  and the inherent capability of the analyte to move in the presence of an electrical field, according to the following equation:

$$ue = ve/E \quad (3)$$

where  $v_e$  is the electrophoretic mobility. Thus increasing the strength of the electrical field, will affect analytes of similar charge differently based on their inherent degree of electrophoretic mobility. Besides affecting the trajectory of charged analytes, the presence of an electrical field can manipulate the molecular orientation of analytes. By molecular orientation, one can achieve selective separation of analytes based on the differences in frictional forces or using membranes that is only permeable to certain orientations.

Electrodialysis is one example of the use of electrical fields for sample preparation [38]. Electrodialysis consists of an electrical field sustained over a semi-permeable membrane. Under the influence of an electrical field, cationic (positively charged) analytes will migrate towards the cathode (negative), while anionic analytes (negatively charged) migrate towards the anode (positive). Together with the membrane, the electrical field in electrodialysis allows selective extraction of compounds based on size and charge.

FFE is a continuous and preparative electrophoresis procedure, performed in a thin buffer film [39]. FFE separates charged particles according to their electrophoretic mobility or isoelectric point. The sample is introduced into a buffer film, which is flowing through a chamber formed by two narrowly spaced glass plates. The electrical field is perpendicular to the buffer flow, and this leads to the movement of charged compounds in the sample towards the respective counter-electrode. The sample and the electrolyte used for the separation enter the chamber at one end and the electrolyte containing different sample components as separated bands fractionate at the other side.

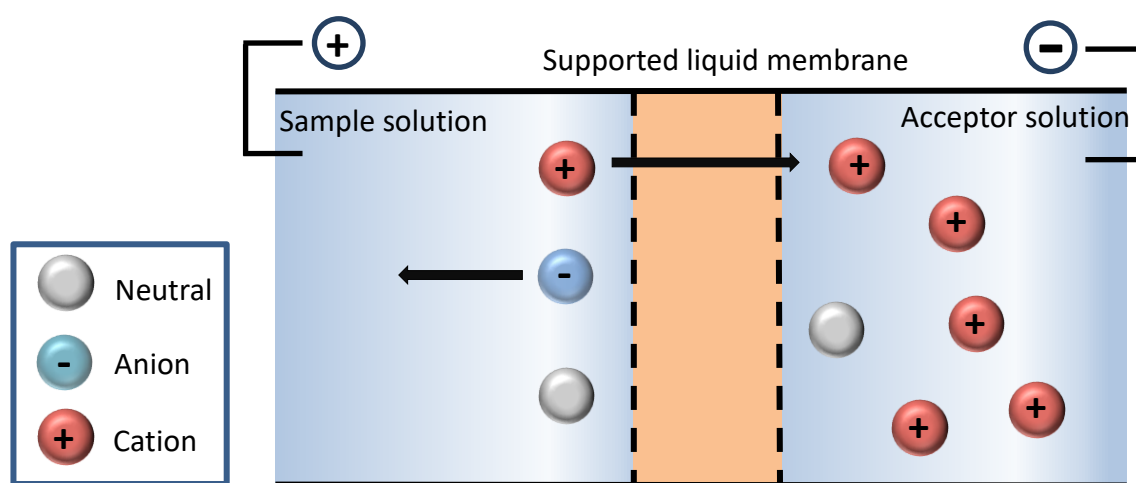
IEF is mostly used for the preparative separation of proteins [40]. The sample is introduced into a gel with a pH gradient, and an electrical field is applied along the gel. Proteins migrate in the gel until they reach pH conditions equal to the isoelectric point. Proteins with different isoelectric point will migrate and stop at different locations in the gel, thereby effectively separating them.

EE can be considered as LLE in combination with an electrical field. First applied to analytical purposes in 1994 [41], it consists of an organic sample phase and an aqueous acceptor phase, with an electrode placed in each solution. The organic sample and the acceptor are immiscible, and one sustains an electrical field over the liquid-liquid interface. Since the introduction, there has been little activity in exploring this principle. Most work has been with samples in ethyl acetate, and with EE taking place in a CE instrument.

### 1.3 Electromembrane extraction

EME was first introduced in 2006 and is a hyphenation of three-phase microextraction and electrophoresis [35]. The extraction configuration is similar to liquid-liquid-liquid extraction with two

aqueous phases (donor and acceptor) separated by an organic solvent immobilized within a porous membrane (SLM) but introduces electrodes in the donor and acceptor solution for the application of an electrical field. By tuning the pH of the aqueous solutions, charged compounds will electrokinetically migrate towards the electrode of opposite polarity (Figure 4). In the first published paper on EME, the authors extracted five hydrophobic basic drugs from water samples, plasma, and urine [35]. The extraction yielded high recovery, enrichment, and shorter extraction time compared to diffusion-based extraction principles. Since 2006, more than 300 scientific research and review articles have been published on EME, mainly about the theoretical understanding of the extraction principle [42], new extraction configurations [43], SLM chemistry [44], and extraction of new compound classes [45].



**Figure 4:** Illustration of EME principle. Charged compounds migrate in accordance with the polarity of the electrical field. Neutral compounds are unaffected by the electrical field.

### 1.3.1 Principle

The mass transfer of compounds in EME is by electrokinetic migration under the influence of an electrical field. Passive diffusion also occurs, but due to the charge of the compounds, diffusion-based migration into the organic SLM is un-favorable and contributes only marginally to the mass transfer, especially for shorter extraction times ( $\leq 15$  min) [46]. Since only charged compounds are influenced by the electrical field, pH of the sample and acceptor needs to be adjusted to a level where the compounds of interest are completely charged (i.e. 2 pH-units below (bases) or above (acids) the  $pK_a$  value of the compound). For the extraction of basic compounds, one adjusts pH to acidic or neutral conditions, and places the anode and cathode in the sample and acceptor solution, respectively. For acidic compounds, one adjusts pH to basic or neutral conditions and reverses the polarity of the electrodes.

Gjeldstad et al described a theoretical model for the mass transfer of charged compounds across an SLM by approximation of the Nernst-Planck equation [47];

$$J_j = -D_j \frac{dc_j}{dx} + \frac{D_j z_j e E c_j}{kT} \quad (4)$$

Where  $J_j$  is the steady-state flux of an ionic compound  $j$ ,  $D_j$  is the diffusion coefficient for the ion,  $c_j$  is the concentration of the ion in the SLM,  $x$  is the distance from the SLM/acceptor interface  $z_j$  is the charge,  $e$  is the elementary charge,  $E$  is the electrical field,  $k$  is the Boltzmann's constant, and  $T$  is the absolute temperature. The first term relates to the diffusion-based migration of the ionic compound and the second term the electro kinetic-based migration. However, Eq.2 is only valid if the membrane thickness is  $\geq 200 \mu\text{m}$ , and if there is no difference in ion concentration between the donor solution compared to the acceptor solution. In most EME setups this is not the case and for these configurations the steady-state flux of a singly charged ion ( $J_i$ ) across the SLM is more accurately described by the following equation:

$$J_i = -\frac{D_i}{h} \left(1 + \frac{v}{\ln \chi}\right) \left(\frac{\chi^{-1}}{\chi - \exp(-v)}\right) (c_i - c_{i0} \exp(-v)) \quad (5)$$

where  $h$  is the membrane thickness,  $\chi$  is the ratio of the total ionic concentration in donor and acceptor phase (termed ion balance), and  $v$  is the dimensionless driving force described by the following equation;

$$v = \frac{z_i e \Delta \phi}{kT} \quad (6)$$

where  $\phi$  is the electrical potential across the SLM. Eq. 5-6 illustrate that membrane thickness is an important equipment-related parameter, and that the magnitude of the electrical potential is an important operational parameter.

### 1.3.2 Parameters affecting EME

#### 1.3.2.1 pH and sample/acceptor composition

As mentioned previously one adjusts pH of the sample and acceptor phase to ensure ionization of target analytes. In most published work, HCl served this purpose for basic analytes [48-54] and NaOH for acidic analytes [55-59]. Sample phase pH has been shown to be less critical for efficient extraction than the acceptor phase [56, 60], making it possible to extract analytes from sample matrices without pH adjustments [61, 62].

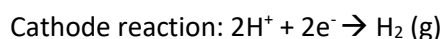
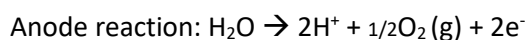
The acceptor phase volume is often less than the donor volume, ensuring the possibility for enrichment of target analytes. In the first EME paper, the donor and acceptor solution volumes were 300 and 30  $\mu\text{L}$ , respectively, achieving an enrichment factor between 7.0 and 7.9 [35]. With the development of

more miniaturized EME configurations, the ratio between acceptor and donor volume has increased making it possible to achieve enrichment factors of hundredfolds [63, 64].

Most samples processed with EME are aqueous. However, certain sampling techniques (e.g dried blood spots) demand a reconstitution of the sample into an organic solvent prior to extraction. Given the organic nature of the solvent in the SLM, the hydrophobicity of the sample needs to be sufficiently low in order to ensure immiscibility with the SLM, and thus a stable extraction system. Seip et al tested EME of sample solutions containing methanol, ethanol, dimethyl sulfoxide, and acetonitrile [65]. With a 50:50 composition of methanol, ethanol or dimethyl sulfoxide mixed with water, the SLM was still stable. The presence of an organic solvent in the sample did not affect steady-state recoveries, but extraction was slower and extraction times increased. Acetonitrile proved to be too hydrophobic to maintain a stable extraction system. With organic solvent in the sample solution, the voltage drop across the SLM is lower, leading to slower analyte kinetics through the SLM compared with pure aqueous samples.

### 1.3.2.2 Voltage and current

An external power supply provides the electrical potential to the EME setup and offers the flexibility to adjust the polarity and magnitude of the electrical field. The polarity can effectively discriminate between extraction of cations and anions, while the voltage enhances or discriminates ions based on their electromigration properties. The electrical current of an EME circuit reflects the flux of analyte and background ions across the SLM and is a function of the applied voltage and the resistance of the SLM. The voltage applied to the EME circuit is thus dependent on the composition of the SLM. Increasing the voltage will lead to higher current, but not necessarily higher extraction recovery of target analytes. Too high current leads to Joule heating, resulting in evaporative losses of the SLM. Furthermore, the current induces electrolysis according to the following reactions [60] :



Under the assumption that the electrode material is inert,  $\text{H}_2$  and  $\text{O}_2$  will form gas bubbles and associated changes of  $\text{H}^+$  will shift the pH of the aqueous solutions. Excessive electrolysis leads to decreased system stability, lower extraction recovery, and reproducibility. As a rule of thumb, the voltage should be tuned so that extraction current is  $\leq 50\mu\text{A}$ , in order to limit excessive electrolysis [45]. There is no golden standard for optimum voltage level, and optimization of voltage is necessary to obtain selective, effective, and stable exactions. In the earliest EME work, voltage of 300 V was used [35], while more recently 9 V has been used for the extraction of basic drugs analytes [62].

### 1.3.2.3 Agitation

Agitation of the EME setup plays an important part in the overall kinetics and efficiency of the extraction. Due to the high resistance of the SLM compared to the aqueous solutions, the majority of the electrical field drop (V/cm) is over the SLM. This makes migration of analytes in the sample slow, while transfer across the SLM is a fast and electro-kinetically driven process. Consequently, the sample/SLM interface quickly depletes for analyte. Convection of the sample by agitation ensures replenishment of analyte to the sample/SLM interface, resulting in faster mass transfer and decreased extraction time to achieve steady-state. Another reason for agitation is to reduce the thickness of the ionic boundary layer that forms in the interface between the sample solution and the SLM. The ionic boundary layer reduces the overall effectiveness of EME extraction, both in terms of kinetics and extraction recovery [35, 56, 66]. For some EME configurations, agitation is necessary to establish and maintain an electrical circuit by ensuring contact between the sample and the SLM [54, 67].

### 1.3.2.4 Extraction time

EME is fast due to electrokinetic migration. Optimal extraction time is when extraction recovery reaches steady-state conditions, and there is no net gain by increasing extraction time. In most published work, steady-state conditions occur after 5-15 minutes [35, 52, 57, 59, 68]. In some cases, an increase of extraction beyond the optimum level results in diminishing recovery, caused by back-diffusion of analytes from the acceptor solution to the SLM [69]. For soft extraction, extraction times down to 1 minute have been reported [62].

## 1.3.3 Supported liquid membrane (SLM)

The SLM is a thin layer of organic solvent immobilized within the pores of a porous membrane and acts as the barrier between the two aqueous phases in the EME system. Furthermore, the SLM is the main resistive component of the electrical circuit [70] and determines the distribution of the electrical field. Thus, the SLM plays a key role in terms of selectivity. The choice of organic solvent is crucial in order to get a stable, efficient, and selective extraction system, and a set of criteria can guide the selection of the SLM. Ideal SLM solvents should be non-volatile to avoid evaporative losses during extraction and have a high degree of water-immiscibility that prevents dissolution into the sample. The SLM should allow penetration and establishment of an electrical current. Furthermore, the solvent should have low viscosity and possess some hydrogen-bonding and/or dipole-dipole interactions in order to facilitate and enhance fast mass transfer into and out of the SLM.

For EME of hydrophobic ( $\log P \geq 2$ ) basic analytes, the nitro-aromatic ethers 2-nitrophenyl octyl ether (NPOE), 2-nitrophenyl pentyl ether, and 1-ethyl-2-nitrobenzene (ENB) have been tested, with NPOE being the most efficient and popular. It possesses all of the aforementioned criteria, including a high

Kamlet-Taft value for dipolarity-polarizability and hydrogen bonding basicity ( $\beta$ ), making it ideal for the extraction of positively charged basic analytes. For extraction of hydrophobic acidic analytes, numerous aliphatic alcohols have been tested, with 1-octanol and 1-nonanol being the best candidates [71]. Aliphatic alcohols possess high hydrogen bonding acidity ( $\alpha$ ) and high to moderate polarizability values. EME of more hydrophilic analytes ( $\log P \leq 2$ ) (i.e. drugs, peptides, metals) with pure organic solvents is challenging, and addition of ion-pair reagents to the organic solvent is often required in order to facilitate efficient mass transfer into the SLM. Interactions between the ion-pair reagent and the hydrophilic analyte results in more hydrophilic complexes, promoting mass transfer into the SLM. Among the commonly used ion-pair reagents, di-(ethylhexyl) phosphate (DEHP), tris-(2-ethylhexyl) phosphate (TEHP), tridecyl phosphate, and dibutyl phosphate have proven to be effective [71]. TEHP added to NPOE in the SLM increased the transfer of medium polar basic analytes [72] and DEHP is the most prevalent additive for extraction of amino acids [73] and peptides [74].

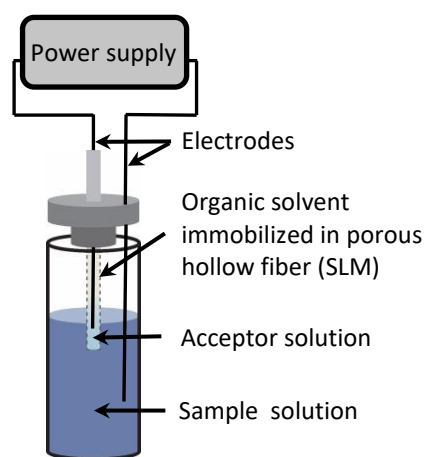
### 1.3.4 Technical configurations

Literature reports on EME in different technical configurations. The following sections discuss a few of these configurations briefly.

#### 1.3.4.1 Hollow fiber

Originally EME emerged in a hollow fiber configuration as illustrated in Figure 5 [35]. This configuration has up to date been one of the most frequently used for EME.

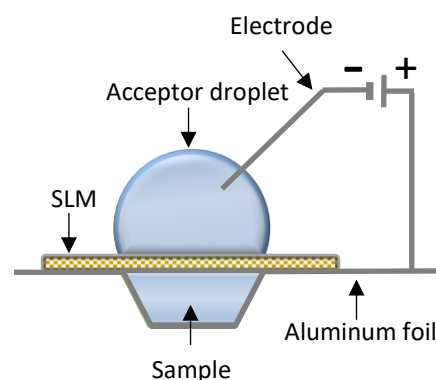
Typically, a piece of porous hollow fiber of polypropylene is used. The length is from 1 to 4 cm, and the hollow fiber is closed in one end by mechanical pressure. The hollow fiber is dipped for a few seconds in the organic solvent used as SLM, and this loads the SLM. Following this, the acceptor solution is injected with a micro-syringe into the lumen of the hollow fiber, and the fiber is placed into the sample. To initiate EME, electrodes coupled to a power supply are placed in the sample and acceptor solution, and a voltage potential is applied. Hollow fibers are inexpensive and normally provide EME systems of high efficiency, as long as the wall thickness does not exceed 200  $\mu\text{m}$ . On the other hand, EME based on hollow fibers may be difficult to commercialize and automate.



**Figure 5:** Schematics of EME in a hollow fiber configuration.

### 1.3.4.2 Drop-to-drop

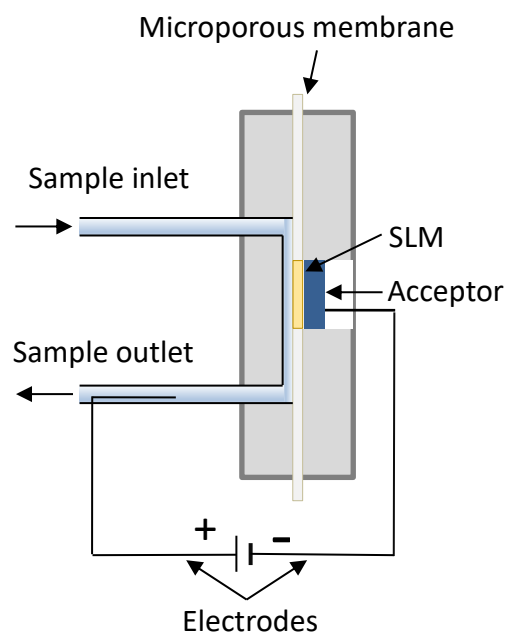
EME in drop-to-drop configuration was developed in 2009 and was utilized for the extraction of basic drugs from a 10  $\mu\text{L}$  sample droplet [75]. The sample was separated from a 10  $\mu\text{L}$  acceptor solution by a 25  $\mu\text{m}$  thick porous polypropylene membrane coated with NPOE. Aluminum foil was used as a compartment for the sample solution and as the anode, while a platinum electrode was used as the cathode in the acceptor solution (Figure 6). The system operated at low voltage (3-20 V) and under stagnant conditions. Due to the short diffusion pathway, extraction recoveries ranged from 33-47 % after only 5 min extraction. The system also showed compatibility with urine and plasma, with low co-extraction of hydrophilic matrix compounds. However, the open nature of the system limited further increase in extraction time due to the risk of evaporative losses of the acceptor phase.



**Figure 6:** Schematics of drop-to-drop EME.

### 1.3.4.3 On-chip

After the pioneering work of the drop-to-drop format, EME was further miniaturized to an on-chip format. The first on-chip EME device was developed in 2010 and comprised a 25  $\mu\text{m}$  thick porous polypropylene membrane bond in between two polymethyl methacrylate (PMMA) plates [43]. The SLM was created locally by filling a portion of the membrane with NPOE. The two PMMA plates functioned as sample channels and acceptor compartment, respectively (Figure 7). The acceptor solution (7  $\mu\text{L}$ , 10 mM HCl) was kept stagnant while the sample solution was pumped through the sample channels at a flow rate between 0.33-3.0  $\mu\text{L}/\text{min}$ . Under optimal conditions using a voltage of 15 V and a sample flow rate of 3.0  $\mu\text{L}/\text{min}$ , extraction recoveries between 20-60 % for the five basic model analytes were achieved after the sample had been in



**Figure 7:** Schematics of EME on-chip device.

contact with the SLM for only 4 seconds. The highly efficient extraction performance demonstrated in this work has led to the development of several new on-chip EME devices [76-79]. EME on-chip devices have also been coupled to online UV or MS detection for continuous measurement of a dynamic



acceptor solution [80]. This concept was further developed for the continuous monitoring of in-vitro metabolism of drug substances by rat liver microsomes [81].

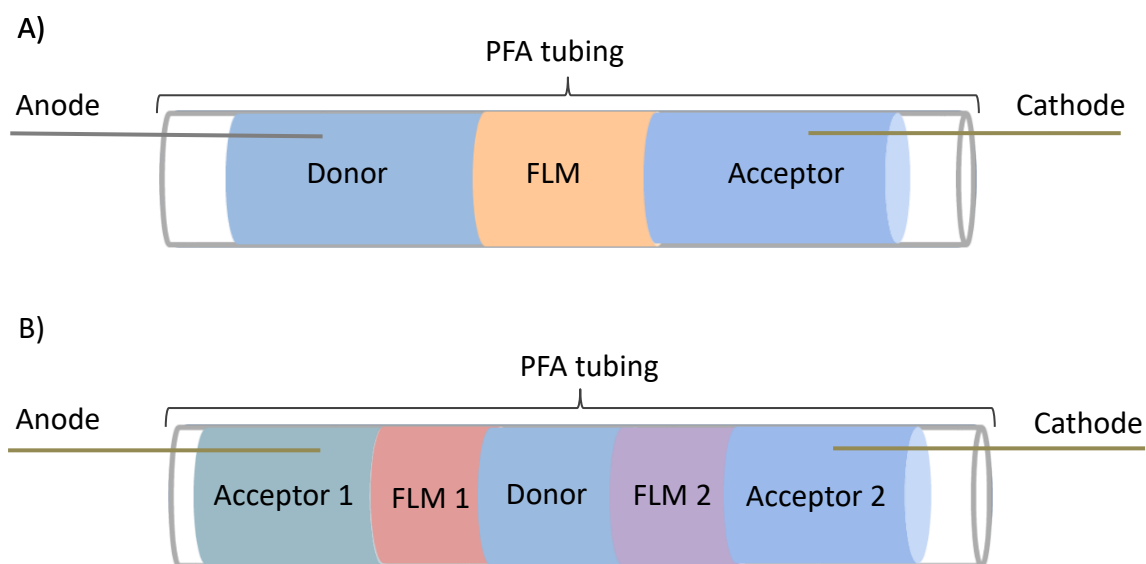
#### **1.3.4.4 96-well format**

EME in a 96-well format was developed in 2014 and offered the potential for higher throughput [67]. A commercially available 96-well plate served as a compartment for the sample solution. The lower part of eight plastic vials was cut out and circular polypropylene flat-membranes were subsequently heat-sealed to the opening. The plastic vial served as a compartment for the acceptor solution and the flat membrane as supporting material for the SLM. Both the walls of the donor and acceptor compartment were coated with aluminum foil, acting as electrodes. Under optimized EME conditions, eight samples of 240  $\mu\text{L}$  undiluted plasma spiked with four basic drugs were selectively extracted into 70  $\mu\text{L}$  formic acid (FA) as acceptor solution, using 3  $\mu\text{L}$  NPOE as SLM, voltage of 70 V, and extraction time of 8 min. The extraction recoveries ranged between 17-33 % and provided acceptable reproducibility (RSDs  $\leq$  15%). The same author further developed the acceptor/SLM setup to allow for 96 extractions simultaneously [82].

Following the trends in sample treatment of lowest possible time consumption per sample, the implementation of EME to a 96-well format is an important development to promote the usage of EME in routine laboratories.

#### **1.3.4.5 Free liquid membrane**

EME with a free liquid membrane (FLM) was introduced in 2014 [83]. A transparent perfluoroalkoxy (PFA) tubing with an inner diameter of 1.0 mm was filed stepwise with 3.0  $\mu\text{L}$  donor solution, 1.0  $\mu\text{L}$  FLM, and 1.5  $\mu\text{L}$  acceptor solution (Figure 8 A). Two 0.25 mm platinum wires were inserted into the donor and acceptor solution, respectively serving as anode and cathode. Three basic drug substances were selectively extracted from urine and serum during 5 minutes using either NPOE or ENB as SLM, and voltage of 300 V and 200 V respectively. In another paper by the same author, a similar setup using 50  $\mu\text{L}$  donor and 0.5  $\mu\text{L}$  acceptor solution resulted in a pre-concentration of  $\text{ClO}_4^-$  with an enrichment factor of 98. Additionally, the transparency of the tubing allowed for visualization of the mass transfer by using the red dye SPANDS as model analyte [84]. Multiple FLMs can be integrated into the same system, allowing for more than one acceptor phase [85] (Figure 8 B). However, the thickness of the FLM is often larger and the surface area smaller than that of an SLM, leading to slower mass transfer. Furthermore, the stability of the FLM is lower than the SLM and agitation is not possible. This further reduces the extraction kinetics and prolonged extraction times are necessary to achieve high recoveries.



**Figure 8:** Schematics of EME with FLM. A) Singel FLM format, B) multiple FLM format

### 1.3.5 EME applications

In theory, EME is applicable for the extraction of all chargeable compounds, which encompasses a large number of potential candidates. In bioanalysis, nearly all compounds of interest (such as drugs, metabolites, peptides, and proteins) can be charged, making EME very applicable for sample treatment from biological matrices. The following gives examples of different EME applications. For a more complete overview, such is found in recent review articles [86].

#### 1.3.5.1 Small-molecule drugs

In the earliest work on EME, loperamide, methadone, pethidine, nortriptyline, and haloperidol (all drug substances) were extracted from acidified human plasma, urine, and water samples [35]. The authors used NPOE as SLM and a voltage of 300 V. After extraction for 5 minutes, the acceptor phase was analyzed on CE-UV. Recoveries of 70-79 % were achieved with RSD  $\leq$  16 %. The effectiveness of EME on monobasic hydrophobic basic drugs has been thoroughly investigated in further work [48-50, 87, 88] and generally, EME provides high recoveries and high precision for such substances. EME of hydrophobic bases with more than one basic group is less straightforward due to low partitioning into the SLM. Fine-tuning sample pH to avoid 100 % ionization, or adding carriers to the SLM is necessary to get high recoveries for substances with multiple charges [72].

The effectiveness of EME for acidic compounds was demonstrated for the first time in 2007 [56]. Diclofenac, fenoprofen, flurbiprofen, gemfibrozil, hexobarbital, ibuprofen, indomethacin, ketoprofen, naproxen, probenecid, and warfarin were extracted from alkalized water solutions using 1-heptanol as SLM and 50 V. Extraction time was 5 minutes. Recoveries ranged from 8-100 %. In another study, six

non-steroidal anti-inflammatory drugs (NSAIDs) were extracted from wastewater samples using 1-octanol as SLM [57]. Using a voltage of 10 V and extraction time of 10 min, recoveries between 55-100 % were achieved. 1-octanol is still the most used SLM solvent for EME of acidic drug compounds [71].

EME of hydrophilic drug molecules ( $\log P < 2$ ) is more challenging due to lower partitioning into the SLM. In the earliest work on hydrophilic basic drugs [60], twenty different basic drug substances with  $\log P$  between -1.3 and 5.7 were extracted from water samples. Using pure NPOE as SLM, the hydrophilic drugs were not extracted. By adding 50 % (w/w) of the ionic carrier DEHP to NPOE, hydrophilic analytes were extracted with recoveries ranging from 7-71%, but the SLM was not capable of simultaneous extraction of hydrophobic drug molecules. The SLM chemistry was optimized by adding 25 % TEHP and reducing DEHP to 25 %, promoting extraction of both hydrophilic and hydrophobic drug molecules, but with lower overall recoveries. DEHP is still the most used carrier for EME of hydrophobic drugs [89-91], albeit the SLM may be somewhat unstable and prone to excessive current. More recently, EME of six hydrophobic drugs (metaraminol, benzamidine, sotalol, phenylpropanolamine, ephedrine, and trimethoprim) from human plasma using 100 % of the non-ionic carrier bis-2-(ethylhexyl) hydrogen phosphite as SLM demonstrated very good stability and lower current profiles than observed for ionic carriers [92].

### **1.3.5.2 Environmental pollutants**

The first publication on EME from environmental water samples focused on degradation products of nerve agents. Methylphosphonic acid, ethyl methylphosphonic acid, isopropyl propyl methylphosphonic acid, and cyclohexyl methylphosphonic acid were spiked to 1  $\mu\text{g/mL}$  level in real river samples and were extracted with 1-octanol as SLM. After 30 min extraction at 300 V, recoveries ranged between 40-57 %. It was demonstrated that the matrix components in the river samples had detrimental effects on the recovery of the target analytes, making it necessary to dilute the sample 10:1 to circumvent this problem [93].

Following the aforementioned study, EME was used to isolate four chlorophenols (4-chlorophenol, 2,4-dichlorophenol, 2,4,6 trichlorophenol and pentachlorophenol (PCP)) from spiked alkalized seawater samples using 1-octanol as SLM and a voltage of 10 V [66]. After 10 min of extraction, recoveries of 74 % and enrichment factors of 23 were achieved for PCP. Further method validation was performed using PCP, demonstrating comparable linearity, reproducibility, and limit of detection (LOD) to existing techniques.

### **1.3.5.3 Metals and metalloids**

The first EME paper on the extraction of metals was performed on lead ions in 2008 [94]. In this feasibility study, Pb<sup>+2</sup> ions were extracted from three different sample matrices (urine, human serum, and amniotic fluid) in a HF-format using toluene as SLM. EDTA was added to the acceptor phase after extraction to complex the lead ions in order to analyze with capillary electrophoresis with UV-detection (CE-UV). The volume ratio between donor and acceptor solution resulted in a pre-concentration factor of 557 after 15 minutes of extraction, and with a LOD of 0.019 mg/L.

Following this feasibility study, more work has been done on metal extraction, such as uranium [95], thorium [96], mercury [97], chromium [98], and arsenic [99] from water samples. The addition of carriers to the SLM is sometimes necessary to facilitate transfer into the SLM, due to the low solubility of metals in pure organic solvents [100].

The scientific research on EME of metals remains active and represents an important branch outside bioanalysis. Due to the hydrophilic nature of metal ions, the development of new SLM solvents and additives will be important for further growth within this branch of EME. These discoveries will also be beneficial for EME of hydrophilic drug compounds.

### **1.3.5.4 Peptides**

EME of peptides was tested for the first time in 2008 [74]. In this feasibility study, three angiotensins, three neurotensins, and one tripeptide were extracted from spiked water samples using 1-octanol mixed with 15 % DEHP as SLM, a voltage of 50 V, and extraction time of 5 min. Recoveries varied, but for angiotensin 3, EME provided a recovery of 61% and an enrichment factor of 9.8. Following this study, EME was performed on angiotensins from acidified human plasma samples and combined with LC-MS analysis [101]. The complexity of human plasma resulted in lower recoveries (25-43 %) compared to water samples, but acceptable linearity ( $r^2=0.989$ ) and RSD (5.6-11.6 %) were achieved, and detection limits were at the pg/mL level. In 2015, extraction of peptides based on their isoelectric point was achieved [102], demonstrating the unique potential of EME in this area.

While EME for peptides is a very interesting direction, it is still in its beginning and further investigation is incentivized. The hydrophilicity of most peptides demands the addition of carrier to the SLM to facilitate penetration and transfer to the acceptor solution. However, this also leads to trapping of peptides within the SLM resulting in lower recoveries. Furthermore, the extraction of peptides with chain-lengths over 13 amino-acids has proven to be challenging [103]. Thus, further studies on SLM chemistry is needed to make EME a viable method for peptide isolation [104].

### 1.3.6 EME towards the future

At the start of this thesis in 2016 around 150 scientific publications related to EME had been published. In 2020, this number has increased to roughly 300 publications with contributions from research groups in Europe, Asia, and Australia. This illustrates the continued and increasing interest and activity on EME in academia. However, EME is still not utilized in the industry or routine laboratories. For EME to be implemented in these areas, the development of core applications that highlight the advantages and uniqueness of EME is required. Furthermore, the need for commercial equipment and a more theoretical understanding of the extraction processes are vital in this pursuit.

In 2016, no commercial equipment was available and EME was performed with laboratory-made equipment. The lack of commercial equipment makes comparisons of results obtained with different devices and standardization more challenging. In 2021 a commercial EME device is expected to be launched by a Norwegian company (<https://www.etn-eme.com>), which will hopefully improve upon these issues.

To develop core applications for EME, it is necessary to demonstrate EME's advantages and competitiveness compared to other existing sample preparation techniques. The core feature of EME is the electrical field, and this has been demonstrated to give rapid extraction kinetics [105, 106] and analyte recoveries of a 100 % [54, 59]. Excellent sample clean-up from complex matrices [66, 88] has been demonstrated, and a high degree of selectivity can be achieved as a function of the SLM and the electrical field [60, 107]. In addition, the minimal use of organic solvent [71] and simple equipment make EME a green and low-cost principle [108]. Most of the work on EME up to this thesis had been performed with samples and compounds related to bioanalysis of pharmaceuticals and where existing sample techniques are successful and preferred. Thus, only a few core applications displaying EME's uniqueness had been developed, where the advantages of EME are much higher than the disadvantages of replacing existing methods [82, 102, 109]. As such, there is an incentive to develop new application principles and investigate EME's applicability in other areas of analytical chemistry.

In order to give EME a proper scientific anchor, a lot of activity has been directed towards expanding the theoretical knowledge about the extraction processes and experimental conditions. In the literature up to the start of this thesis, investigation on experimental conditions, such as SLM composition [110-112], pH conditions [72, 113], voltage [51, 114], and convection [115, 116], had provided useful insights towards optimization of EME performance. In addition, the development of mathematical models and equations had increased the fundamental understanding of the extraction processes in EME [47, 70, 117]. However, more investigation into SLM composition, pH conditions, and fundamental aspects is necessary to improve the foundation of EME as a sample preparation platform. An exploration into new SLM solvents, additives, and materials could expand the applicability of EME

towards new compound classes and application areas. Complete control over pH conditions will result in a more stable extraction system, give more predictability, and provide higher selectivity.

## 2 Aim of the study

The aim of this study has been to expand upon the theoretical understanding of EME and to develop applications in new areas, in order to move the concept further towards bioanalytical laboratories and expand the analytical scope of the technique. The major focus has been on fundamental aspects regarding EME of hydrophobic basic analytes under different pH conditions, investigate the applicability of EME for sample purification, and simplifying the extraction setup. In line with the demand for high throughput, the development of multi-well EME systems has been an underlining factor in this work. The following are the major areas that have been investigated in this thesis:

- Effects of buffers and pH in EME of hydrophobic basic analytes (**paper I, V**)
- Relationship between extraction recovery and  $pK_a$  and  $\log P$  for hydrophobic basic analytes (**paper V**)
- Optimization of extraction parameters for EME under stagnant conditions (**paper III**)
- EME in “matrix removal mode” for selective removal of matrix components (**paper II, IV**)
- EME from highly concentrated analyte solutions (**paper IV**)

## 3 Results and discussion

In the following text, results from **paper I-V** are presented and discussed. Only key findings are included and readers are referred to the individual papers for further details.

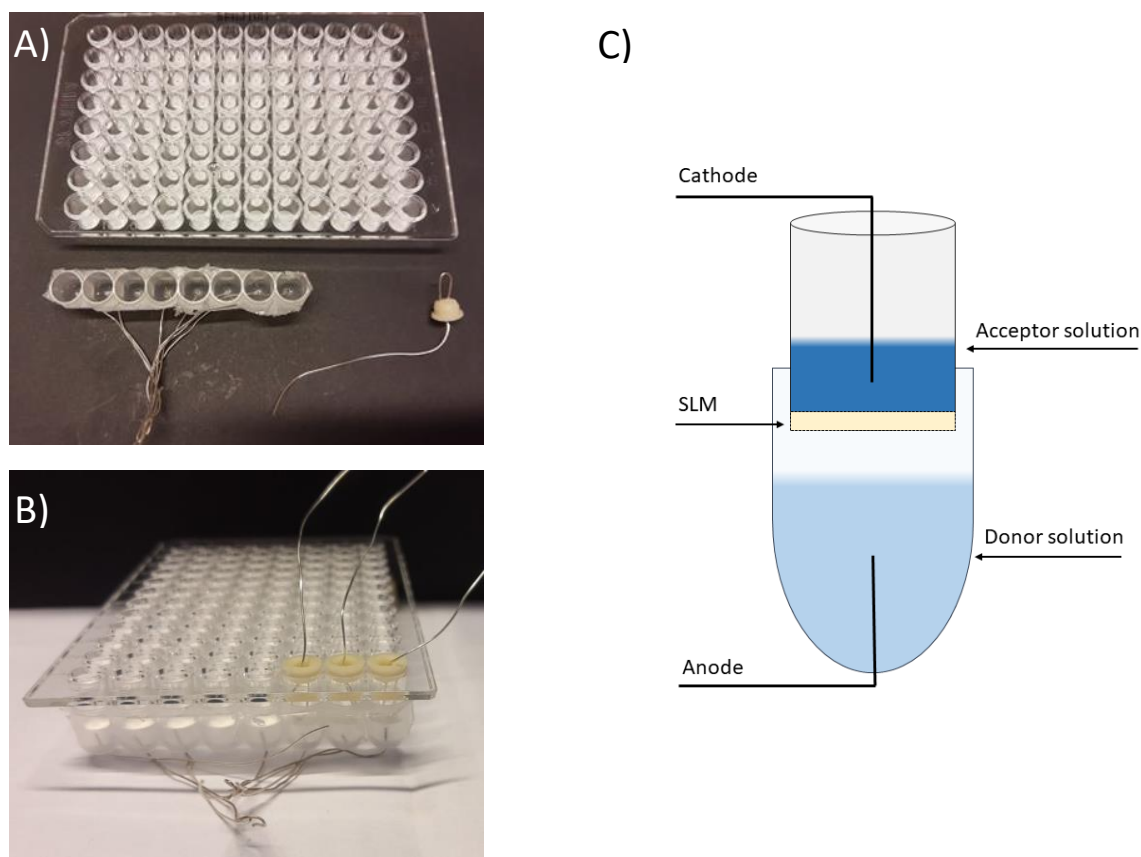
### 3.1 EME configurations and experimental conditions

#### 3.1.1 Parallel EME (Pa-EME)

In **paper I**, EME was performed in a parallel format. The equipment is presented in Figure 9. A row of eight wells from an Agilent 96-well polypropylene plate was cut out and served as compartment for the donor solution. Each well was punctured with a 1.0 mm syringe needle and a 1.0 mm silver electrode, functioning as the anode, was inserted and glued in place. A 96-well Multiscreen-IP filter plate with 100  $\mu\text{m}$  thick polyvinylidene fluoride (PVDF) membranes (pore size: 0.45  $\mu\text{m}$ ) was used to make the SLM and as compartment for the acceptor solution. A 1.0 mm silver electrode glued to a plastic septa seal was fastened to the top of the 96-well filter plate and functioned as cathode.

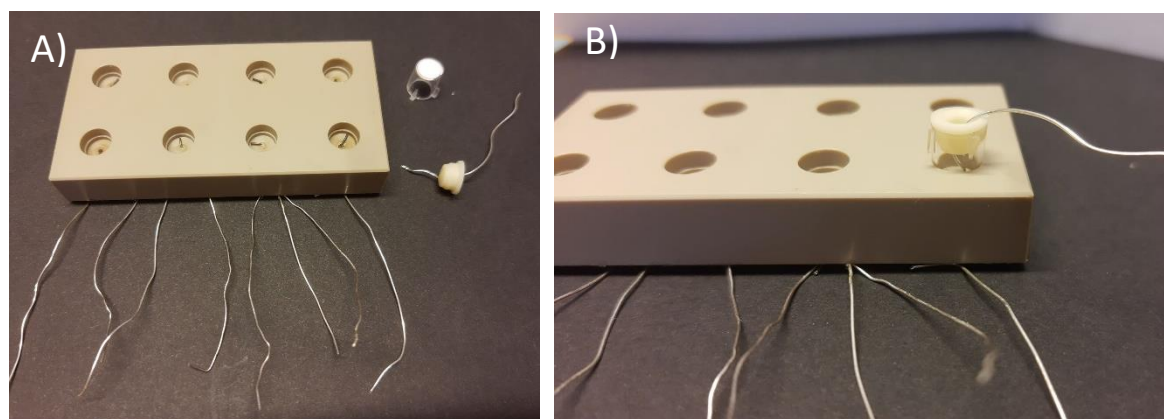
Preparation for EME was performed by pipetting 200  $\mu\text{L}$  of a buffer solution spiked with five basic analytes into a compartment of the donor plate. Subsequently, 3  $\mu\text{L}$  NPOE was pipetted on to the membrane of the filter plate, establishing the SLM. 100  $\mu\text{L}$  of a buffer solution was pipetted into the acceptor compartment and the cathode was inserted. The plates were clamped and transferred to an agitator board (Heidolph, model: Vibramax 100). The electrodes were connected to a DC power supply (Delta Elektronikca BV, model: ES 0300-0.45) and electrokinetic migration was initiated by simultaneous application of agitation (900 rpm) and voltage (20-80 V). After 10 minutes of extraction, the acceptor solution was transferred to a vial and analyzed by high performance liquid chromatography with UV detection (HPLC-UV).





**Figure 9:** Equipment used for Pa-EME: A) 96-well filter plate, sample plate, and electrode glued to plastic septum, B) assembled setup, C) schematic illustration of setup.

The equipment for EME under stagnant conditions (**paper III**) is depicted in Figure 10. A laboratory-made 8-well plate of polyoxymethylene with four different well depths served as a compartment for the sample solution. A 0.5 mm hole was drilled in the bottom of each well and a 0.5 mm silver electrode (anode) was inserted and fixed with glue. 3.0  $\mu\text{L}$  NPOE was pipetted onto the membrane of a single well cut out from a 96-well filter plate, and allowed to immobilize. Subsequently, 50  $\mu\text{L}$  of acceptor solution was transferred to the compartment of the single well, and a 0.5 mm silver electrode (cathode) glued to a plastic septa seal was placed in contact with the acceptor solution. EME was initiated by applying voltage (0-100 V) and performed with varying extraction times (2-60 min). After extraction, the acceptor solution was transferred to vials and analyzed by HPLC-UV (buffer solutions) or LC-MS (whole blood samples).



**Figure 10:** Equipment used for stagnant EME. A) Sample plate, single filter well, and electrode. B) Assembled setup.

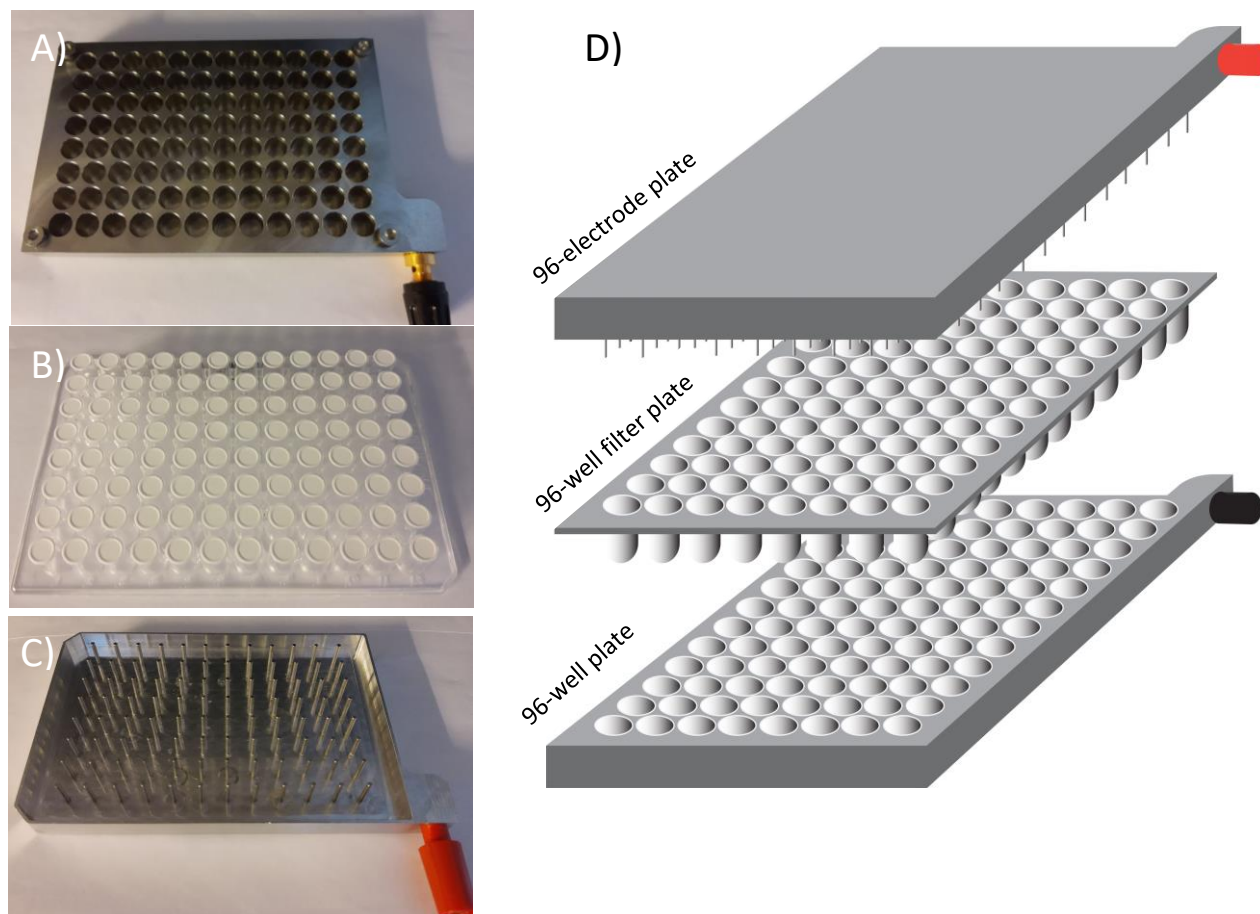
### 3.1.2 96-well EME

Equipment for EME in 96-well format is depicted in Figure 11. A conducting laboratory-made 96-well plate of stainless steel (A), with the same dimensions as the Agilent 96-well polypropylene plate, was utilized as a compartment for the sample (**paper V**) or waste solution (**paper II** and **IV**). The same 96-well PVDF filter plate (3.1.1) was also used in this format (B). A laboratory-made aluminum plate with 96 rods was used as lid and electrodes (C).

The 96-format was performed in two different modes; *extraction mode* and *removal mode*. In removal mode (**paper II** and **IV**), 200  $\mu\text{L}$  of 10 mM NaOH waste solution was transferred to a well in the conducting 96-well plate. Then 3.0  $\mu\text{L}$  of A336/1-octanol (**paper II**) or A336/1-nonanol (**paper IV**) was pipetted on the filter membrane, and allowed to immobilize within the pores. Finally, 100  $\mu\text{L}$  of sample solution was transferred into a well of the 96-membrane plate, and all three plates were clamped and fastened to an agitator. The 96-well plate and the 96-rod plate were connected to an external DC-power supply, functioning as anode and cathode, respectively. Extraction was performed with a voltage of -100 to +100 V (**paper II**) or 0 to +5 V (**paper IV**), and agitation of 900 rpm. After EME, the sample solution was collected and analyzed by Taylor dispersion analysis (TDA) (**paper II**) or by UV-spectrophotometry (**paper IV**).

In extraction mode (**paper V**), the conducting 96-well plate was used as a compartment for the sample solution. First, 200  $\mu\text{L}$  buffer solution spiked with twelve basic analytes was transferred to a well in the 96-well plate. The SLM was created in the same manner as in removal mode but with 3.0  $\mu\text{L}$  NPOE as the organic solvent. Finally, 100  $\mu\text{L}$  of acceptor solution was transferred to the well of the 96-membrane plate, before all plates were clamped. In this setup, the 96-well plate was used as the anode

and the 96-rod plate as the cathode. Extraction was performed with 100 V, and after 10 minutes, the acceptor phase was removed and analyzed by HPLC-UV.



**Figure 11:** Equipment used for EME in a 96-well format. A) 96-electrode plate, B) 96-well filter plate, C) 96-well plate, D) schematic illustration.

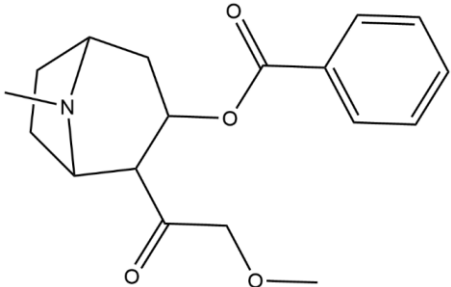
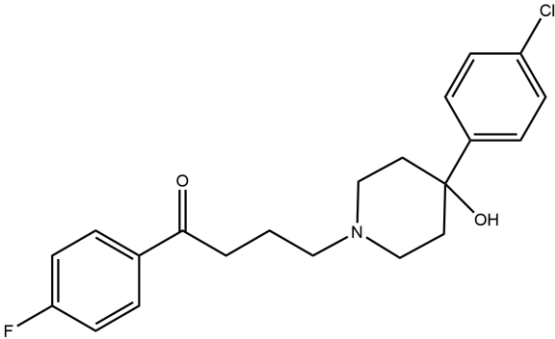
## 3.2 Model analytes

The model analytes used in this thesis can be divided into four categories; small molecular basic drugs (**paper I, III and IV**), fluorescent dye (**paper II**), ionic detergent (**paper IV**), and proteins (**paper II**).

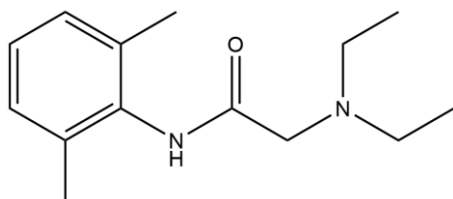
### 3.2.1 Small molecular basic drugs

The selection of the model analytes was based on their physicochemical properties, focusing on the dissociation constant ( $pK_a$ ) and the hydrophobicity ( $\log P$ ). In **paper I and III**, EME was performed on five hydrophobic basic drugs with a  $pK_a$  and  $\log P$  range of 7.84-10.00 and 2.19-4.15, respectively. In **paper IV**, seven additional hydrophobic basic drugs were added to expand the  $pK_a$  and  $\log P$  range. EME of these model analytes have been previously investigated in the literature [35], allowing for easier comparison of results and observations achieved in this thesis with previous work.

**Table 3.1:** Overview of selected small molecular basic drugs and their molecular structure,  $pK_a$  value, and  $\log P$ .

Analyte	Structure <sup>a</sup>	$pK_a^b$	$\log P^b$	Paper
Cocaine		8.97	2.28	<b>V</b>
Haloperidol		8.04	3.76	<b>I, III, V</b>

Lidocaine

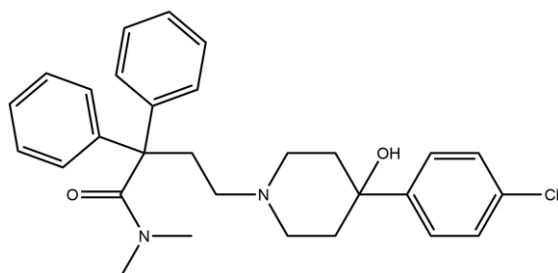


7.96

2.20

**V**

Loperamide

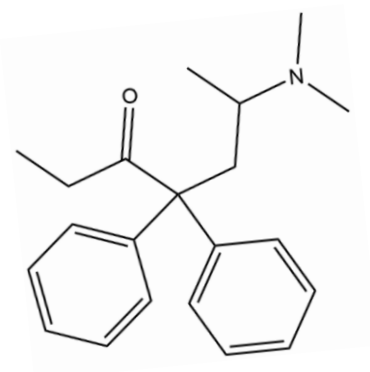


7.76

4.15

**I, III, V**

Methadone

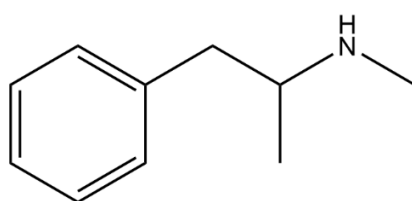


9.05

3.93

**I, III, V**

Methamphetamine

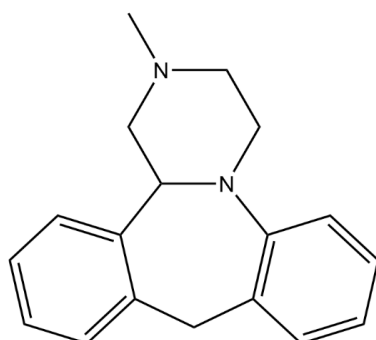


10.38

2.20

**V**

Mianserin

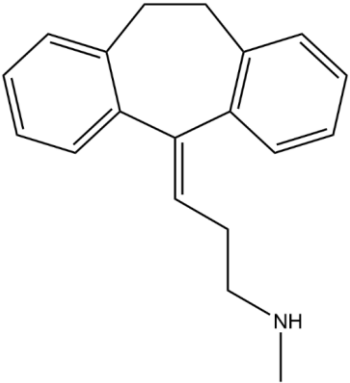
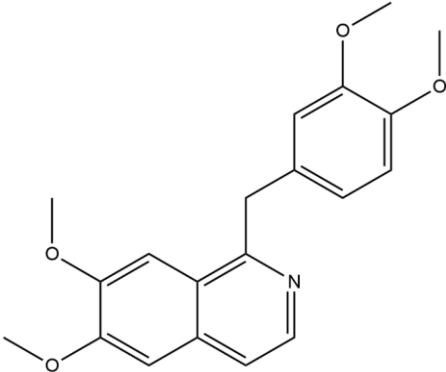
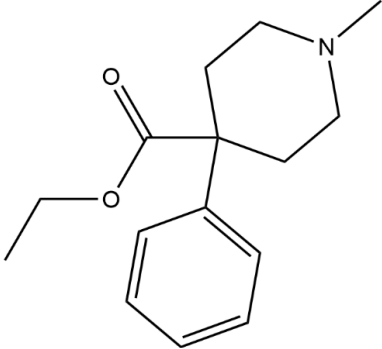
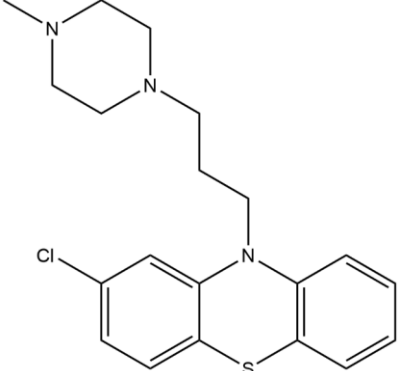


8.26

3.83

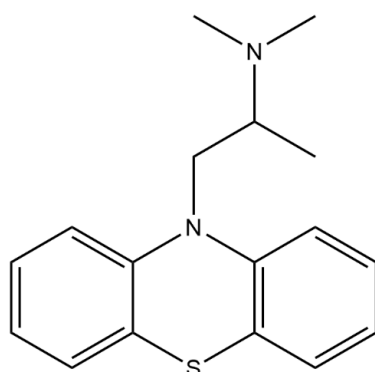
**V**

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Nortriptyline		10.00	3.97	I, III, V
Papaverine		6.32	2.93	V
Pethidine		7.84	2.19	I, III, V
Prochlorperazine		7.66	4.64	V

---

Promethazine



8.98

4.89

V

<sup>a</sup> Structures made in Chemdraw 19.1 <https://perkinelmer.flexnetoperations.com/>

<sup>b</sup> pK<sub>a</sub> and log P values obtained from Scifinder (www. Scifinder.com, accessed 06.06.2020)

### 3.2.2 Fluorescent dye

The fluorescein derivate fluorescein isothiocyanate (FITC) was selected as matrix component in **paper II** based on its frequent use [118], and its physiochemical properties. Structure, pK<sub>a</sub>, and log P value is found in Table 3.2.

**Table 3.2:** Structure, pK<sub>a</sub> and log P of fluorescein isothiocyanate (FITC).

Analyte	Structure <sup>a</sup>	pK <sub>a</sub> <sup>b</sup>	log P <sup>b</sup>	Paper
Fluorescein isothiocyanate		6.40	4.70	II

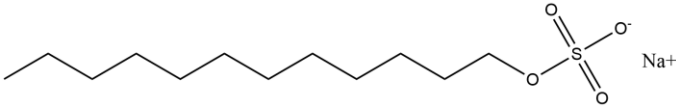
<sup>a</sup> Structures made in Chemdraw 19.1 <https://perkinelmer.flexnetoperations.com/>

<sup>b</sup> pK<sub>a</sub> and log P value obtained from Scifinder (www. Scifinder.com, accessed 06.06.2020)

### 3.2.3 Ionic surfactant

The anionic surfactant sodium dodecyl sulfate (SDS) was selected as matrix component in **paper IV** based on its frequent use in and high solubility in aqueous solution [119]. Structure and log P value is found in Table 3.3.

**Table 3.3:** Structure and log P value of sodium dodecyl sulfate (SDS).

Analyte	Structure <sup>a</sup>	pK <sub>a</sub>	log P <sup>b</sup>	Paper
Sodium dodecyl sulfate		-	1.60	IV

<sup>a</sup> Structures made in Chemdraw 19.1 <https://perkinelmer.flexnetoperations.com/>

<sup>b</sup> log P value obtained from Scifinder (www. Scifinder.com, accessed 06.06.2020)

### 3.2.4 Proteins

Proteins represented targets of interest during EME in “matrix removal mode”. Selection of model proteins were made to represent variety in terms of molecular weight and isoelectric point. Relevant physicochemical properties for the selected proteins are found in table 3.4.

**Table 3.4:** Relevant physicochemical properties for the selected model proteins.

Protein	M <sub>w</sub> <sup>a</sup>	pI <sup>b</sup>	AA <sup>c</sup>	Hydrophobicity index <sup>d</sup>	Paper
Cytochrome C (horse heart)	12 kDa	10.5	105	-0.354	II
Human serum albumin	66.5 kDa	5.8	609	-0.840	II
Myoglobin (bovine heart)	17 kDa	6.7, 7.2	154	-0.381	II

<sup>a</sup> Molecular weight and <sup>b</sup> isoelectric point [120-122]

<sup>c</sup> Number of amino acids

<sup>d</sup> ExPASy (<https://web.expasy.org/protparam>, accessed 24.07.20)



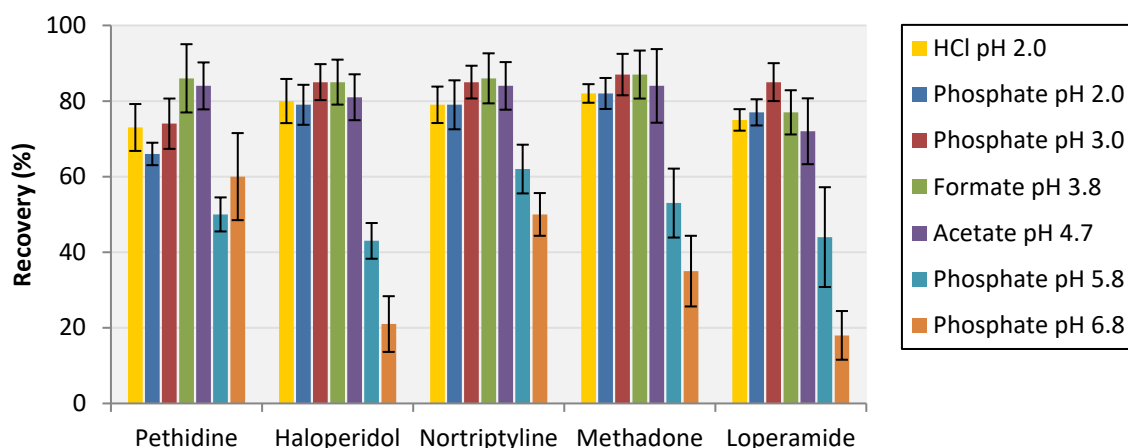
### 3.3 Investigation of buffer and pH selectivity in EME

Optimization and control of all major operational parameters in EME are essential in order to achieve efficient and reproducible extraction. Prior to this thesis, control of pH in the donor and acceptor solution was usually achieved with acids (e.g HCl, FA) or bases (e.g NaOH). The use of buffers was at that time only investigated in a few cases [123, 124], and therefore a more systematic investigation was performed in this thesis. Primarily, buffers are more resistant to pH changes caused by electrolysis, making it possible to increase extraction time and current, without losing control over donor and acceptor pH. Furthermore, and unlike strong acid and bases, the ionic concentration and buffer strength of a buffer solution can be increased or decreased without altering the pH. Lastly, buffer systems offer pH-control within a large pH-window (pH 2-12), making it possible to fine-tune the pH for selective extraction based on the  $pK_a$  values of the target analytes.

#### 3.3.1 Extraction performance with buffer composition

##### 3.3.1.1 Extraction recovery

In **paper I**, a systematic investigation of the effects of buffers was performed by extracting five hydrophobic basic analytes from either phosphate, formate or acetate buffer solutions with varying pH (2.0-6.8) and buffer strength (10-50 mM). Extractions from solutions of 10 mM HCl were also performed for comparison. With pH between 2.0-4.7 extraction performance (recovery and reproducibility) was mostly independent of the buffer type and buffer strength, and was comparable to results obtained from 10 mM HCl samples (Figure 12). At  $pH \geq 5.8$ , an unexpected drop in recovery and reproducibility was observed. The performance drop was initially unclear since all analytes should be completely ionized at pH 5.8 (discussed in section 3.3.2). However, the performance drop was to some degree correlated with the analytes  $pK_a$  values, as recoveries for nortriptyline ( $pK_a = 10.47$ ) were considerably higher than for haloperidol ( $pK_a = 8.05$ ).



**Figure 12:** Recoveries obtained after EME of five basic drugs as function of pH in donor and acceptor solution (**paper I**). Only results using buffer strength 10 mM are shown. Errors bars: SD

### 3.3.1.2 Extraction current

The extraction current is an important parameter in terms of system stability and performance. The current is a picture of the total flux of analyte and background ions across the SLM. Since the concentration of background ions normally is much higher than target analytes, their affinity towards the SLM should be low. Due to the more organic nature of buffer components used in **paper I-III** and **V**, compared to HCl and NaOH, it was initially hypothesized that this could lead to a higher flux of background ions across the SLM, and thus a higher current. This would require a reduction of voltage in order to avoid system instability, potentially reducing the optimal voltage for the extraction of the target analytes. Extraction current was measurement at a frequency of 12 Hz, using a multimeter that was coupled in serial with the extraction device. In **paper I, III, and IV** current profiles at optimum extraction voltage were characterized by an initial spike (6-20  $\mu\text{A}$ ), followed by a small and stable current (2-5  $\mu\text{A}$ ). In **paper I**, current measurements were performed with buffer concentrations of 10 mM or 50 mM. No difference in extraction currents was observed at 50 mM compared to 10 mM, indicating that the flux of buffer ions across the SLM is low and that steady-state flux is reached at buffer concentration  $\leq 10$  mM.

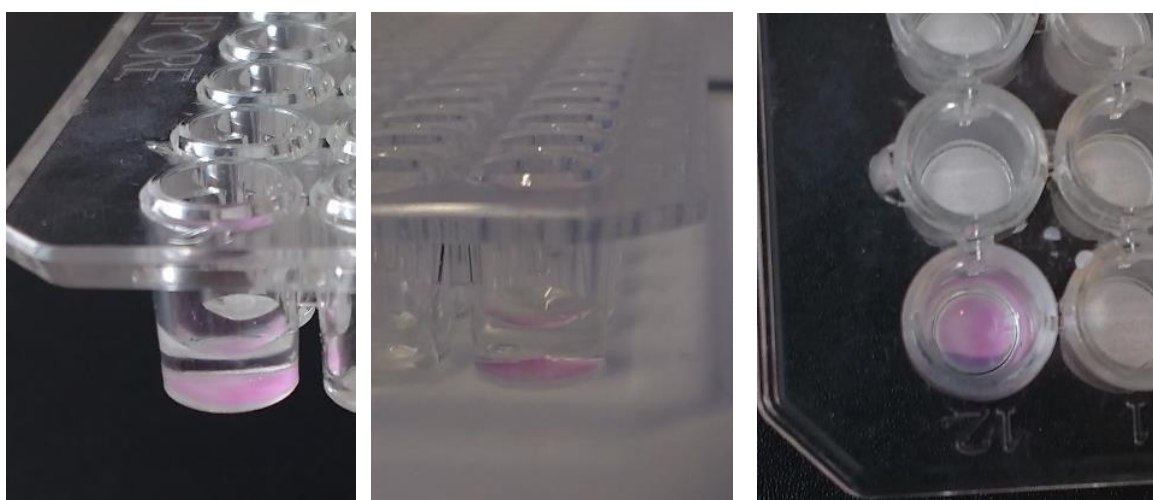
### 3.3.1.3 pH stability

One key reason to use buffer is to stabilize the pH of the donor and acceptor solution. It has previously been shown that pH can be stabilized by using acids in high concentrations (e.g 500 mM FA, 100 mM HCl) [113]. However, this limits the pH flexibility of the system. In **paper I**, the pH stability of phosphate, formate, and acetate buffer were evaluated. The pH of the donor and acceptor solution was measured by a digital pH-meter prior to extraction and after 10 minutes of EME with a voltage of 50V. Measured

pH shift after EME was  $\leq 0.1$  and similar results were obtained independent of buffer strength (10 mM and 50 mM). The low extraction currents (Section 3.3.1.1) obtained when using buffers, thus limiting electrolysis, further provided pH stability. Since optimal extraction performance in **paper I, III, and V** were obtained at low extraction currents, a stress test of the buffer systems using excessive currents and prolonged extraction time was not performed. For most EME applications, however, the results obtained in **paper I, III, and V** indicate a high degree of system stability using buffer systems as pH modification.

### 3.3.2 Acceptor boundary layer

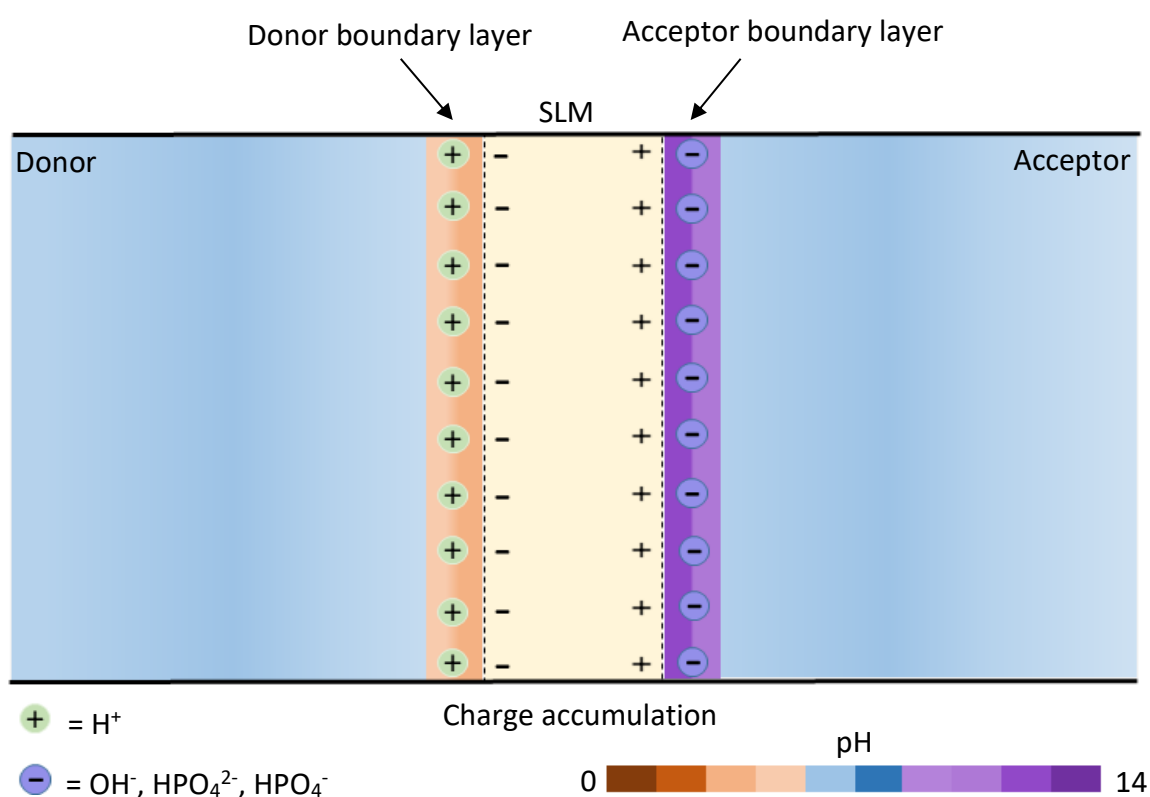
The decline in recovery when sample and acceptor pH was above 5.8 (Figure 12) was hypothesized to be caused by the formation of a boundary layer with elevated pH conditions in the interface between the SLM and acceptor solution. Charged analytes migrating through the SLM towards the acceptor solution will deionize in contact with the boundary layer, resulting in back-extraction into the SLM and reduced recoveries. To visualize the presence of elevated pH conditions, EME was performed with the addition of the pH indicator phenolphthalein in the acceptor solution. Phenolphthalein is colorless at  $\text{pH} \leq 8$  and converts to a pink/red color at  $\text{pH} 8-10$ . After 60 seconds of extraction using phosphate buffer pH 6.8 as donor and acceptor, a clear pink color was visible close to the SLM, while the bulk acceptor phase remained colorless throughout the extraction (Figure 13). This observation substantiates the hypothesis of elevated pH conditions in the acceptor boundary layer. It is likely that a similar boundary layer with decreased pH conditions is formed in the interface between the donor phase and the SLM, promoting ionization of the analytes, however, this was based on theoretical assumptions and was not visualized experimentally. Boundary layers give a plausible explanation as to



**Figure 13:** Visualization of elevated pH conditions in the acceptor boundary layer using phenolphthalein as pH indicator (**paper I**).

why pH in the donor solution is less critical for efficient extraction, in contrast to the acceptor solution where pH 3-4 units below  $pK_a$  are necessary for the extraction of basic analytes.

A theoretical model explaining the formation of the boundary layer was proposed. It has previously been shown that the SLM possesses both resistive and capacitive properties and this was further verified in **paper I** by impedance measurements. The measured relationship between frequency and impedance correlated with a parallel coupling of a resistor and capacitor. During EME of basic analytes positive charge accumulates in the SLM on the acceptor side and correspondingly negative charge accumulates on the donor side. Electroneutrality is maintained by electrolyte stacking of negatively charged ions in the acceptor solution;  $OH^-$ ,  $HPO_4^{2-}$  and  $H_2PO_4^-$ , resulting in an increase in pH (Figure 14). The exact mechanism was not fully understood, and could also involve flux of these ions across the SLM. Tuning the experimental parameters, such as buffer strength, agitation, and voltage did not affect the formation of the boundary layer. The formation of boundary layers is most likely inherent to the EME procedure, but in **paper II-IV** where EME was performed with low pH in the acceptor solution, the effects were not noticeable. Nonetheless, it is clear that the elevated pH conditions in the acceptor boundary layer represent a major obstacle towards the objective of pH selective extraction of analytes based on their  $pK_a$  values.



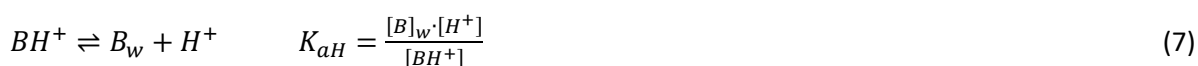
**Figure 14:** Illustration of suggested mechanism for the formation of donor and acceptor boundary layer at the SLM interface. The SLM function as a capacitor, causing electric charge to accumulate in at the intersections of the SLM. To establish electroneutrality, ions in the sample and acceptor accumulate at the SLM interface, leading to altered pH conditions compared to the bulk solutions.

### 3.3.3 Relationship between pK<sub>a</sub> and log P

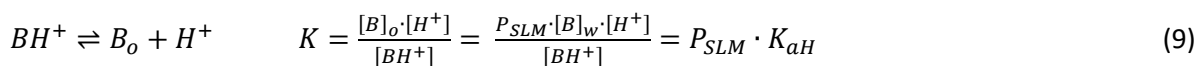
In **paper V**, a theoretical model to predict EME performance of hydrophobic basic analytes based on the relationship between pK<sub>a</sub> and log P was developed. Twelve basic analytes with varying pK<sub>a</sub> (6.32-10.38) and log P (2.19-4.64) values were extracted and evaluated according to the model.

#### 3.3.3.1 Theoretical considerations

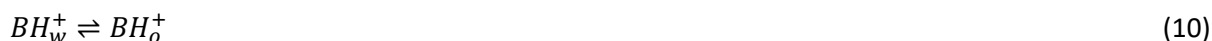
The experimental results in **paper I** demonstrated that extraction performance could not be predicted based on analyte pK<sub>a</sub> and system pH alone. A more accurate model for prediction needs to take in to account the acid-base equilibria occurring within the aqueous phases and the SLM as well as coupled equilibria associated with partitioning between the aqueous phases and the SLM. The following equilibria and corresponding equations represent the base dissociation and partitioning for a given hydrophobic monobasic analyte (Figure 15) :



Here BH<sup>+</sup> and B represent the basic analyte in a charged state and uncharged state, and B<sub>w</sub> and B<sub>o</sub> denote if the analyte is in an aqueous solution (i.e donor and acceptor), or an organic solution (i.e SLM), respectively. Eq.7 describes the base dissociation equilibrium present in the sample/acceptor solution, while Eq.8 describes the partitioning equilibrium of the basic analyte in an uncharged state between the sample/acceptor solution and the SLM. The coupled relationship between the two equilibria at the SLM interfaces can be represented by the following equilibrium and corresponding equations:



In the presences of an extraction potential, BH<sup>+</sup> will partition (electro-assisted) between sample/acceptor solution and the SLM by the following equilibrium:



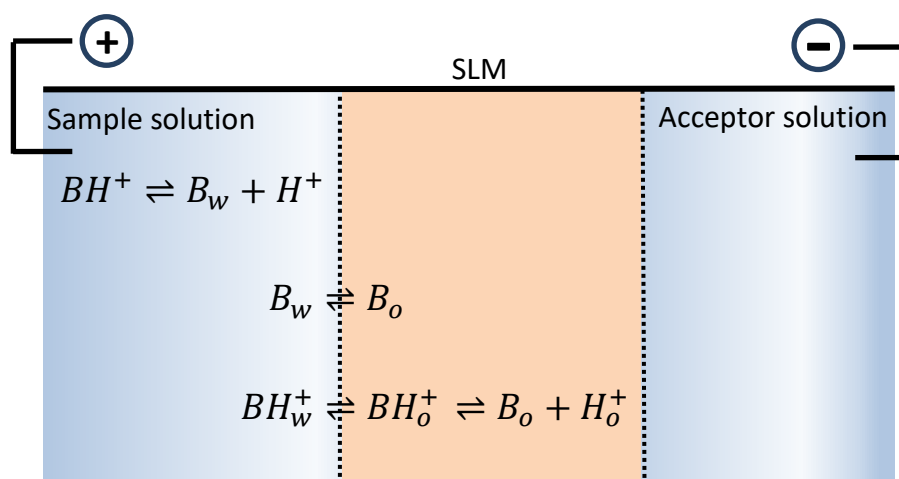
In the SLM, deprotonation of  $BH^+$  is expected to occur to some extent. The extent of deprotonation is dependent on the possible stabilization of  $H_o^+$  by co-extracted ions in the SLM. The base dissociation in the SLM is described by the following equilibrium and corresponding equation:



Considering the coupled relationship between these equilibria, equipartition of a basic analyte (B) between donor/acceptor and the SLM occur according to the following equation:

$$\frac{[B]_o}{[BH^+]_w} = \frac{K}{[H^+]_w} = \frac{K}{10^{-pH}} = \frac{P_{SLM} \cdot K_{aH}}{10^{-pH}} = 1 \Rightarrow pH = pK_{aH} - \log P_{SLM} \quad (12)$$

From Eq. 12, extraction performance for a given basic analyte can be divided into three different kinetic patterns based on the pH conditions. If  $pH \gg pK_{aH} - \log P_{SLM}$ , the basic analyte exists predominantly in the SLM in an uncharged state (B) leading to slow diffusion-based extraction kinetics. Reversely, efficient EME of basic analytes based on electrokinetic mass transfer is assumed to occur if  $pH \ll pK_{aH} - \log P_{SLM}$ . With  $pH \sim pK_{aH} - \log P_{SLM}$  the extraction mechanism will be in a mixed-mode between EME and LPME.



**Figure 15:** Illustration of base dissociation ( $pK_{aH}$ ) and partitioning ( $\log P$ ) equilibria of hydrophobic basic analytes during EME.

### 3.3.3.2 Experimental verification

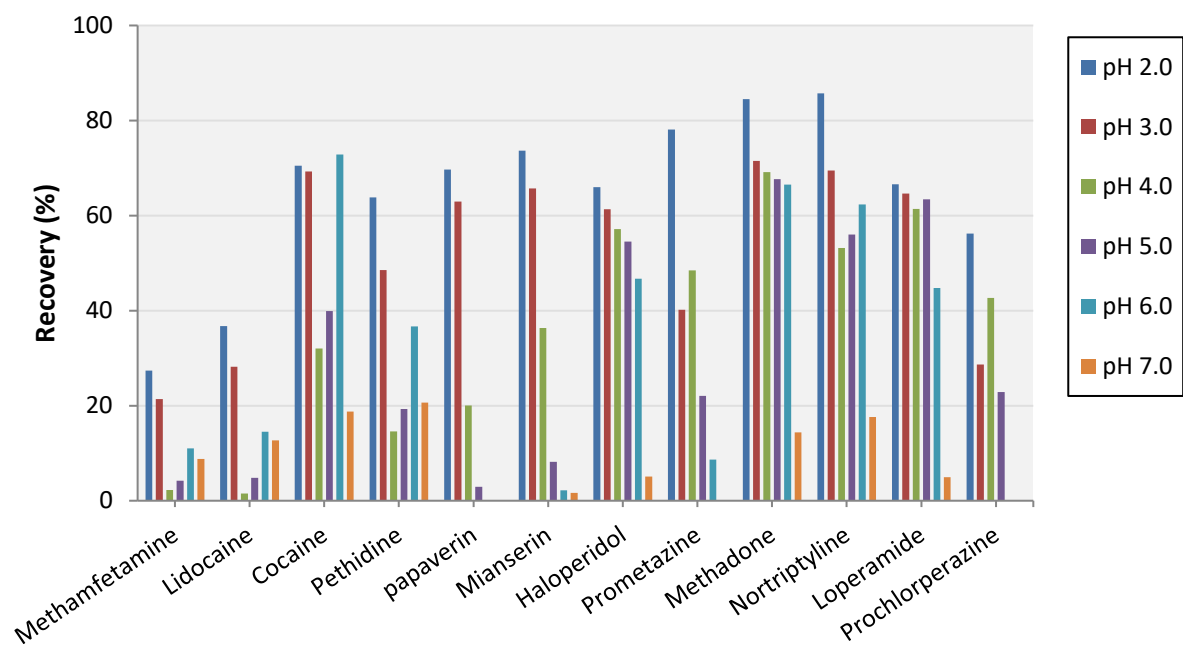
EME of twelve basic model analytes was performed under similar conditions as in **paper I** and with sample and acceptor pH ranging between 2.0-7.0. Physicochemical properties and calculated  $pK_{aH} - \log P$  values are listed in Table 3.5.

**Table 3.5:** Log P,  $pK_{aH}$ , and calculated  $pK_{aH} - \log P$  for the selected hydrophobic basic analytes.

Analytes	log P	$pK_{aH}$	$pK_{aH} - \log P$
Methamphetamine	2.20	10.38	8.18
Cocaine	2.28	8.97	6.69
Nortriptyline	3.97	10.00	6.03
Lidocaine	2.20	7.96	5.76
Pethidine	2.19	7.84	5.65
Methadone	3.93	9.05	5.12
Mianserin	3.83	8.26	4.43
Haloperidol	3.76	8.04	4.28
Promethazine	4.89	8.98	4.09
Loperamide	4.15	7.76	3.51
Papaverine	2.93	6.32	3.39
Prochlorperazine	4.64	7.66	3.02

Considering extraction recoveries of  $\geq 40\%$  to represent efficient extraction, cocaine, nortriptyline, methadone, mianserin, promethazine, papaverine, and prochlorperazine were in accordance with the theoretical model (Figure 16). These analytes were effectively extracted when  $pH \ll pK_{aH} - \log P_{SLM}$ , and less effective as  $pH \sim pK_{aH} - \log P_{SLM}$ . Methamphetamine, lidocaine, and pethidine deviate considerably from the theoretical prediction. This was surprising, as they possessed among the highest  $pK_{aH} - \log P$  values and should, therefore, extract well under the tested pH conditions. The explanation for this was considered to be related to these analytes low log P values. So while the theoretical model was assumed to be valid for analytes with  $\log P_{SLM} > 1$ , the experimental work revealed it to be only valid for analytes with  $\log P_{SLM} > 2-3$ . Furthermore, it was hypothesized that the extraction performance of these three analytes was influenced by deprotonation inside the SLM (Eq. 11) as well as stabilization of the protonated form by ion-pairing with buffer ions inside the SLM. In this situation the analytes are

not influenced by the applied electrical field and extraction time of 10 minutes is suboptimal for efficient extraction performance.



**Figure 16:** Extraction recovery of hydrophobic bases as a function of sample and acceptor pH (**paper V**).



## 3.4 Optimization of EME for purification of samples

To highlight the versatility of EME, the work in **paper II** and **IV** was focused on adapting EME towards purifying sample solutions of certain matrix components. This is highly relevant in many bioanalytical settings, where removal of matrix components from the sample is a necessary step prior to further analysis. EME tuned towards purification has only briefly been investigated [125, 126], and further investigation can potentially expand the applicability of EME in this area. Conceptually, the principle remains the same as in extraction mode, but the main objective is the removal of matrix components from the sample, rather than extracting analyte over to the acceptor solution. Thus, the acceptor solution was considered a “waste solution” in this EME mode. In **paper II**, the concept was investigated with FITC as a matrix component and proteins as targets of interest. In **paper IV**, further investigation was done from highly concentrated samples of SDS, where SDS was considered a matrix compound.

### 3.4.1 Clearance

The efficiency of the purification was evaluated based on the clearance of the matrix component from the sample after EME, as defined by the following equation:

$$\text{Clearance} = \left( 1 - \frac{[\text{matrix component}]_{\text{sample,after EME}}}{[\text{matrix component}]_{\text{sample,before EME}}} \right) * 100 \% \quad (13)$$

#### 3.4.1.1 Sample Conditions

In **Paper II**, initial testing was performed on 100  $\mu\text{L}$  samples of either FITC, myoglobin, cytochrome C, and human serum albumin (HSA) using 50 mM borate buffer pH 9.20 as background ions. Under these pH conditions, FITC was negatively charged, HSA and myoglobin net positively charged and cytochrome C net negatively charged. In the evaluation step, FITC labeled HAS (HSA/FITC) in a reaction buffer (50 mM borate buffer pH 9.20 and 150 mM NaCl) represented a real sample. In **paper IV**, EME was performed on three different concentrations of SDS (0.1 %, 0.5 %, and 1.0 %) with 10 mM NaOH as background ions.

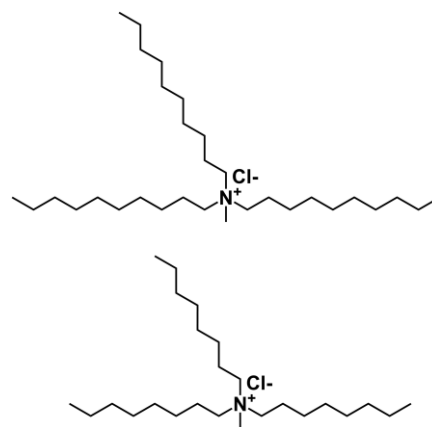
#### 3.4.1.2 SLM

Four different pure organic solvents were tested as SLMs; NPOE, 1-octanol, 1-nonanol, 1-decanol. Besides NPOE, these solvents possess strong hydrogen donor properties, which is ideal for extraction of anionic compounds. However, initial experiments with 1-octanol (**paper II**) and 1-nonanol (**paper IV**) provided suboptimal clearance of the matrix component. Further experiments were conducted with the addition of the ionic carrier Aliquat 366 (A336) to the SLM. A336 is a mixture of  $\text{C}_8$  and  $\text{C}_{10}$  tetra-alkyl ammonium chlorides and facilitates the transfer of anionic compounds into the SLM (Figure 17) [127]. The addition of A336 reduced the electrical resistance of the SLM, requiring a reduction of

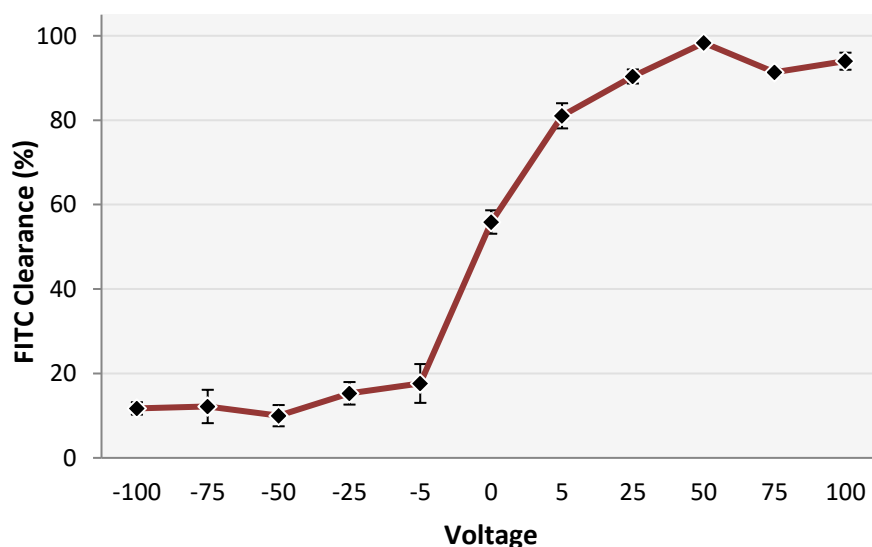
voltage in order to avoid excessive current and system instability. The optimal concentration of A336 in the SLM with regards to clearance and system stability was 0.1 % (w/w) (**paper II**) and 1.0 % (w/w) (**paper IV**).

### 3.4.1.3 Voltage

In **paper II**, FITC clearance with 0.1 % A336/1-octanol as the SLM was initially performed with different applied voltages (-100 – 100 V) (Figure 18). Surprisingly, even at 0 V, a clearance of around 55 % was achieved, demonstrating a clear effect of passive diffusion into the SLM. This was also observed with SDS (**paper IV**), where substantial clearance (dependent on sample concentration) was achieved at 0 V. The presence of A366 in the SLM clearly made partitioning into the SLM, even in charged form, more favorable. However, the application of an electrical field greatly affected the extraction performance, and both extraction kinetics and total clearance increased with voltage compared to zero voltage. For FITC, increased clearance was observed with increasing voltage up to 50 V. Any further increase did not result in higher clearance but caused system instability due to excessive extraction current. Reversing the polarity of the electrical field (negative voltage) also demonstrated the impact of the electrical field and clearance could be limited to 10 % at -50 V. In **paper IV**, the electrical field had less impact on the extraction kinetics but resulted in a significant increase in extraction clearance compared to zero voltage conditions. This demonstrated that partitioning into the SLM was enhanced by the presence of the electrical field. However, due to the high A336 content in the SLM, the voltage was limited to 5 V in order to avoid excessive extraction currents.



**Figure 17:** Structure of Aliquat 366.

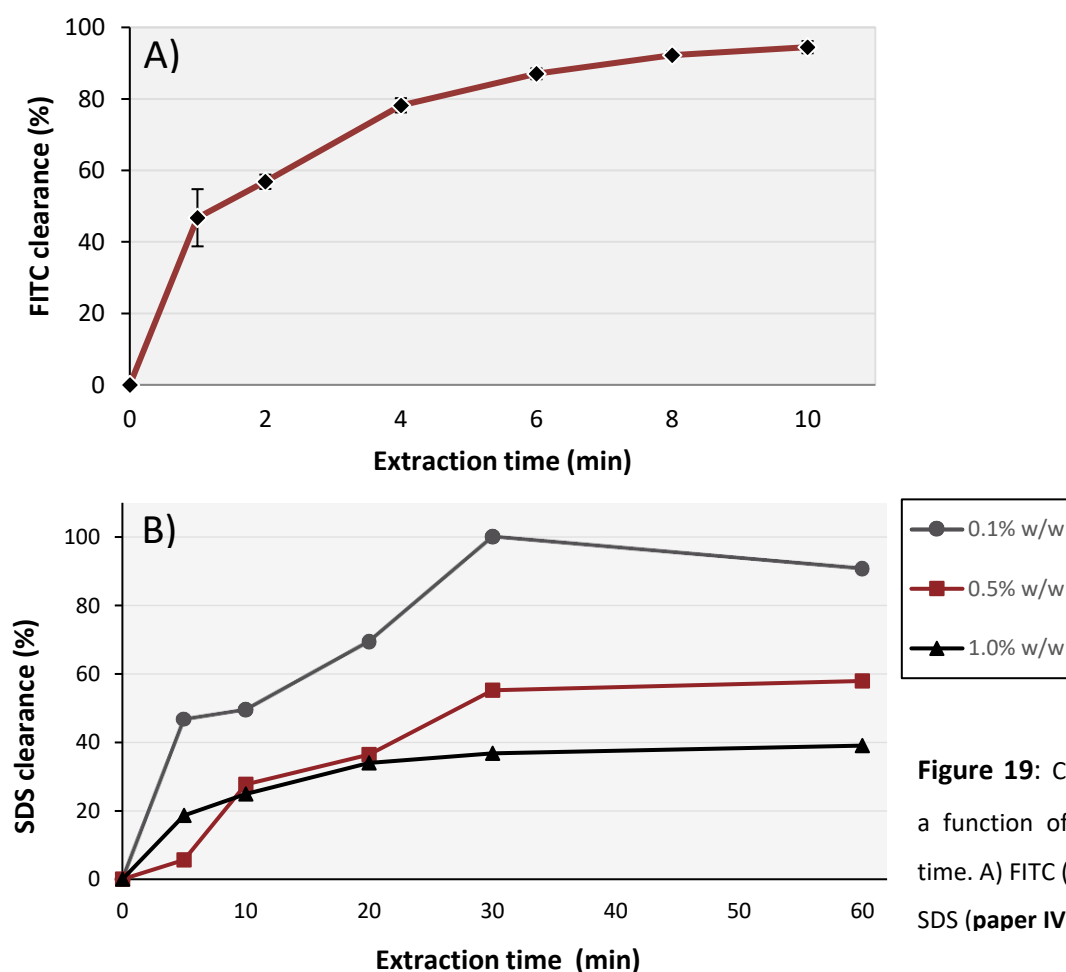


**Figure 18:** FITC clearance as a function of voltage (**paper II**).

### 3.4.1.4 Extraction time

In **paper II**, clearance was evaluated by performing EME for 1, 2, 4, 6, 8, and 10 min (Figure 19 A). Already after 1 min extraction, average FITC clearance reached 47 %, demonstrating rapid kinetics. Further increase in clearance was observed with extended extraction time and after 10 min steady-state conditions were reached, with an average FITC clearance of 98 %. The rapid extraction kinetics in combination with the 96-well format demonstrated the high throughput potential of EME in purification mode.

In **paper IV**, clearance was evaluated by performing EME for 5, 10, 20, 30, and 60 min (Figure 19 B). Similar to the results in **paper II**, extraction clearance after 5 minutes demonstrated very rapid extraction kinetics. Considering the high SDS concentration in the samples, the 20 % clearance achieved from 0.5 % (5 mg/ml) samples after 5 min extraction can be calculated to an average removal flux of  $0.71 \mu\text{g}/\text{min}/\text{cm}^2$ . For comparison, the average removal flux of FITC in **paper II** was calculated to  $0.006 \mu\text{g}/\text{min}/\text{cm}^2$ . With prolonged extraction time clearance increased, and for all concentrations steady-state conditions were reached after 30 minutes extraction. However, after the initial 5-10 minutes, extraction kinetics was considerably slower and 100 % clearance after 30 minutes was only achieved from 0.1 % SDS samples.



**Figure 19:** Clearance as a function of extraction time. A) FITC (**paper II**) B) SDS (**paper IV**)

### 3.4.1.5 Mass balance

The distribution of the target analytes between the sample, SLM, and waste solution after EME was investigated. After the extraction a cloudy white (SDS) and orange (FITC) discoloration of the SLM was observed, leading to the assumption that most of the target analytes were trapped in the SLM. This was further verified in **paper IV**, where no SDS could be detected in the sample or the waste solution after EME of 0.1 % SDS samples. Small amounts of SDS was found in the waste solution after EME from 0.5 % and 1.0 %, but this was considered to be caused by diffusion from the SLM rather than electrokinetic migration. Thus, the waste solution had little impact on the mass transfer of target analytes and functioned primarily as an electrolytic solution to establish electric contact. From highly concentrated (5-10 mg/ml) SDS solutions, the SDS accumulation exceeded the SLMs capacity and impeded further mass transfer. In, **paper II** the accumulation of FITC in the SLM was not considered problematic, as the capacity of the SLM was not exceeded, and thus not limiting the mass transfer. Since the capacity of the SLM was the limiting factor towards complete clearance of SDS, EME purification with extended membrane area or replenishment of the SLM halfway through the extraction was performed. In the former setup, the membrane was extended from 28 cm<sup>2</sup> to 43 cm<sup>2</sup> and the SLM volume was increased to 10 μL. Under these conditions, a clearance of 100 % was achieved from 0.5 % SDS samples with an extraction time of 30 min. In the latter setup, extraction time had to be extended to 120 minutes with replenishment of the SLM after 60 min, in order to get 100 % clearance from 0.5 % SDS samples. Clearance from 1.0 % SDS samples increased in both modified setups, but 100 % clearance could not be achieved. Based on these observations the applicability of the developed EME purification setup using A336 as ionic carrier in the SLM is reduced for highly concentrated solutions, and further investigation in SLM composition is needed to enhance the mass transfer.

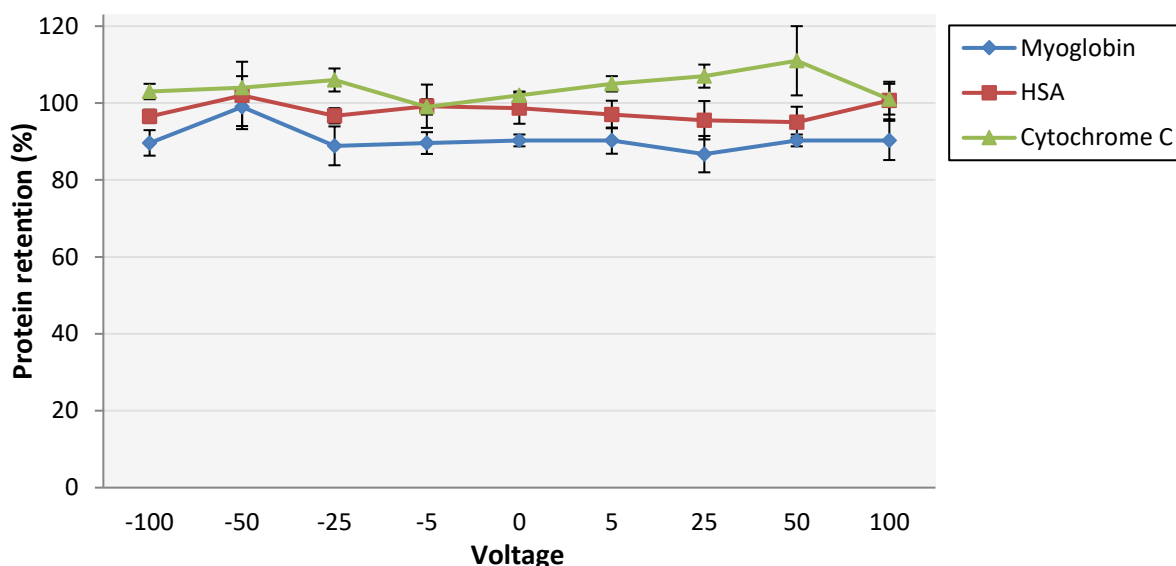
### 3.4.2 Protein retention and functionality

In **Paper II**, the selectivity of EME purification was investigated with three proteins (myoglobin, cytochrome C and HSA) as targets of interest and was evaluated based on how much protein was left in the sample after EME purification (protein retention), as defined by the following equation:

$$\text{Protein retention} = \left( \frac{[\text{Protein}]_{\text{sample,after EME}}}{[\text{Protein}]_{\text{sample,before EME}}} \right) * 100 \% \quad (14)$$

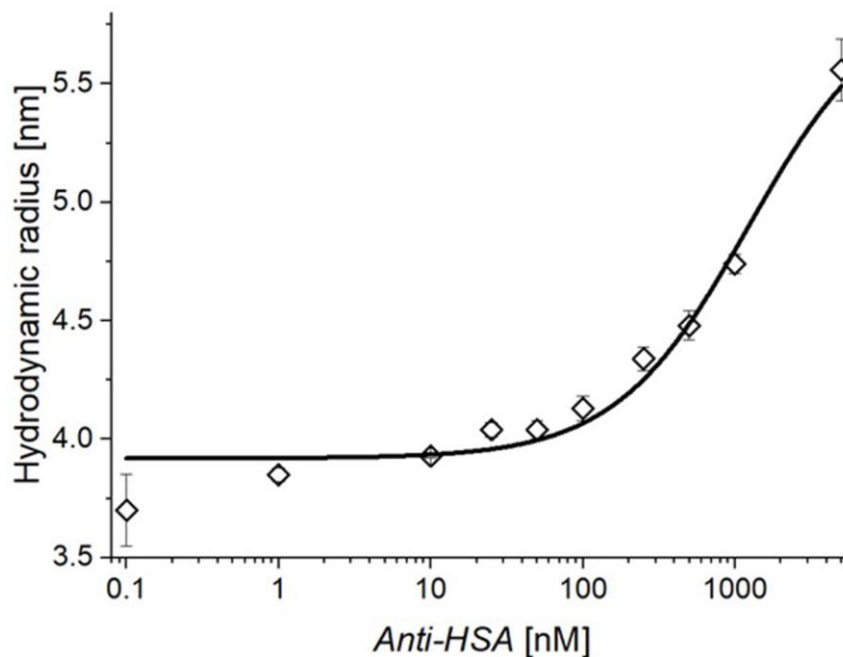
After optimization of FITC clearance, protein retention was evaluated under the same EME conditions. As shown in Figure 20, high protein retention (90-110 %) was achieved for all proteins. No correlation between the polarity of the electrical field and the net charge of the proteins in relation to protein retention was observed. This observation was explained in a follow-up experiment, where equal

volumes of the protein sample and the SLM solvent were mixed in order to measure protein partitioning into the organic solvent. The results demonstrated that the proteins were insoluble in 0.1% A336/1-octanol mixture, making the SLM an effective barrier. This was in agreement with previously published EME work on protein-rich matrices [81]. While this was beneficial in the context of high protein retention, novel SLM compositions are necessary in order for EME to be applicable for protein extraction.



**Figure 20:** Protein retention of myoglobin, HSA and cytochrome C as a function of voltage (**paper II**). Error bars: SD

To test if the protein retention achieved from pure buffer solutions was transferable to a more complex sample solution, EME purification was performed on solutions of FITC labeled HSA. The fluorescent labeling was performed with a molar ratio of 10:1 between FITC and HSA. After EME purification, the quantity of the labeled complex (HSA/FITC) was determined by TDA and compared against an unpurified solution. The protein retention of HSA/FITC after EME purification was slightly lower (79 %) than that of HSA from buffer solution (92 %). This was considered to be caused by partial degradation of the FITC/HSA complex and adsorption to the walls of the 96-well filter plate.



**Figure 21:** Binding curve for the interaction between HSA/FITC and monoclonal anti-HSA antibody (**paper II**)

To further evaluate the applicability of EME purification on real samples, the preserved functionality of the HSA/FITC complex in terms of binding affinity after EME purification was assessed by an antibody study using anti-HSA in combination with flow-induced dispersion analysis (FIDA). With increasing concentration of anti-HSA added to the EME purified HSA/FITC solution, the apparent hydrodynamic radius of the HSA/FITC complex increased, thus demonstrating preserved binding affinity (Figure 21). This was further supported by the calculated dissociation constant ( $K_d$ ) and hydrodynamic radius, which was comparable with previously reported values for HSA/FITC.

### 3.5 Optimization of EME under stagnant conditions

In **Paper III**, a robust non-agitated (stagnant) EME system was developed to simplify the equipment needed to perform EME. This was done in order to address some of the limitations in previously developed stagnant EME systems, such as evaporation of the acceptor solution [75] or applicability only for very small sample volumes (1-5  $\mu\text{L}$ ) [128]. This work was therefore focused on developing a fully closed extraction system, allowing for prolonged extraction times without potential evaporative losses of the sample/acceptor phase. Due to the reduced extraction kinetics under stagnant conditions, longer extraction times are necessary to get high extraction recoveries in stagnant. This is related to the distribution of the electrical field. As demonstrated by the impedance measurements in **paper I**, the voltage drop across the SLM when using NPOE was 5000 V/cm, whereas the voltage drop in the donor and acceptor solution is approximately 0.25 V/cm. Thus, electro-kinetic migration of analytes mainly takes place across the SLM, while transfer in the aqueous phases is mainly driven by passive diffusion. To avoid that the overall extraction kinetics are bottlenecked by mass transfer in the sample, traditional EME systems are agitated, to ensure convection in the sample.

#### 3.5.1 Theoretical considerations

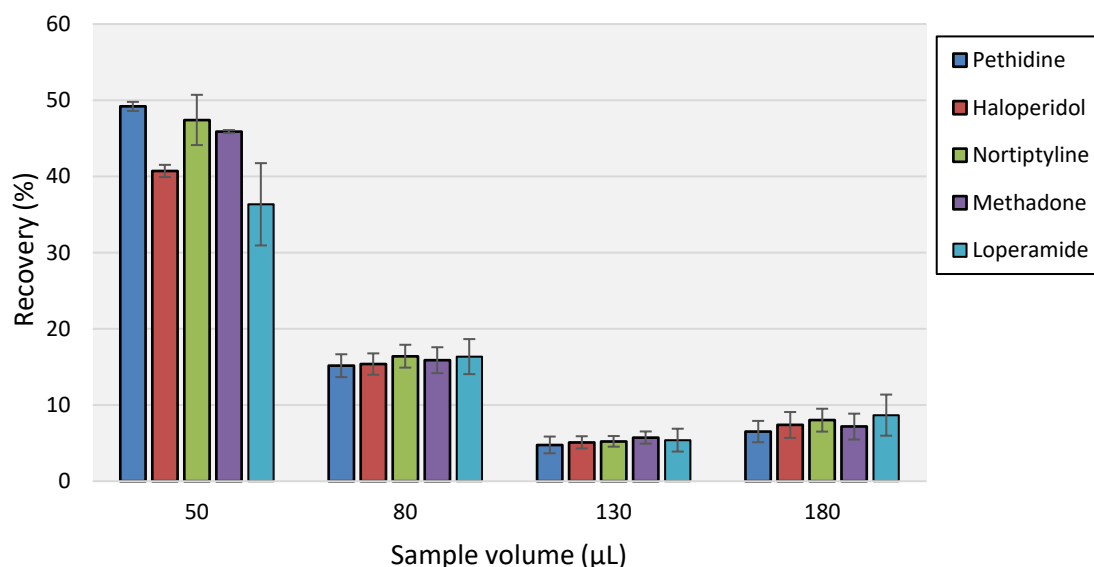
In order to increase the extraction performance under stagnant conditions, system optimization with respect to extraction recovery ( $R_i$ ) was based on a theoretical equation developed in previous work [75]:

$$R_i(t) = 100\% \cdot \frac{2A}{V_s} \sqrt{\frac{D_i}{\pi}} t \quad (15)$$

Here  $A$  is the surface area of the SLM,  $V_s$  is sample volume,  $D_i$  is the diffusion coefficient of the analyte, and  $t$  is the extraction time. As  $D_i$  is inherent to the analyte, only  $A$ ,  $V_s$ , and  $t$  can be considered operational parameters. While an increase in  $A$  was expected to enhance extraction recoveries, the filter membrane used as SLM in this work limited the surface area to 28 mm<sup>2</sup>. Therefore optimization was directed towards  $V_s$  and  $t$ . In EME under stagnant conditions, analytes in the sample/SLM intersection are quickly extracted by the electrical field, while analytes in the bulk sample need to migrate towards the SLM by diffusion. By reducing the sample volume, the average diffusion distance (distance from the center of the sample volume and to the SLM) is reduced, thereby improving the extraction kinetics. Even with reduced sample volumes, increased extraction times compared to EME under convection conditions were expected to be necessary to achieve high or exhaustive recoveries.

### 3.5.2 Sample volume

Optimization of sample volume was performed by using a sample plate with wells depths of 6.4, 4.6, 2.9, and 1.8 mm. As the cross-sectional area of the wells was constant (28 mm<sup>2</sup>), the different well depths corresponded to sample volumes of 180, 130, 80, and 50  $\mu$ L, respectively. The extraction performance was tested on five hydrophobic model analytes from a 20 mM phosphate buffer pH 2.7 solution, using a voltage of 75 V for 10 minutes. (Figure 22). The obtained results were in agreement with the theoretical foundation for Eq. 15. Analyte recoveries were highly affected by the reduction in sample volume, and with a sample volume of 50  $\mu$ L, extraction recoveries ranged between 36-49 %. Further reduction in sample volume was not found viable using this device, as lower sample volumes than 50  $\mu$ L did not cover the whole surface area of the SLM, thus eliminating the potential benefits of a shorter diffusion distance.



**Figure 22:** Extraction recoveries as a function of sample volume in stagnant EME (paper III). Error bars: SD

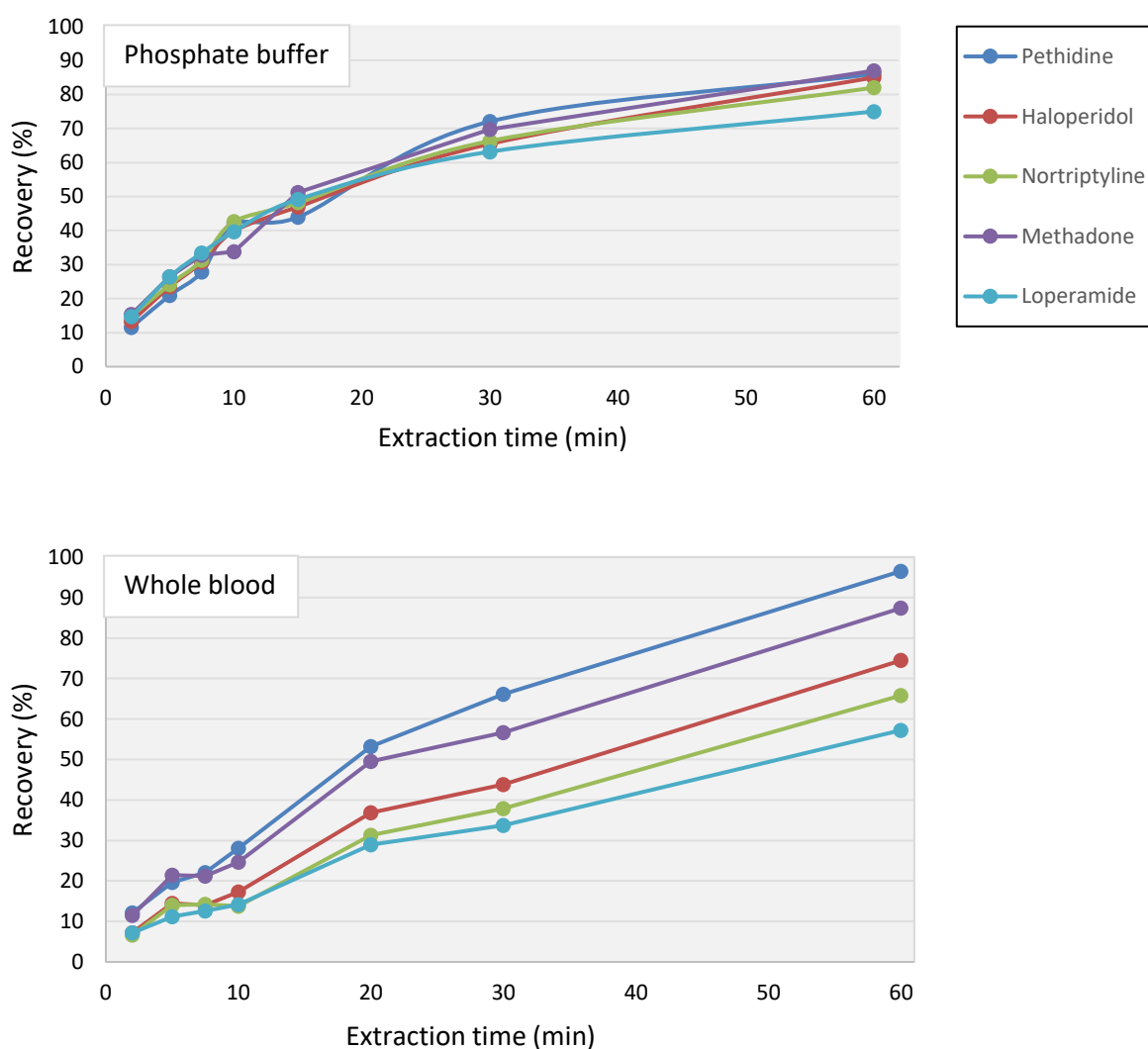
### 3.5.3 Extraction time

Extraction times were investigated by performing EME for 2, 5, 7.5, 10, 15, 30, and 60 minutes (Figure 23). Initial extraction kinetics from both buffer and whole blood samples were fast as analytes in the sample/SLM intersection were quickly extracted over to the acceptor phase. This was followed by slower extraction kinetics controlled by diffusion of analytes in the bulk sample towards the SLM. Extraction kinetics from whole blood samples were slower than from buffer solutions and this was assumed to be caused by higher viscosity and analytes binding to matrix proteins. The latter also



explained the compound-to-compound variability in recovery, since the degree of protein binding was different between the analytes.

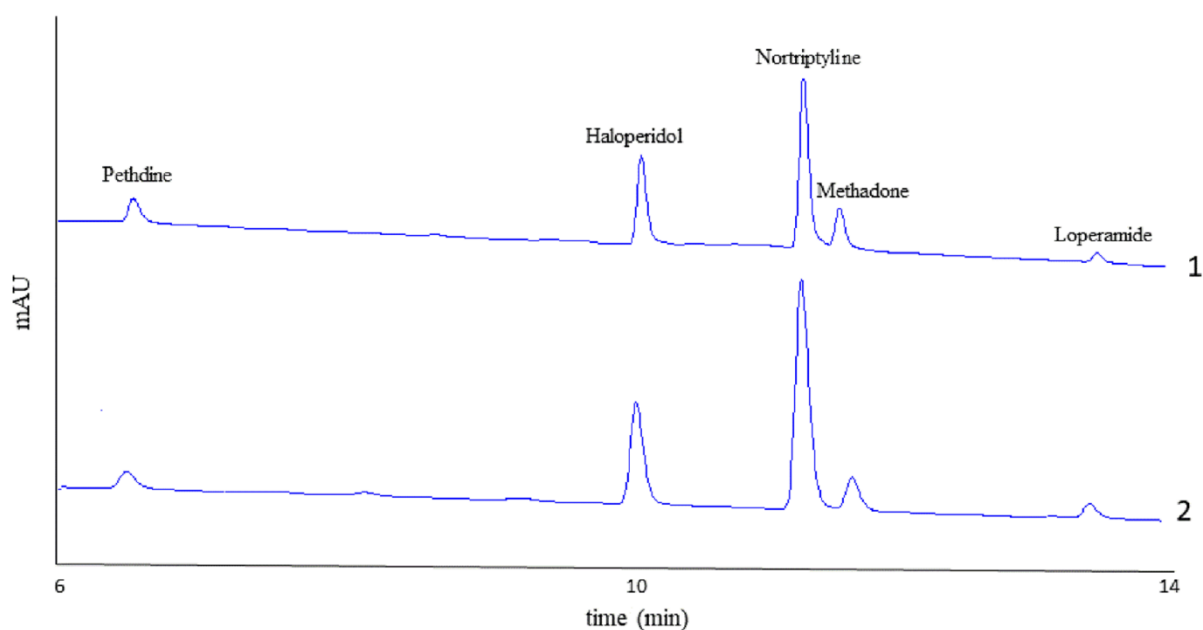
Extraction recoveries increased with prolonged extraction times, and after 60 minutes, recoveries from buffer solutions and whole blood samples varied between 75-87 % and 57-96 %, respectively. Extraction times of 60 minutes are considerably longer than needed in **Paper I, III, and V**, and illustrates the impact of sample convection on extraction kinetics. However, considering the simplicity of the stagnant system, minimal sample handling needed, the potential for in-field operation, and no need for further sample preparation, the technique is competitive with other sample preparation techniques.



**Figure 23:** Extraction recoveries from buffer solutions and whole blood as a function of extraction time (paper III).

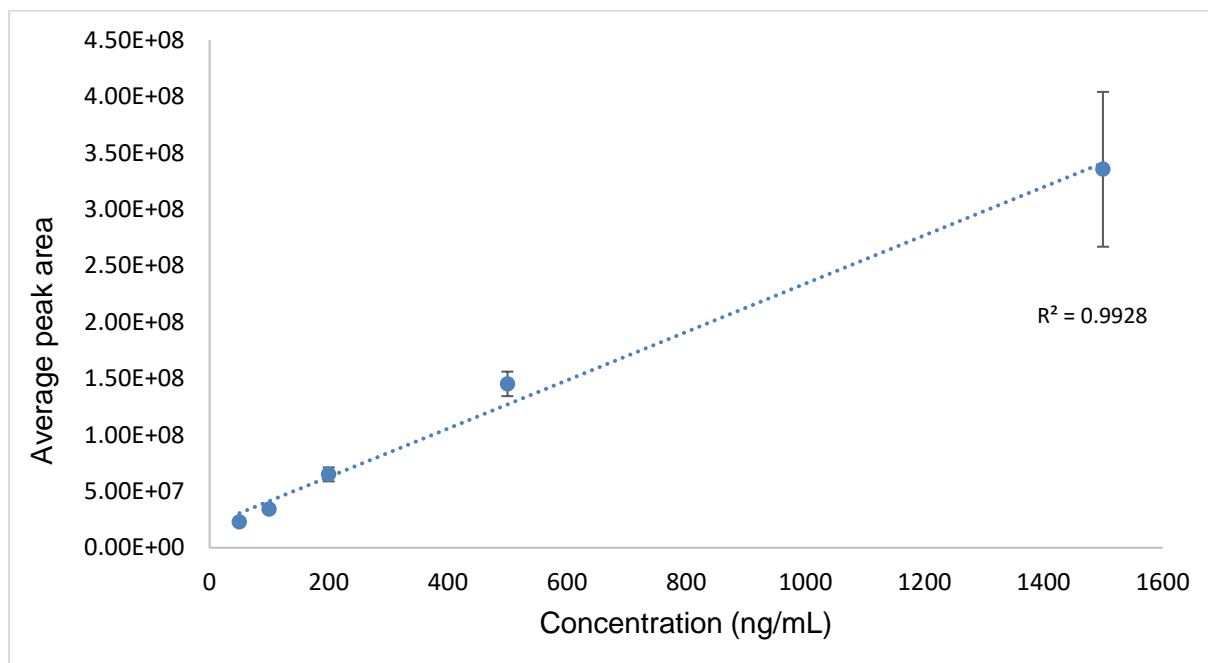
### 3.5.4 Evaluation

The extraction performance of the stagnant EME device was demonstrated to be selective towards the basic model analytes. A comparison between the HPLC-UV chromatograms from buffer solutions and whole blood showed no additional peaks in the latter (Figure 24) and similar chromatographic noise. LODs and limits of quantification (LOQs), were calculated using a signal-to-noise (S/D) ratio of 3:1 and 10:1. Both LODs (0.2-0.9  $\mu\text{g/ml}$ ) and LOQs (0.8-2.8  $\mu\text{g/ml}$ ) from whole blood were similar to that obtained from buffer solutions (LODs: 0.2-0.9  $\mu\text{g/ml}$ , LOQ: 0.5-2.8  $\mu\text{g/ml}$ ).



**Figure 24:** HPLC-UV chromatograms of the five model analytes from whole blood samples (1) and phosphate buffer solution (2) (paper III).

The excellent clean-up properties of the stagnant EME system was further demonstrated by an evaluation for the determination of methadone from whole blood samples in combination with LC-MS/MS. Methadone was spiked into whole blood samples in five different concentrations (50-1500 ng/ml) and the EME performance was evaluated based on linearity, reproducibility, LOD, and LOQ. The obtained calibration curve was linear within the chosen concentration window with a correlation of  $R^2=0.99$  (Figure 25). Reasonable reproducibility was obtained with RSD values ranging between 7-20%. LOD and LOQ were calculated to 0.4 ng/ml and 1.3 ng/ml using S/D ratio of 3:1 and 10:1, respectively



**Figure 25:** Peak signal to methadone concentration in sample after 60 min EME from diluted whole blood (paper III). Error bars: SD.

## 4 Concluding remarks and future perspective

The work in this thesis has focused on expanding the general knowledge on electromembrane extraction (EME) and highlighting the versatility of the extraction principles. This was achieved by increasing the theoretical understanding of the extraction processes in EME and developing new extraction principles and configurations. Most of the work in this thesis was performed from a fundamental standpoint and was aimed to increase further research in the investigated areas. The major findings in the five papers that comprise this thesis are presented below:

- Stable pH conditions and extraction currents can be achieved using buffer solutions as sample and acceptor phase. Extraction recoveries of hydrophobic basic drugs were independent of buffer composition but sensitive to pH in the acceptor solution.
- The presence of a boundary layer in the SLM/acceptor interface with elevated pH-conditions was visualized and characterized. This was hypothesized to be caused by the accumulation of buffer ions at the SLM/acceptor interface due to charge accumulation within the SLM.
- A theoretical model to predict the extraction performance of hydrophobic basic analytes under different pH conditions based on coupled relationships between  $pK_a$  and  $\log P$  was developed. Successful predictions were achieved for the more hydrophobic analytes, while less hydrophobic analytes (close to  $\log P = 2$ ) deviated from the model.
- EME tuned towards the removal of anionic matrix components from sample solutions. Complete removal of fluorescein isothiocyanate (FITC) was achieved with the addition of the ionic carrier Aliquat 366 (A336) to the supported liquid membrane (SLM). Removal from highly concentrated sodium dodecyl sulfate samples was somewhat limited by excessive trapping inside the SLM. Selective removal was demonstrated on FITC samples containing proteins, thus demonstrating the potential applicability for real samples.
- Development of a robust stagnant EME system for extractions of hydrophobic basic analytes from buffer solution and whole blood samples. The fully closed experimental system allowed for prolonged extraction times without evaporative losses of the acceptor solution, resulting in high extraction recoveries.

The increased knowledge and new principles presented in this thesis will hopefully be beneficial for the further development of EME as a sample preparation platform. The demonstrated pH stability and flexibility with buffer solutions might encourage the replacement of strong acid and bases traditionally used in EME. However, more work should be focused on the characterization and understanding of local pH effects at the SLM interface, in order to achieve predictable extraction performance and increase the potential for pH selective extraction. EME as a sample purification principle demonstrates a new way to use EME and has broadened its potential applicability. Hopefully, this will stimulate further investigation of EME in this direction or inspire investigation into other application principles. At this point, EME is still an academic discipline and a further investigation into SLM solvents and fundamental theory are expected to be required before it can be implemented in routine settings. Considering the high scientific activity and the already demonstrated advantageous of EME, this vision should be within reach.

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## Comprehensive study of buffer systems and local pH effects in electromembrane extraction



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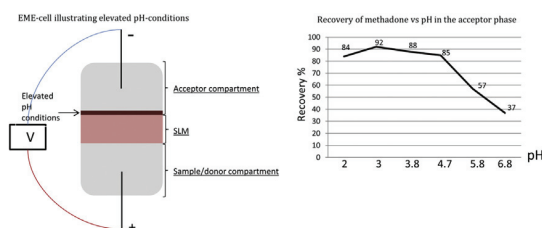
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### HIGHLIGHTS

- Buffers in EME provide stable pH, low current and high recoveries.
- Elevated pH conditions at the acceptor/SLM interface affect the extraction.
- Elevated pH conditions proved by visual inspection using the pH indicator phenolphthalein.
- Indicates why acceptor phase pH is more critical to EME than the donor phase.

### GRAPHICAL ABSTRACT



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### ABSTRACT

Different phosphate-, acetate- and formate buffers in the pH range 2.0–6.8 were tested for electro-membrane extraction (EME) in a 96-well system. The five basic drugs haloperidol, loperamide, methadone, nortriptyline, and pethidine were selected as model analytes. The EME performance was tested with respect to extraction recovery, extraction current and pH-stability. The analytes were extracted from 200  $\mu$ L buffer, through a 100  $\mu$ m thick supported liquid membrane (SLM) of 2-nitrophenyl octyl ether (NPOE) immobilized in the pores of filters in a 96-well plate, and into 100  $\mu$ L buffer acceptor phase. The extraction voltage was 50 V and the extraction time was 10 min. The acceptor phase was analyzed by HPLC-UV. The extraction current was  $\leq 6 \mu$ A with all buffers, and pH was effectively stabilized during EME using buffers as donor (sample) and acceptor phase. For buffers with pH  $\leq 4.8$  as acceptor phase, the extraction recoveries were in the range 66–97% and with RSD  $< 15\%$ . With pH in the range 5.8–6.8 in the acceptor phase, the extraction recoveries decreased and were in the range 21–62%. This was attributed to elevated pH conditions in the acceptor/SLM interface. The presence of elevated pH conditions was visualized with phenolphthalein as pH sensitive color indicator. Increasing the buffer strength from 10 to 500 mM in an attempt to offset the elevated pH conditions gave no improvement, and elevated pH conditions remained. Elevated pH conditions in the acceptor/SLM interface were also observed when voltage was increased, and when NPOE was replaced with tributyl phosphate as SLM. The presence of

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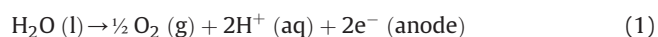
elevated pH conditions close to the SLM in EME was discussed for the first time, and this information is highly important for future development of EME.

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## 1. Introduction

Electromembrane extraction (EME) was introduced in 2006 [1] as a novel microextraction technique. EME was developed from hollow-fiber liquid-phase microextraction (LPME) [2], which in turn evolved based on the idea of solid-phase microextraction (SPME) [3], single-drop microextraction (SDME) [4], and supported liquid membrane extraction [5]. EME is performed in a three-phase system comprising two aqueous phases (donor (sample) and acceptor) separated from each other by an organic supported liquid membrane (SLM). The SLM is a thin film of an organic solvent which is immobilized in the pores in the wall of a porous hollow fiber. EME is based on electrokinetic migration of analytes in an electrical field sustained across the extraction system. This is in contrast to LPME which is based on diffusion of the analytes. EME provides faster extraction compared to LPME, while maintaining all the benefits of a microextraction system, such as (a) efficient sample clean-up, (b) high enrichment, (c) very low consumption of hazardous organic solvents, and (d) potential for automation and high-throughput. With an electrical field as the driving force for mass transfer, EME offers a unique possibility of tuning the extraction selectivity by the direction (polarity) and magnitude (voltage applied) of the electrical field [6]. For efficient EME, target analytes have to be charged. This means that for extraction of basic or acidic compounds, pH in the donor and acceptor phases has to be adjusted to a level where they are ionized. Up to date, pH adjustment has in most cases been accomplished using strong acids and bases (typically HCl and NaOH). In the area of pharmaceutical analysis, EME has been used to extract acidic drugs [7], basic drugs [8], and peptides [9] from different samples such as whole blood [10], plasma [11], urine [12], breast milk [13], and saliva [14]. Recent EME applications also include extraction of environmental pollutants [15], metal ions [16], and inorganic anions [17] related to environmental and food analysis applications.

While EME has been studied for 10 years and close to 250 EME papers have been published (Scopus), there are still fundamental questions and challenges that remain unanswered. One very important challenge is the occurrence of electrolysis in the donor and acceptor phase upon application of the electrical field. Thus, pH may decrease at the anode (Equation (1)) and may increase at the cathode (Equation (2)):



Changes in pH have been reported in several EME papers, and due to the small volumes involved the changes may be significant [18,19]. The level of electrolysis is determined by the extraction current, which is carried by mass transfer of analyte and background ions across the SLM, and by the extraction time. The extraction current in turn, is mainly determined by the applied voltage and by the chemical composition of the SLM. Therefore the level of electrolysis can normally be controlled by careful selection of experimental conditions. As a consequence of electrolysis, EME will always be associated with pH changes (especially the acceptor phase), even under low extraction current conditions (<50  $\mu\text{A}$ ) [20].

In most papers published up to date, dilute solutions of HCl and NaOH have been used as donor and acceptor phase. In some of those papers pH changes have most probably been relatively small, whereas in others major pH changes may have challenged the extraction performance. Nevertheless, for future development of EME, focus should be directed towards stabilizing pH. This is considered important in order to enhance the reliability and robustness of EME procedures, and also for the general acceptance and implementation of the concept. Analytical scientists will prefer operating an extraction system under constant pH conditions, as long as it is obvious that performance is pH dependent.

In a few EME papers, special attention has been directed towards stabilizing pH in the acceptor phase. In one recent paper, the basic drug substances procaine, nortriptyline, and papaverine were extracted using 500 mM formic acid as acceptor phase [21]. Even after 40–80 min of EME, pH in the acceptor phase was still within 0.2 units from its origin, and due to the buffer capacity analyte molecules were not back-extracted into the SLM. While 10 mM HCl may be prone to pH shift, 100 mM HCl also provided stable pH in the acceptor phase, and after 80 min of operation the shift in pH was only 0.1 [21]. Successful use of very strong solutions of mineral acid was reported in another paper related to  $\mu$ -EME. In this case pH changes up to eight units were observed using 1–10 mM HCl as acceptor phase, whereas pH stabilized when 100 mM HCl was used [19]. In the  $\mu$ -EME system, the volume of acceptor phase was only 1.5  $\mu\text{L}$ . However, electrolytically induced pH changes up to 8.5 pH units were also observed in experiments performed in a traditional EME system based on a hollow fiber, where the volume of acceptor phase was 20  $\mu\text{L}$  [18]. In a paper by Huang et al., acetate buffers were tested as acceptor phase for selective EME of peptides based on their isoelectric point [22]. In that work, 75 mM acetate buffer pH 5.2 was stable within 0.1 pH units during EME.

The use of different buffer systems in EME applications is not new and has already been investigated by different research groups. However, the challenge lies in choosing the right one for the application in terms of recovery, pH stability and extraction current. In the first part of this work we investigated different buffer systems for use in the pH range 2.0–6.8. During testing with different buffers, we discovered a layer of elevated pH in the acceptor boundary layer in close contact with the SLM. Although several publications have discussed on formation of electrical double layers at the SLM surfaces [23–27], we are to the best of our knowledge the first group to address the effect of pH difference between the SLM and the bulk acceptor phase. Because this layer of elevated pH has major impact on the performance and mass transfer, it was investigated systematically for the first time in the second part of this paper. The layer of elevated pH may better explain data already published in the literature, and knowledge about it is very important for future EME work.

## 2. Experimental

### 2.1. Chemicals and materials

All chemicals were of high analytical grade (>95%) Haloperidol, loperamide, methadone, nortriptyline, pethidine and ammonium molybdate were all purchased from Sigma Aldrich (St Louis,

Missouri, USA). Phosphoric acid, sodium dihydrogen phosphate, disodium hydrogen phosphate, sodium acetate, acetic acid, sodium formate, formic acid, phenolphthalein, 2-nitrophenyl octyl ether (NPOE), tributyl phosphate (TBP) acetonitrile, sulphuric acid and nitric acid were purchased from Merck (Darmstadt, Germany). Ethanol was obtained from Arcus (Oslo, Norway) and water (18 MΩ cm) was obtained from a Milli-Q water purification system (Molsheim, France).

## 2.2. Preparation of standard and sample solutions

A stock solution of haloperidol, loperamide, methadone, nortriptyline, and pethidine at concentration of 1 mg/mL was prepared in ethanol, and stored at 4 °C protected from light. The stock solution was diluted with appropriate buffers to make HPLC standards and samples with concentration of 10 µg/mL and 4 µg/mL, respectively. A solution of phenolphthalein was prepared by dissolution of 0.1 g substance in 80 mL ethanol and diluted to 100 mL with water. Ammonium molybdate was prepared by dissolving 2.5 g ammonium molybdate in 33 mL sulphuric acid and diluting to 100 mL with water.

## 2.3. Equipment for EME

A single row comprising eight wells was cut out from an Agilent 96-well polypropylene plate with 0.5 mL wells (Agilent, Santa Clara, CA, USA) and served as donor compartment. The eight wells were punctured in the bottom with a needle, and silver wires (1 mm diameter, K.A. Rasmussen, Hamar, Norway) were inserted and glued. The silver wires served as anodes. A 96-well MultiScreen-IP filter plate with polyvinylidene fluoride (PVDF) filter membranes with 0.45 µm pore size (Merck Millipore Ltd., Carrigtwohill, Ireland) was used as acceptor compartment. A model ES 0300–0.45 (Delta Elektronika BV, Zierikzee, Netherlands) was used as power supply, and a Vibramax 100 Heidolph shaking board (Kellheim, Germany) was used to agitate the extraction system. The pH in the donor and acceptor phase before and after EME, was measured with a micro pH-meter (110 ISFET pH Meter, Beckman Coulter, Brea, CA, USA).

## 2.4. EME set up and procedure

EME was performed according to the following procedure: a) 200 µL donor phase was pipetted into the donor compartment, b) 3 µL NPOE was pipetted into the filter membranes (SLM), and c) 100 µL buffer was pipetted into the acceptor compartment. The anode was located in the donor compartment, and the cathode was located in the acceptor compartment. Prior to extraction, the donor and acceptor compartments were clamped together. The extraction time was 10 min, the voltage was 50 V, and agitation was 900 rpm.

## 2.5. Instrumentations

### 2.5.1. HPLC-UV

The chromatographic system comprised a 3000 Ultimate HPLC-UV (Thermo Fisher, Waltham, MA, USA). The chromatographic separation was accomplished with a 150 mm × 2 mm ID Gemini C-18 column (Phenomenex Torrance, CA, USA) with 5 µm particle size. Mobile phase A consisted of 20 mM formic acid and acetonitrile (95:5, v/v), and mobile phase B consisted of 20 mM formic acid and acetonitrile (5:95, v/v). The injection volume was set to 20 µL with a mobile phase flow of 0.4 mL/min. A linear gradient was used from 100% to 60% mobile phase A during 15 min. This composition was kept constant for 2 min before the system was flushed with 80% mobile phase B for 2 min. Finally, the gradient was reestablished at 100% mobile phase A for 6 min prior to a new injection. The column

temperature was set to 60 °C. UV detection was performed at 214 nm. All HPLC measurements achieved signal-to-noise ratios exceeding 10:1.

### 2.5.2. Impedance measurements

Impedance measurements were performed to investigate the resistive and capacitive properties of the EME system, using a Solartron 1260 + 1294 frequency response analyzer (AMETEK, PA, USA) connected to the EME-electrodes in a two-electrode setup. The frequency range was 10 Hz - 1 MHz with an applied amplitude of 100 mV rms. The data interpretation was done with the ZView software (Scribner Assoc., NC, USA).

## 2.6. Calculations

The EME recovery was calculated using the following equation:

$$\text{Recovery (\%)} = \frac{C_{ap} V_{ap}}{C_{dp} V_{dp}} \times 100 \quad (3)$$

where,  $C_{ap}$  is the final concentration of the analyte in the acceptor phase,  $C_{dp}$  is the initial analyte concentration in the donor phase,  $V_{ap}$  is the volume of the acceptor phase and  $V_{dp}$  is the volume of the donor phase.

## 3. Results and discussion

In order to study the use of buffers systematically from a fundamental point of view, different phosphate, formate, and acetate buffers were tested as donor and acceptor phases. The buffers were in the pH range 2.0–6.8 with buffer strengths of 10 and 50 mM. Five different nonpolar monobasic drug substances were selected as model analytes, namely pethidine, nortriptyline, methadone, haloperidol, and loperamide. The analytes were extracted from 200 µL donor phase and into 100 µL acceptor phase. EME was performed at 50 V for 10 min under strong agitation (900 rpm). The SLMs were 3 µL of NPOE immobilized in PVDF filter membranes. NPOE was selected due to its excellent performance for EME of basic drugs [28]. This is due to 1) strong hydrogen bond acceptor properties, 2) very low water solubility, and 3) very low volatility.

### 3.1. Extraction current and pH measurements

For all buffer systems tested, the extraction current was recorded continuously (12 Hz). A typical current profile is illustrated in Fig. 1, where current was recorded during extraction from 50 mM phosphate buffer pH 2.0 as donor, and into the same buffer as acceptor phase. Following a short initial peak current (discussed below), the current stabilized around 2–3 µA. This was a low current, and provided a highly stable extraction system. All the other buffer systems (phosphate, acetate, and formate) provided comparably low currents ( $\leq 6$  µA after the initial peak current) and current profiles. Also, the current was independent of the buffer pH and the buffer molarity. The low current level in all tested systems was beneficial from a system stability point of view. Thus, with low current and with buffer capacity in both the donor and acceptor phase, no significant pH changes were measured during EME using a digital pH-meter. In conclusion, with 10 or 50 mM phosphate, acetate, and formate buffers in the pH-range 2.0–6.8, pH was highly stable in the donor and acceptor phases using NPOE as the SLM.

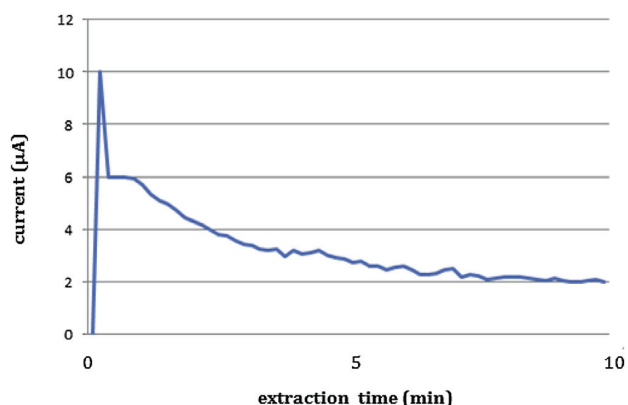


Fig. 1. Extraction current as function of extraction time.

### 3.2. Recovery of analytes with different buffers

The extraction recoveries for the model analytes obtained with the different buffer systems are shown in Table 1. In the pH range 2.0–4.7, the recoveries were relatively high and ranged from 66% to 97%. Recoveries in this pH range were independent of buffer type, pH, and buffer molarity. Also, with all buffer systems tested (pH 2.0–4.7), the repeatability was acceptable and within 15% RSD. The performance data with the different buffer systems were comparable to the use of 10 mM HCl, which may be considered as standard conditions for EME of nonpolar basic drugs (Table 1).

With buffers in the pH range 5.8–6.8, recoveries were lower and RSD-values increased, even though the basic model analytes still should be fully protonated. In view of this performance drop, we hypothesize that pH is elevated in the acceptor boundary layer

during EME as illustrated in Fig. 2. This acceptor boundary layer, which is located at the acceptor/SLM interface, has most likely a pH higher than in the bulk acceptor phase. The analyte molecules transfer the SLM as protonated species, but when entering the acceptor/SLM interface, they become partly deprotonated and therefore electro-kinetic migration is reduced.

In order to visualize elevated pH in the acceptor boundary layer, the pH sensitive color indicator phenolphthalein was added to the acceptor phase prior to EME. Phenolphthalein is colorless at pH conditions below 8.0, and converts to red/pink color between pH 8.0–10.0. EME was performed under the same conditions as before with phosphate buffer pH 6.8 as both donor and acceptor phase. The acceptor phase was spiked with 20 µL phenolphthalein solution. After 60 s of EME a clear pink color appeared close to SLM in the acceptor phase, while the bulk acceptor phase remained colorless as illustrated in Fig. 3. This gave a clear proof of elevated pH in the acceptor boundary layer.

### 3.3. Theoretical model for elevated pH in the acceptor boundary layer

The SLM in the EME cell can be considered an electrical circuit as illustrated in Fig. 4 [29]. The major electrical resistance in the system is located in the SLM. The resistance of the SLM ( $R_{SLM}$ ) can be divided into two independent contributions, namely a membrane resistance ( $R_{solvent}$ ) and a charge transfer resistance ( $R_{CT}$ ).  $R_{solvent}$  is the resistance of the organic solvent used as SLM (typically very high), whereas  $R_{CT}$  is the resistance related to the presence and transfer of back-ground ions and analyte ions in the SLM. The electrical resistance in the donor ( $R_{donor}$ ) and acceptor ( $R_{acceptor}$ ) phase are small compared to  $R_{SLM}$  and  $R_{CT}$ , because both are aqueous. Thus, the major voltage drop in the system occurs across the SLM. The SLM also exposes a capacitive component, working in parallel with the resistive component. The capacitive properties

Table 1

Recoveries and RSD with different buffers as donor and acceptor phase. All extractions were accomplished at 50 V for 10 min with NPOE as SLM.

Conditions	Recovery (%) (RSD %)				
	Pethidine	Haloperidol	Nortriptyline	Methadone	Loperamide
Donor: 10 mM HCl Acceptor: 10 mM HCl	73(8.5)	80(7.3)	79(6.1)	82(3.0)	75(3.8)
Donor: 10 mM phosphate pH 2.0 Acceptor: 10 mM phosphate pH 2.0	66(4.5)	79(6.7)	79(8.2)	82(5.0)	77(4.5)
Donor: 50 mM phosphate pH 2.0 Acceptor: 50 mM phosphate pH 2.0	72(7.3)	84(5.0)	76(9.2)	86(5.0)	79(7.2)
Donor: 10 mM phosphate pH 3.0 Acceptor: 10 mM phosphate pH 3.0	77(9.0)	85(5.6)	85(5.1)	87(6.3)	85(5.9)
Donor: 50 mM phosphate pH 3.0 Acceptor: 50 mM phosphate pH 3.0	74(7.5)	90(4.5)	81(4.5)	97(3.3)	96(5.3)
Donor: 10 mM formate pH 3.8 Acceptor: 10 mM formate pH 3.8	86(10.5)	86(7.0)	87(7.7)	89(7.3)	86(7.6)
Donor: 50 mM formate pH 3.8 Acceptor: 50 mM formate pH 3.8	86(5.3)	85(7.5)	86(7.2)	87(7.0)	77(11.1)
Donor: 10 mM acetate pH 4.7 Acceptor: 10 mM acetate pH 4.7	84(7.4)	81(7.5)	84(7.5)	84(11.6)	72(12.1)
Donor: 50 mM acetate pH 4.7 Acceptor: 50 mM acetate pH 4.7	79(6.1)	77(11.0)	80(10.4)	85(2.7)	72(6.4)
Donor: 10 mM phosphate pH 5.8 Acceptor: 10 mM phosphate pH 5.8	50(9.0)	43(11.0)	62(9.5)	53(17.2)	44(30.0)
Donor: 50 mM phosphate pH 5.8 Acceptor: 50 mM phosphate pH 5.8	50(16.0)	50(6.0)	67(7.3)	61(4.2)	48(10.0)
Donor: 10 mM phosphate pH 6.8 <sup>a</sup> Acceptor: 10 mM phosphate pH 6.8	60(21.1)	21(19.2)	50(26.2)	35(26.7)	18(35.8)
Donor: 50 mM phosphate pH 6.8 <sup>a</sup> Acceptor: 50 mM phosphate pH 6.8	63(19.2)	24(35.1)	58(11.3)	38(22.9)	21(52.9)

n = 5.

<sup>a</sup> Similar results was obtained with phosphate buffer pH 2 as donor phase.



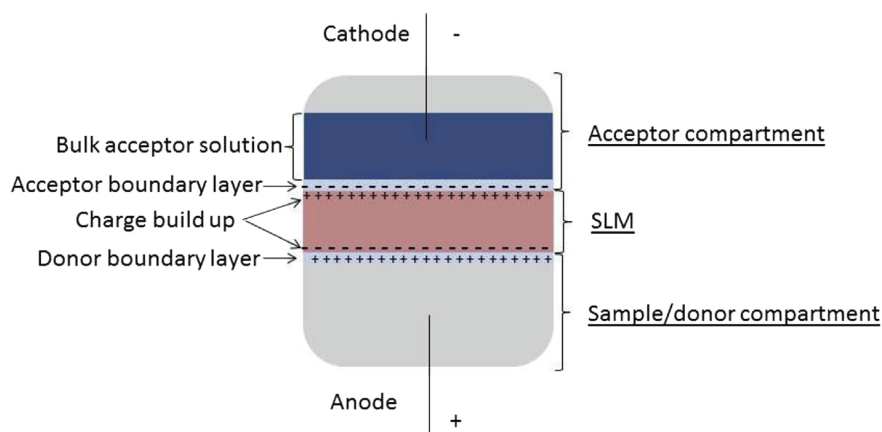


Fig. 2. Schematic illustration of acceptor boundary layer.

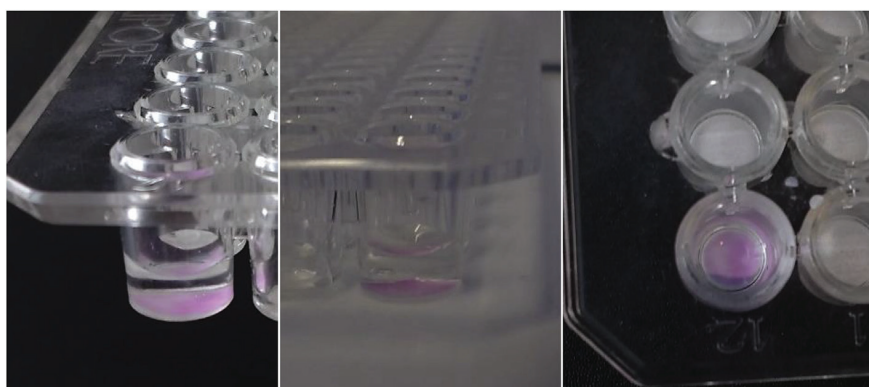


Fig. 3. Visualization of elevated pH conditions in the acceptor boundary layer by using phenolphthalein.

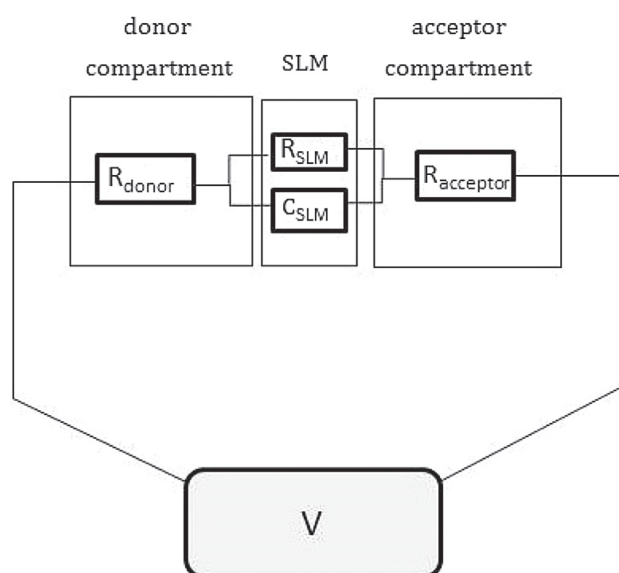


Fig. 4. Schematic illustration of the electrical circuit of the EME system.

arise from two serial capacitors, one in the SLM boundary layer at the donor interface ( $C_{\text{donor}}$ ) and another in the SLM boundary layer at the acceptor interface ( $C_{\text{acceptor}}$ ). The overall behavior of the SLM

as a parallel coupling of a resistor and a capacitor has been discussed previously [29]. This was confirmed in the present work by experimental impedance measurements as shown in Fig. 5, where the impedance is plotted versus frequency. The measured relationship between impedance and frequency is characteristic for a parallel coupling of a resistor and a capacitor [30].

The nature of the electrical circuit affected the current profile during EME (Fig. 1). Initially, the current was high due to the charging of the capacitors ( $C_{\text{donor}}$  and  $C_{\text{acceptor}}$ ). Thus, the SLM was charged during the initial phase of the extraction, and charge was instantaneously accumulated internally in the SLM boundary layer at the donor and acceptor interfaces. When the SLM was fully charged after short time, the current decreased and became solely dependent on the resistive properties of the system ( $R_{\text{SLM}}$  and  $R_{\text{CT}}$ ). The current was then principally caused by the flux of analyte ions and background ions across the SLM. Based on the impedance experiments (Fig. 5), the total capacitance of the SLM was measured to be 28.9 pF for NPOE and 20.5 pF for tributyl phosphate (TBP). Thus, more charge was accumulated in the SLM with NPOE than with TBP. With NPOE as SLM, the resistance across the SLM ( $R_{\text{solvent}} + R_{\text{CT}}$ ) was measured to approximately 2 M $\Omega$  based on the impedance experiments, whereas the total resistance in the aqueous phases ( $R_{\text{donor}} + R_{\text{acceptor}}$ ) was in the order of 2 k $\Omega$ . Based on these resistances and based on the dimensions of the EME system, the voltage drop across the SLM was calculated to be 5000 V/cm, whereas the voltage drop in the donor and acceptor phase was approximately 0.25 V/cm each.

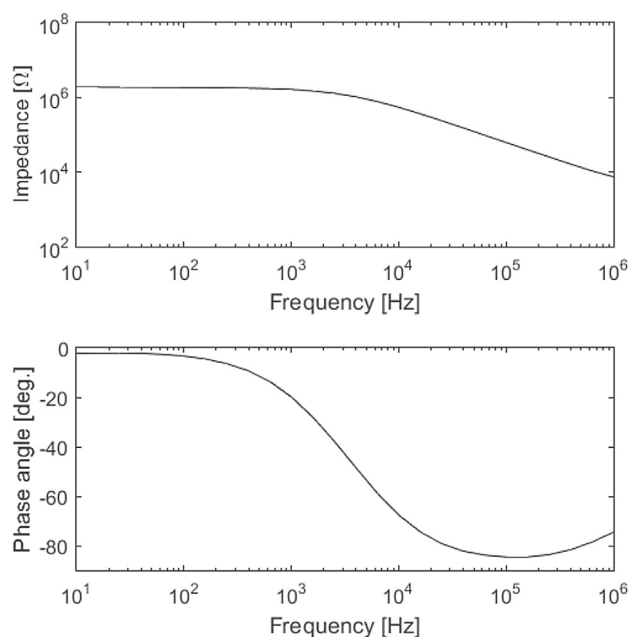
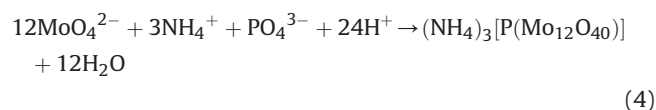


Fig. 5. Read-out from impedance measurement.

In the EME system tuned for basic analytes, the cathode is located in the acceptor phase. Under these circumstances, positive charge is expected to accumulate in the SLM boundary layer at the acceptor interface. Correspondingly, negative charge is expected to accumulate in the SLM boundary layer at the donor interface. Instantaneously, electrical double layers are established. In the acceptor boundary layer,  $\text{OH}^-$ ,  $\text{HPO}_4^{2-}$ , and  $\text{H}_2\text{PO}_4^-$  ions are attracted to maintain electro-neutrality in the interfacial region as illustrated in Fig. 2. The thickness of this electrical double layer was calculated to be less than 10 nm [31]. Theoretically, pH in this electrical double layer will differ from pH in the bulk acceptor phase [32], but according to our calculations, this effect is not significant in our EME set-up based on the measured capacitance of the SLM.

After the initial formation of the electrical double layer,  $\text{OH}^-$ ,  $\text{H}_2\text{PO}_4^-$ , and  $\text{HPO}_4^{2-}$  migrate from the acceptor boundary layer and towards the SLM interface. To some extent, the anions are transferred to and cross the SLM, but the flux is low. This statement is supported by the fact that currents were only a few  $\mu\text{A}$  in all systems tested, and were almost independent of the acceptor phase pH and buffer molarity. Elevated pH in the boundary layer arises either because the flux of  $\text{H}_2\text{PO}_4^-$  across the SLM is higher than the flux of  $\text{OH}^-$  and  $\text{HPO}_4^{2-}$  ions and  $\text{H}_2\text{PO}_4^-$  ions are depleted (electrolyte depletion), and/or because  $\text{OH}^-$  and  $\text{HPO}_4^{2-}$  ions have higher flux towards the SLM interface than  $\text{H}_2\text{PO}_4^-$  and are stacked (electrolyte stacking). The exact mechanism is currently unclear, and it may be dependent on experimental conditions, but clearly elevated pH was established in the acceptor boundary layer at pH values above 5.8 in the bulk acceptor phase. The change of pH causing the elevated pH layer is a gradual process, but even in the initial phase of the extraction, mass transfer is affected. In the bulk acceptor phase there is convection, and therefore the effect is only local. Thus, the pH in the bulk acceptor phase is not affected. From a practical point of view, however, the elevated pH layer affects the electrokinetic transfer across the SLM/acceptor interface. The hypothesis also explains very well many experimental observations in the literature, namely that pH in the acceptor phase is highly critical, and should normally be 3–4 units below the pKa-value of the target analytes.

The hypothesis above implies that  $\text{H}_2\text{PO}_4^-$  ions cross the SLM, at least under electrolyte depletion conditions. To verify this, a simple experiment was set up. EME was performed under similar conditions as before, but with a pure 50 mM phosphate buffer pH 6.8 as acceptor phase and 10 mM HCl as donor phase. After 60 min of extraction, the donor was transferred to a vial and a few drops of nitric acid and 1 mL ammonium molybdate solution were added. The donor phase was then placed in a heater for 10 min at 50 °C. In the presence of phosphate ions, ammonium molybdate will make a yellow precipitation in accordance with the following reaction:



The donor did not produce visible precipitation, but still had a yellow colorization. This supported that phosphate ions to some extent migrated from the acceptor phase into the donor phase. The same experiment was done with 10 mM HCl used both as donor and acceptor phase, and this gave no colorization.

### 3.4. Additional experiments

While it was difficult to fully investigate and explain the exact mechanism of formation of the elevated pH conditions in the acceptor boundary layer, a few additional experiments were conducted to investigate the phenomenon. In a first set of experiments, the impact of the buffer strength of the acceptor phase was studied. The experiments were performed with phosphate buffer pH 6.8, and testing buffer strength of 10, 50, 100, 250 and 500 mM as both donor and acceptor phase. All other conditions were identical to those used in previous experiments. Two noticeable results were extracted from this experiment. First, recoveries were not improved by increasing the buffer strength (data not shown), and therefore the elevated pH conditions in the acceptor boundary layer were not suppressed using high buffer strength in the acceptor phase. Second, the extraction current was independent of the buffer strength, and this may indicate that at high buffer strength, electrolyte stacking maintained elevated pH in the acceptor boundary layer.

In a next set of experiments, NPOE was replaced with tributyl phosphate (TBP) as SLM. The voltage was now set at 20 V based on earlier experience [20]. The experiments were conducted with (a) 10 mM phosphate buffer pH 2.0 as donor and acceptor phase, and with (b) 10 mM phosphate buffer pH 6.8 as donor and acceptor phase. The data are summarized in Table 2 (top two lines). At pH 2.0, the model analytes were extracted with recoveries in the range 76–94%, except for pethidine (20%). When the same experiment was repeated with pH 6.8 in donor and acceptor phase, recoveries for haloperidol and loperamide decreased significantly, whereas the drop in performance was less with the other model analytes. Thus, elevated pH in the acceptor boundary layer also appeared to affect mass transfer when using TBP as SLM, but the impact was compound dependent.

In a final set of experiments, the impact of the extraction voltage was investigated. The experiments were done at 20 and 80 V, with both NPOE (Table 3) and TBP (Table 2, bottom line), and with 10 mM phosphate buffer pH 6.8 as both donor and acceptor phase. For TBP the increase of voltage from 20 V to 80 V did not improve recoveries, except for pethidine, which was generally extracted with low efficiency in all experiments using TBP. Thus, with TPB, reduced mass transfer due to high pH in the boundary layer appeared to be almost unaffected by the applied voltage above 20 V. This voltage is corresponding to the level where EME of non-polar bases with TBP

**Table 2**

Recoveries and RSD with different pH and voltage. All extractions were accomplished with 10 min extraction time and TBP as SLM.

Conditions	Recovery (%) (RSD %)				
	Pethidine	Haloperidol	Nortriptyline	Methadone	Loperamide
Buffer: 10 mM phosphate pH 2.0 Voltage: 20 V	20(19.2)	82(6.0)	94(1.9)	76(6.7)	84(5.6)
Buffer: 10 mM phosphate pH 6.8 Voltage: 20 V	19(15.7)	7(2.6)	71(19.0)	70(6.3)	12(5.6)
Buffer: 10 mM phosphate pH 6.8 Voltage: 80 V	51(3.5)	7(19.1)	75(18.0)	73(4.2)	10(30.0)

n = 5.

**Table 3**

Recoveries and RSD with different voltage. All extractions were accomplished with 10 min extraction time and NPOE as SLM.

Conditions	Recovery (%) (RSD %)				
	Pethidine	Haloperidol	Nortriptyline	Methadone	Loperamide
Buffer: 10 mM phosphate pH 6.8 Voltage: 20 V	15(4.6)	17(3.6)	31(15.2)	51(15.6)	33(10.5)
Buffer: 10 mM phosphate pH 6.8 Voltage: 80 V	98(4.2)	38(12.6)	71(2.6)	55(10.4)	33(12.8)

n = 5.

is no longer voltage limited [20]. With NPOE, the pattern was slightly different, and in this case the effect of elevated pH conditions in the acceptor boundary layer was reduced by increasing the voltage from 20 to 80 V. However, especially for haloperidol and loperamide, mass transfer was still affected even at 80 V. We have currently no explanation why haloperidol and loperamide were more sensitive to elevated pH in the acceptor boundary layer than the other model analytes. Explanation based on simple parameters like  $pK_a$  and  $\log P$  was not clear. The most striking difference between haloperidol and loperamide and the other model analytes are the more linear and less rigid molecular structures of the former, and this will be investigated in more details in future work.

#### 4. Conclusions

The present work has demonstrated that use of phosphate, acetate, and formate buffers in EME provides beneficial extraction conditions in terms of pH stability, extraction current, and recoveries for non-polar basic drug substances. With the buffers tested, pH in the donor and acceptor phase was highly stable during EME, and the current was less than 6  $\mu A$ . Recoveries were high for selected basic drugs ( $8.1 < pK_a < 10.5$ ) at  $pH < 4.8$  in the acceptor phase, but were reduced at acceptor phase  $pH > 4.8$ . This was attributed to elevated pH conditions in the boundary layer between the acceptor phase and the SLM. The presence of elevated pH was verified by the pH-sensitive color indicator phenolphthalein. The elevated pH layer arose due to stacking of  $OH^-$  and  $HPO_4^{2-}$  ions in the stagnant acceptor/SLM boundary layer, or due to depletion of  $H_2PO_4^-$  ions from the same area. Tuning parameters such as buffer strength, voltage and organic solvent did not alleviate the effects of the elevated pH conditions.

The occurrence of elevated pH conditions in the stagnant acceptor/SLM boundary layer is an important observation, and explains a fundamental question in the literature, namely why EME is much more sensitive to pH in the acceptor phase than in the donor phase. Further understanding of this phenomenon is needed in order to develop robust and reliable EME methods and systems, and will undoubtedly be addressed in future research.

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III





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## Towards exhaustive electromembrane extraction under stagnant conditions

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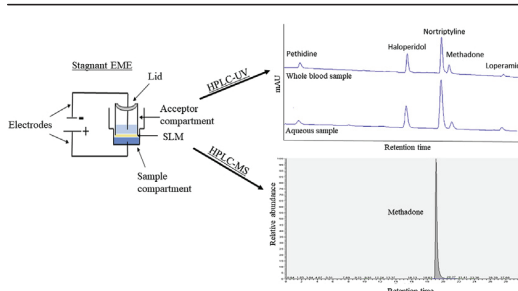
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### HIGHLIGHTS

- Electromembrane extraction under totally stagnant conditions.
- Extraction from whole blood.
- Very simple device with potential for in-field operation.

### GRAPHICAL ABSTRACT



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 Whole blood

### ABSTRACT

Electromembrane extraction (EME) in small, stagnant and chip-like devices has the potential for future in-field operation. Literature briefly discuss such systems, but performance suffered from evaporative losses of sample and acceptor. To address this, the current paper reports electromembrane extraction (EME) of five basic drugs (model analytes) from aqueous buffer solutions and whole blood samples under stagnant conditions in a completely closed system. A laboratory-made polyoxymethylene (POM) well plate served as compartment for the sample solution, while a commercially available well filter plate was used to immobilize 2-nitrophenyl octyl ether (NPOE) as supported liquid membrane (SLM) and as closed compartment for the acceptor solution. Major design parameters (sample compartment and electrode geometry) and operational parameters (sample volume, voltage and extraction time) were investigated and optimized. Electrode geometry was not very critical, but extraction efficiency increased with decreasing sample volume. Extraction from 50  $\mu\text{L}$  aqueous buffer solution for 60 min and with a voltage of 75 V was considered exhaustive (sample was depleted), with recoveries ranging between 75% and 87% for loperamide, haloperidol, methadone, nortriptyline, and pethidine (RSD: 2–12%). Extraction from whole blood samples under optimized conditions yielded slightly lower recoveries, ranging between 57 and 96% (RSD: 3–12%). Stagnant EME was evaluated in combination with liquid chromatography-mass spectrometry (LC-MS) as a highly specific instrumental method, and provided evaluation data on methadone from blood samples in accordance with regulatory requirements (LOD: 0.4 ng/mL, LOQ:

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1.4 ng/mL, RSD: 6–20%). This work has improved upon the design of stagnant EME, moving it further towards a viable in-field operation device.

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## 1. Introduction

Electromembrane extraction (EME) was introduced in 2006 as a combination of conventional liquid-liquid microextraction and electrophoresis [1]. The EME system comprises three liquid phases: the aqueous sample (donor) solution, the supported liquid membrane (SLM, i.e. an organic solvent immobilized in the pores of a filter membrane), and the aqueous acceptor solution. Analytes are extracted from the sample solution to the acceptor solution by an electrical field, which is applied to the system by placing an electrode in each of the aqueous solutions. The electrodes are coupled to an external power supply. In order to promote electrokinetic migration, sample and acceptor solution pH is adjusted to keep the analytes charged. For extraction of basic analytes, pH should be neutral or acidic, and the cathode is placed in the acceptor solution. For acids, EME occurs under neutral or alkaline conditions, and the direction of the electrical field is reversed. The choice of organic solvent in the SLM is critical in order to get an efficient, stable, and selective extraction system. The solvent should have a high degree of water immiscibility and be non-volatile at operational temperature [2]. For basic analytes the solvent should have strong hydrogen bond acceptor properties, to facilitate the transfer of protonated substances [3]. For non-polar basic analytes, 2-nitrophenyl octyl ether (NPOE) has been the solvent of choice, as it fulfils the aforementioned criteria and yields excellent extraction recoveries [1].

While maintaining all of the benefits of a microextraction system, such as microliter solvent usage (3–10  $\mu\text{L}$ ), compatibility with small sample volumes, and high extraction selectivity, the use of an electrical field adds flexibility to EME. With an electrical field as the main driving force, extraction time can be significantly shortened compared to diffusion-based techniques [4]. The possibility to tune the magnitude (voltage) and direction (polarity) of the electrical field gives another dimension of selectivity. Up to date EME has been utilized for the extraction of a multitude of different compounds, such as hydrophobic/hydrophilic basic and acidic drugs [1,5], environmental contaminants [6] peptides [7], metals [8,9] and inorganic ions [10] from environmental waters [11] and biological matrices including urine [12], plasma [13] and whole blood [14]. EME has also been further miniaturized as an on-chip device for sample clean up and isolation in online analysis [15], and for continuous in-vitro drug metabolism measurements [16].

In order to get fast extraction kinetics and high analyte recovery, the EME system needs to be agitated. Strong convection improves mass transfer significantly and ensures contact between the sample solution, SLM and acceptor solution [17]. While the benefits of agitation are important, the agitation equipment reduces the simplicity of the system. EME has potential for in-field operation, because equipment is sizeable to very small dimensions and because the power supply can be a battery, but the agitation is a challenge unless EME is performed under stagnant conditions. In EME without agitation (stagnant EME), the sample solution requires continuous contact with the SLM [14]. Due to a lack of convection in the sample solution, extraction kinetics are slow and therefore, sample volume needs to be reduced or extraction times increased, as compared to EME under convective conditions. Three different approaches to stagnant EME have previously been

published. In one approach, five non-polar basic drugs were extracted by stagnant EME from 10  $\mu\text{L}$  urine and plasma samples in an open drop-to-drop setup. Extractions were limited to 5 min due to the open nature of the system, and longer extractions suffered from evaporative losses of acceptor solution [18]. In a second approach, four basic drugs were extracted from 70  $\mu\text{L}$  plasma in a hollow-fibre setup. The system suffered from diminishing recoveries with extraction times exceeding 5 min, limiting the possibility for exhaustive extraction [19]. The third approach is micro-EME using sample and acceptor volumes of 1–5  $\mu\text{L}$ . This approach shows great potential and offers sample clean-up, high recoveries and short extraction times (<10 min), but is limited to very small sample volumes [20].

In the present work, a fully closed experimental system for stagnant EME was developed and optimized to investigate the potential for exhaustive extraction. Extraction performance was evaluated with the five non-polar basic analytes loperamide, haloperidol, nortriptyline, methadone, and pethidine from aqueous buffer solutions and whole blood. The stagnant EME setup was evaluated in combination with LC-MS/MS as a highly specific instrumental method to initially verify the reliability of the concept.

## 2. Experimental

### 2.1. Chemicals

NPOE, 2-nitrophenyl pentyl ether (NPPE), acetonitrile (ACN), formic acid (FA), loperamide hydrochloride, methadone hydrochloride, nortriptyline hydrochloride, pethidine hydrochloride, haloperidol hydrochloride, phosphoric acid, sodium dihydrogen phosphate and sodium chloride (NaCl) were purchased from Sigma Aldrich (St Louis, MO, USA). Pooled drug-free whole blood samples were obtained from four volunteers at the University of Oslo, Department of Pharmacy (Oslo, Norway). Deionized water was obtained from a Milli-Q water purification system (Molsheim, France).

A stock solution of haloperidol, loperamide, methadone, nortriptyline, and pethidine was prepared by dissolving 10 mg of each analyte in 10 mL ethanol. The stock solution was diluted with phosphate buffer or phosphoric acid to a concentration of 5 or 10  $\mu\text{g/mL}$  of each analyte.

### 2.2. Whole blood

Pooled frozen whole blood samples were obtained from four healthy subjects at the University of Oslo. The authors state that they have obtained appropriate institutional review board approval and have followed the principles outlined in the Declaration of Helsinki for all human experimental investigations. Subsequently, after thawing, the blood samples were diluted 1:1 with 10  $\mu\text{g/mL}$  of the five basic analytes in either 20 mM phosphate buffer or 100 mM phosphoric acid. For the evaluation experiments, the blood samples were diluted 1:1, making concentrations of methadone of 1.5, 0.5, 0.2, 0.1 or 0.05  $\mu\text{g/mL}$ .

### 2.3. High-performance liquid chromatography with UV-detection (HPLC-UV)

HPLC-UV conditions have been described previously [21]. In brief, the chromatographic separation was accomplished with a Phenomenex (Torrance, CA, USA) column (Gemini 5u C18 110A (inner diameter (ID): 1.0 mm, length (L): 150 mm, particle size (dp): 5.0  $\mu\text{m}$ ). Mobile phase A consisted of 20 mM FA and ACN (95:5, v/v), and mobile phase B consisted of 20 mM FA and ACN (5:95, v/v). The injection volume was set to 20  $\mu\text{L}$  with a mobile phase flow of 0.4 mL/min. The chromatographic separation was performed over 15 min with a linear gradient from 0% to 40% mobile phase B. This composition was kept constant for 2 min before the system was flushed with 80% mobile phase B for 2 min. Finally, the gradient was re-established at 0% mobile phase B for 6 min prior to a new injection. The column temperature was set to 60 °C. The UV detection was performed at 214 nm.

### 2.4. High performance liquid chromatography-mass spectrometry (HPLC-MS)

The chromatographic separation was carried out on 5  $\mu\text{L}$  sample at 50  $\mu\text{L}/\text{min}$  with a Dionex (Sunnyvale, CA, USA) Ultimate 3000 pump module coupled to a Phenomenex (Torrance, CA, USA) Gemini 5u C18 110A (ID: 1.0 mm, L: 150 mm, dp: 5.0  $\mu\text{m}$ ). The chromatographic separation was performed over 11 min with a linear gradient from 2 to 90% mobile phase B (ACN/water/FA:95/5/0.1 v/v/v) followed by 5 min wash and a subsequent reconditioning for 10 min with mobile phase A (ACN/water/FA:5/95/0.1 v/v/v). Detection was performed with a Thermo Fischer TSQ Quantum Access operated in SRM with positive mode electrospray ionization (ESI). The applied voltage was set to 4 kV, heated capillary: 270 °C, CID at 20 eV, collision gas pressure to 1.7 mTorr and 0.5 s scan intervals. The Q1 and Q3 resolution were both set to 0.7 at full width at half maximum (FWHM). The MS transitions used for methadone were  $m/z$  310.20  $\rightarrow$  219.45,  $m/z$  310.20  $\rightarrow$  223.13, and  $m/z$  310.20  $\rightarrow$  265.10. The system was operated by Dionex Chromeleon Xpress and Thermo Fischer Xcalibur 2.2 (version: SP 1.48).

### 2.5. Electromembrane extraction

The EME equipment used is shown in Fig. 1. A laboratory-built 8-well plate made from polyoxymethylene (POM) served as sample compartment. Each well had a radius of 3.3 mm, a depth of 1.80 mm, and a volume of 50  $\mu\text{L}$ . For the optimization of sample volume, a similar POM plate was used, with a radius of 3.3 mm and four different wells with depths of 1.8, 2.9, 4.6, and 6.4 mm. A hole with 0.5 mm width (W) was drilled in the bottom of each well and silver wire (W: 0.5 mm) electrodes (K.A. Rasmussen, Hamar, Norway) was inserted through the holes. The electrodes were glued to the bottom of the plate to avoid any leakage of the sample solution.

A 96-well MultiScreen-IP filter plate with polyvinylidene fluoride (PVDF) filter membranes (100  $\mu\text{m}$  thickness and 0.45  $\mu\text{m}$  pore size, Merck Millipore Ltd., Carrigtwohill, Ireland) was cut into single wells and served as both the membrane for the SLM and compartment for the acceptor solution. An ES 0300-0.45 (Delta Elektronika BV, Zierikzee, The Netherlands) was used as the power supply.

Firstly, the sample solution was pipetted into a well of the 8-well plate. Secondly, 3  $\mu\text{L}$  of NPOE was pipetted on the filter of a single SLM/acceptor compartment and allowed to immobilize (5–10 s). Thirdly, 50  $\mu\text{L}$  acceptor solution was pipetted into the SLM/acceptor compartment. A silver electrode (W: 0.5 mm) was inserted through and glued to a septa seal (Thermo Scientific, Waltham, MA, USA), which fitted exactly to the top of the SLM/acceptor compartment and functioned both as lid and cathode. Prior to extraction, the electrodes were connected to an external power supply and coupled to a Metrahit X-tra multimeter (Gossen-Metrawatt, Nürnberg, Germany) in order to measure the current. After extraction, the acceptor solution was pipetted and transferred to HPLC glass vials.

### 2.6. Calculations

The EME recovery was calculated using the following equation:

$$\text{Recovery}(\%) = \frac{C_{ap} V_{ap}}{C_{dp} V_{dp}} \times 100 \quad (1)$$

where  $C_{ap}$  is the final concentration of the analyte in the acceptor phase,  $C_{dp}$  is the initial analyte concentration in the donor phase,  $V_{ap}$  is the volume of the acceptor phase and  $V_{dp}$  is the volume of the donor phase.

## 3. Results and discussion

As illustrated in Fig. 1, both the sample and acceptor solution was in closed compartments designed to avoid evaporation, which has been a limitation in previous work [18]. The performance of the system was evaluated based on extraction recovery, accuracy, and precision of the model analytes. The impact of design and operational parameters, including sample well depth, electrode geometry, voltage and time, were investigated in regards to performance. Optimization was performed stepwise; first from aqueous buffer solutions and subsequently from whole blood samples.

The five model analytes haloperidol, loperamide, methadone, nortriptyline, and pethidine were selected as ideal candidates for EME (mono-basic and  $\log P > 2$ ) and enabled comparison of performance with EME in other technical configurations. The analytes were dissolved in aqueous solutions of phosphate buffer (pH 2.7) to ensure complete ionization and to avoid pH changes due to electrolysis [1]. The same buffer was also used as acceptor solution in

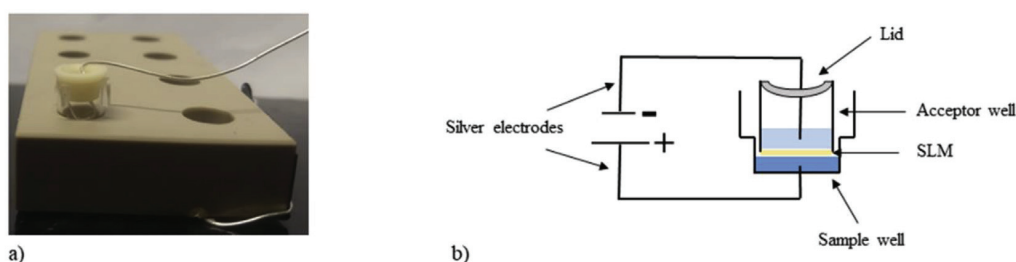


Fig. 1. Extraction setup. a) Picture of the 8-well sample plate and acceptor well clamped, b) schematic illustration of an EME-cell.

order to promote the dissolution of analytes from the SLM. NPOE was chosen as SLM because of excellent extraction performance for non-polar basic compounds [1]. To promote the extraction of positively charged basic analytes from the sample to the acceptor solution, the negative electrode (cathode) was placed in the acceptor solution and the positive electrode (anode) in the sample solution.

It should be emphasized that the current work is conceptual, and experiments were conducted in a model system to study fundamental aspects. The analytes were selected as model substances only, to provide data comparable with previous publications. All measurements were by HPLC-UV and HPLC-MS, and these are not in-field instruments. However, they were used as highly reliable instrumental methods, to investigate the reliability of the concept of stagnant EME. From the current proof-of-concept, future work should be focused on combinations with portable analytical devices (PADs) and applications where PADs provide sufficient selectivity and sensitivity.

### 3.1. Theory

Previously published work on stagnant EME performed in a drop-to-drop system developed the following equation for extraction recovery as a function of time ( $R_i(t)$ ) [18]:

$$R_i(t) = 100\% \cdot \frac{2A}{V_s} \sqrt{\frac{D_i}{\pi}} t \quad (2)$$

where  $A$  is the surface area of the SLM,  $V_s$  is sample volume,  $D_i$  is the diffusion coefficient of the analyte, and  $t$  is the extraction time. Increasing  $A$  enhances the extraction recoveries, but in this work  $A$  was constant and determined by the dimensions of the membrane (28 mm<sup>2</sup>).  $D_i$  was inherent for each analyte, leaving only  $V_s$  and  $t$  to be operational parameters. From Eq. (2), decreasing the sample volume and increasing the extraction time will theoretically increase the extraction recovery.

Even though extraction in EME is driven by an electrical field, the major voltage drop is across the SLM [21], and therefore mass transfer in bulk sample is mainly by passive diffusion. In traditional EME performed under convective conditions, agitation enhances this mass transfer. In stagnant EME, however, the analyte concentration in the sample/SLM intersection is depleted rapidly, and this seriously affects extraction kinetics. In order to improve the kinetics, the average diffusion distance (distance from the centre of the sample volume and to the SLM) should be as short as possible. Therefore, the depth of the sample wells (sample compartment geometry) was optimized. Even though temperature of the sample and acceptor solution has been reported to increase the diffusion of analyte [22], all extractions were performed at room temperature to keep the EME system simple.

### 3.2. Initial optimization experiments

#### 3.2.1. Sample compartment geometry and volume

Four different sample well depths were tested (1.8, 2.9, 4.6 and 6.4 mm). As the cross-sectional area of the sample wells was constant and identical to the area of the SLM, the corresponding sample volumes were 50, 80, 130 and 180  $\mu$ L, respectively. In all cases, the sample wells were loaded to maximum where the sample was in contact with the entire cross-sectional area of the SLM. Extractions were performed for 10 min at 75 V, yielding a current of >5  $\mu$ A with NPOE as SLM, and with 50  $\mu$ L of 20 mM phosphate buffer (pH 2.7) as acceptor solution. A similar experiment was conducted with NPPE as organic solvent in the SLM. However, recoveries were lower with NPPE and consequently,

NPOE was chosen as organic solvent in further experiments. As the results in Fig. 2 clearly demonstrate, extraction recoveries increased as a function of reduced well depth and  $V_s$ , in accordance with the theoretical model presented in Eq. (2). As sample well depth and volume decreased, the average diffusion distance decreased and the electrical field in the sample (V/cm) increased. These effects concurrently yielded higher extraction recoveries. Therefore, 50  $\mu$ L was selected as the optimal sample volume and 1.5 mm as the optimal well depth for the further experiments. Because acceptor volume was 50  $\mu$ L, no enrichment was achieved in current setup, but as discussed later, the system provided excellent sample clean up. The sample filled the entire well without any air bubbles present. Therefore, experiments with mechanical movement of the device, manually and by a laboratory agitator, induced neither convection nor improved mass transfer.

#### 3.2.2. Electrode geometry

In order to test the impact of electrode geometry on the electrical field distribution and extraction performance, extraction with three different cathodes (rod, u-shaped, and coil) were tested. All electrodes were placed with the same distance to the SLM, and extractions were performed with similar conditions as in section 3.2.1. Neither recovery, reproducibility, nor extraction current was significantly affected by the difference in electrode geometry. The electrical field of the three different electrodes was modeled in Comsol Multiphysics® 5.4 (COMSOL Inc., USA) which revealed that in all three configurations, less than 0.1% of the total applied voltage was lost to the acceptor and donor solutions (Fig. 3). Since the membrane has a conductivity that is approximately five orders of magnitude lower than that of the liquid phases [21], it dominates the resistance of the system and hence the shape of the electrodes is not very crucial for the performance. The rod-shaped electrode was selected for further experiments, based on its simplicity.

#### 3.2.3. Voltage

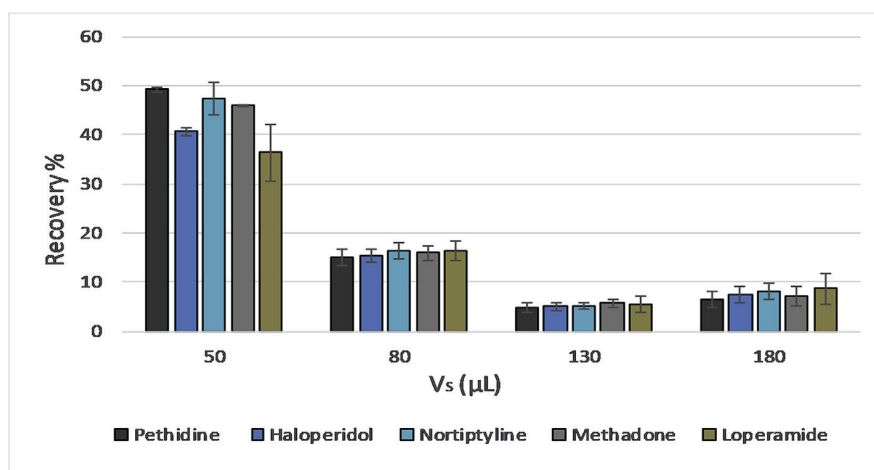
Different voltages in the range 0–100 V were tested and the recoveries after 10 min of extraction are presented in Fig. 4. As expected, no analytes were detected in the acceptor solution with 0 V. Thus, no mass transfer occurred across the SLM without voltage. Increasing the voltage up to 10 V provided recoveries between 30 and 45%, while further increases in voltage yielded only modest increases in recovery. Lower overall RSDs were observed at higher voltage; therefore, 75 V was selected for extraction from aqueous buffer solutions. In all experiments, the current was initially 10–20  $\mu$ A when the voltage was applied, followed by a quick decline to a stable current at 1–2  $\mu$ A throughout the rest of the extraction. This current profile is typical for EME of non-polar basic drugs using NPOE as SLM [23].

#### 3.2.4. Extraction time

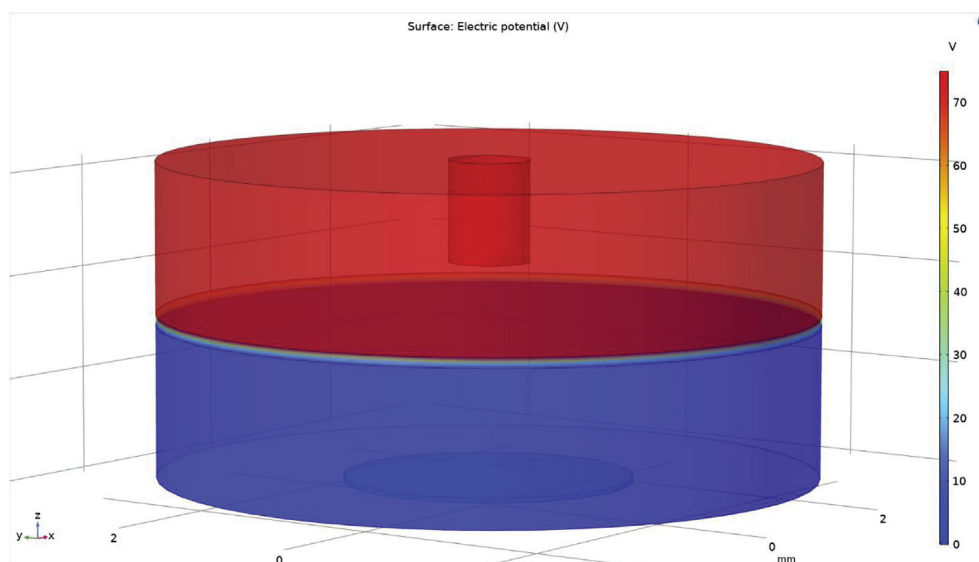
Extraction times between 2 and 60 min were tested, from 50  $\mu$ L sample using a voltage of 75 V (Fig. 5). After 2 min, approximately 15% recovery was achieved for the model analytes. This is in agreement with previously published experiments, where analyte in the sample/SLM intersection was extracted quickly over to the acceptor solution [18]. Because the current EME system was totally closed, extraction time was not limited, and experiments up to 60 min were conducted successfully. Recoveries increased with extraction time up to 60 min. Recoveries after 60 min ranged between 75 and 87% (Table 3), and are considered as exhaustive or near-exhaustive extraction. This was based on analysis of the sample solution after extraction, which was depleted. The extraction recoveries were superior to the data reported with previous stagnant EME systems [18,19].

In additional experiments, the acceptor solution was weighed





**Fig. 2.** Extraction recovery versus sample volume ( $V_s$ ) with error bars (SD). Sample: 5  $\mu\text{g}/\text{mL}$  of pethidine, haloperidol, nortriptyline, methadone and loperamide in 10 mM phosphate buffer (pH 2.7), acceptor solution: 50  $\mu\text{L}$  10 mM phosphate buffer (pH 2.7), SLM: 3  $\mu\text{L}$  NPOE, voltage: 75 V and extraction time: 10 min  $n = 3$ .



**Fig. 3.** Plot from Comsol Multiphysics® showing potential distribution for rod-shaped electrode.

before and after extraction. The measurements, presented in Table 1, revealed that no significant evaporative losses of acceptor solution were observed even after 60 min of operation. Clearly, the current closed extraction system was much more reliable as compared to the open drop-to-drop extraction systems reported previously for stagnant EME [18]. The robustness of the closed system provided a possibility for long-term operation, which was required under stagnant conditions in order to obtain high recoveries.

### 3.2.5. Ion balance

In previous work, the steady-state flux of an ion across the SLM was described by the following equation (Eq. (3)) [24].

$$J_i = \frac{D_i}{h} \left(1 + \frac{v}{\ln \chi}\right) \left(\frac{\chi - 1}{\chi - \exp(-v)}\right) (c_i - c_{i,0} \exp(-v)) \quad (3)$$

where  $D_i$  is the diffusion coefficient of the ion,  $h$  is the thickness of

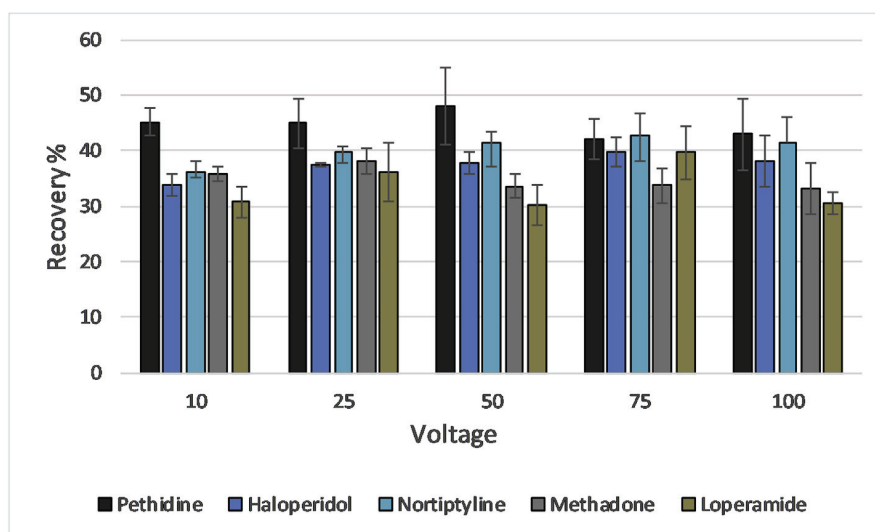
the SLM,  $v$  is the dimensionless driving force and  $\chi$  the ion balance. Ion balance ( $\chi$ ) is defined as the ratio of total ionic concentration in the sample divided by the total ionic concentration in the acceptor solution:

$$\chi = \frac{\sum_i c_{i,cations} + \sum_k c_{k,anions}}{\sum_i c_{i,0,cations} + \sum_k c_{k,0,anions}} \quad (4)$$

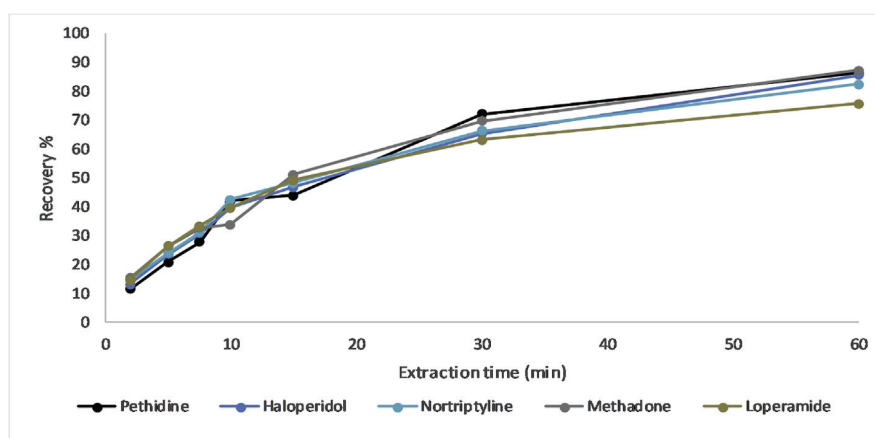
Theoretically, the flux of ions across the SLM increases with increasing ion balance. The main contribution of ions in both the sample and acceptor solution will be the buffer ions since the contribution from dissolved analytes is negligible. Consequently, the experiments in section 3.4.1–3.4.4 were performed with  $\chi = 1$  since 10 mM phosphate buffer was utilized both as sample and acceptor solution.

To evaluate the possible effect of ion balance, extractions were performed with  $\chi$ -values of 0.04, 0.4, and 1 with voltage of 75 V and 10 min extraction time. As the results summarized in Table 2 demonstrates, neither recoveries nor repeatability was affected





**Fig. 4.** Extraction recovery versus extraction voltage with error bars (SD). Sample: 50  $\mu$ L of 5  $\mu$ g/mL pethidine, haloperidol, nortriptyline, methadone and loperamide in 10 mM phosphate buffer (pH 2.7), acceptor solution: 50  $\mu$ L 10 mM phosphate buffer (pH 2.7), SLM: 3  $\mu$ L NPOE and extraction time: 10 min n = 3.



**Fig. 5.** Extraction recovery versus extraction time. Sample 50  $\mu$ L of 5  $\mu$ g/mL pethidine, haloperidol, nortriptyline, methadone and loperamide in 10 mM phosphate buffer (pH 2.7), acceptor solution: 50  $\mu$ L 10 mM phosphate buffer (pH 2.7), SLM: 3  $\mu$ L NPOE, voltage: 75 V n = 3.

**Table 1**

Measurement of acceptor solution volume before and after EME.

Condition	Mean (n = 3) $\pm$ SD
Before EME	50.2 $\pm$ 0.2 $\mu$ L
After 10 min EME	46.4 $\pm$ 2.0 $\mu$ L
After 60 min EME	47.4 $\pm$ 1.7 $\mu$ L

significantly by the ion balance. We also tested 2.5% (w/w) NaCl in the sample, but again recoveries were not affected. This further supports that the limiting step in stagnant EME is not the flux of analytes across the SLM, as is the case in EME under convective conditions, but rather the mass transfer in the sample.

### 3.3. Extraction from whole blood

The optimized EME conditions used with the aqueous buffer solutions were tested on thawed whole blood samples to investigate capability with real samples. The blood samples were diluted

**Table 2**

Extraction recoveries as a function of ion balance.

Ion balance	Recovery (%) $\pm$ SD (n = 3)		
	X = 1 <sup>a</sup>	X = 0.4 <sup>b</sup>	X = 0.04 <sup>c</sup>
Pethidine	43 $\pm$ 0.3	45 $\pm$ 2.2	48 $\pm$ 2.2
Haloperidol	40 $\pm$ 2.3	39 $\pm$ 3.1	42 $\pm$ 2.1
Nortriptyline	41 $\pm$ 1.7	42 $\pm$ 2.5	43 $\pm$ 1.7
Methadone	41 $\pm$ 0.8	42 $\pm$ 3.6	46 $\pm$ 1.1
Loperamide	40 $\pm$ 2.5	37 $\pm$ 3.1	44 $\pm$ 2.8

<sup>a</sup> Sample: 10 mM, acceptor: 10 mM.

<sup>b</sup> Sample: 10 mM, acceptor: 25 mM.

<sup>c</sup> Sample: 1 mM, acceptor: 25 mM.

1:1 with 10  $\mu$ g/mL of the five model analytes in either 20 mM phosphate buffer or 100 mM phosphoric acid to a final analyte concentration of 5  $\mu$ g/mL. pH of the diluted whole blood samples were measured to be 6.8 and 3.5, respectively. In the initial experiments, a voltage of 75 V was applied for 10 min, yielding recoveries in the range 10–20%. Increasing the voltage to 125 V nearly doubled

**Table 3**

Analytical data from HPLC-UV analysis of EME extracts after 60 min extractions.

Analyte	Buffer solution					Whole blood				
	Pet	Hal	Nor	Met	Lop	Pet	Hal	Nor	Met	Lop
ER (%)	86	85	82	87	75	96	74	65	87	57
RSD (n = 3)	3	8	2	6	12	11	12	7	12	3
LOD ( $\mu\text{g/mL}$ )	0.9	0.1	0.2	0.3	0.2	0.9	0.5	0.4	0.3	0.2
LOQ ( $\mu\text{g/mL}$ )	2.8	0.5	0.7	1.1	0.8	2.8	1.6	1.5	1.1	0.8

Pet-pethidine, Hal-haloperidol, Nor-nortriptyline, Met-methadone, Lop-loperamide.

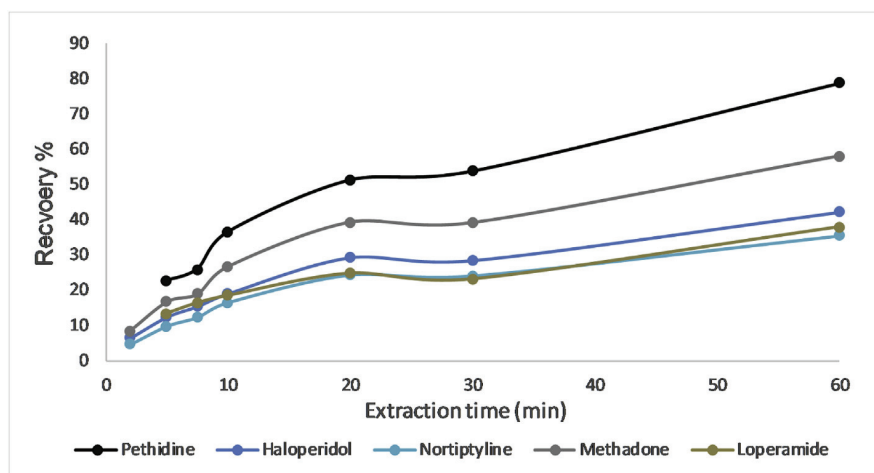
ER: extraction recovery.

LOD: limit of detection, LOQ: limit of quantification.

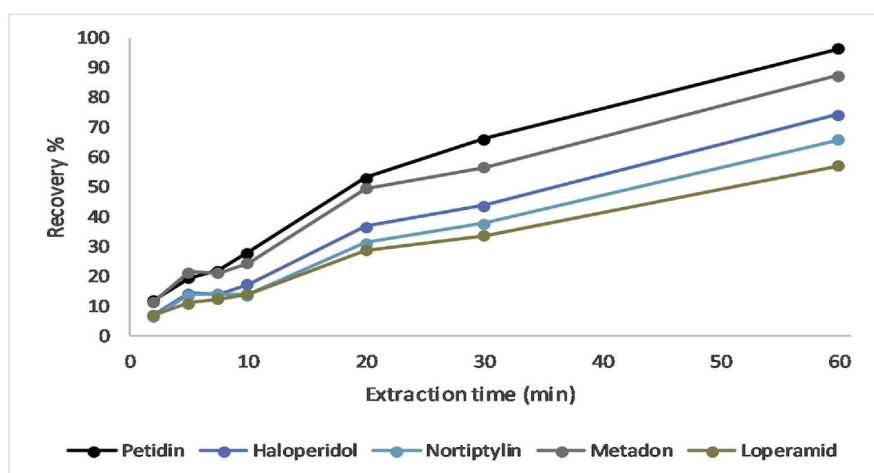
the recoveries (18–38%) and current profiles were still stable ( $\leq 5 \mu\text{A}$ ). Therefore, a potential of 125 V was used as optimal voltage for whole blood samples. Compared to aqueous buffer solutions, the extraction kinetics from whole blood samples with pH 6.8 were considerably slower and displayed larger compound-to-compound variability. Thus, after 60 min, the recoveries for pethidine and

nortriptyline were 79% and 35%, respectively (Fig. 6).

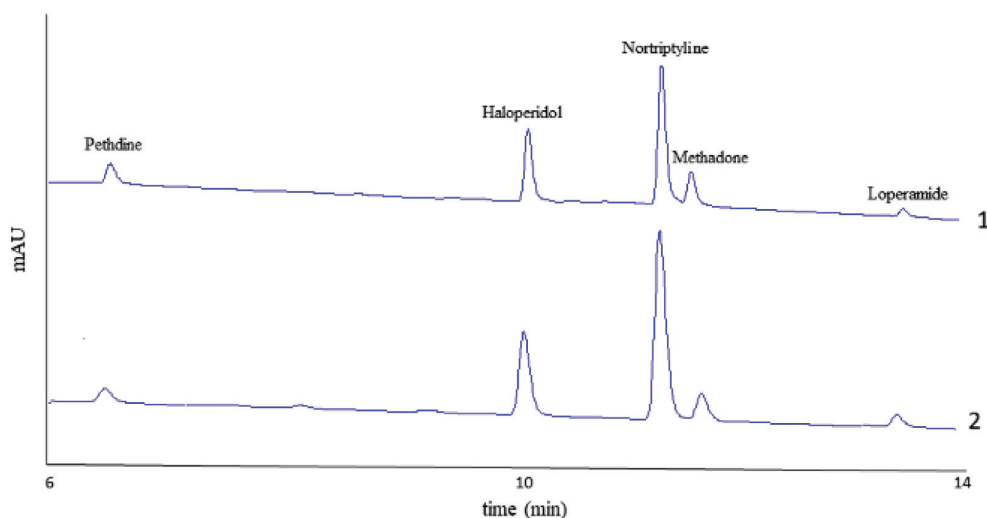
Since the diffusion of analytes in the sample solution is considered an essential parameter in stagnant EME, the higher viscosity of whole blood [25] compared to aqueous buffer solution, contributed to slower kinetics. Based on reported diffusion coefficients for the model analytes in water, where methadone ( $10.3 \cdot 10^{-6} \text{ cm}^2/\text{s}$ ) and nortriptyline ( $8.0 \cdot 10^{-6} \text{ cm}^2/\text{s}$ ) have the highest values [18], the differences in extraction kinetics were not clearly correlated with diffusivity. Apparently, the differences in extraction kinetics more correlated with reported values for protein binding [26], pH of 6.8 is near physiological pH and the protein binding equilibrium is to a large degree retained. Pethidine has a relatively low protein binding of 58%, while the other model analytes range between 89 and 97%. With low protein binding, more unbound analyte is available for electrokinetic migration. Extraction recoveries from the acidified blood samples (pH 3.5) were considerably higher than for pH 6.8 blood samples, with extraction recoveries ranging from 57 to 96% after 60 min (Table 3). The diffusivity remains the same, but the extraction kinetics were higher (Fig. 7), due to a lower degree of protein binding, resulting in



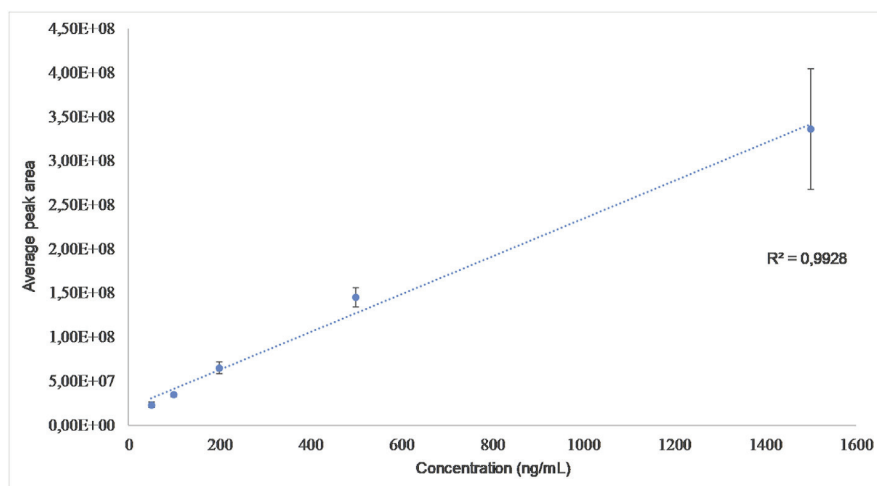
**Fig. 6.** Extraction recoveries versus extraction time from whole blood diluted 1:1 with 20 mM phosphate buffer. Sample 50  $\mu\text{L}$  of 5  $\mu\text{g/mL}$  pethidine, haloperidol, nortriptyline, methadone and loperamide in 1:1 diluted whole blood, acceptor solution: 50  $\mu\text{L}$  10 mM phosphate buffer (pH 2.7), SLM: 3  $\mu\text{L}$  NPOE, voltage: 125 V n = 3.



**Fig. 7.** Extraction recoveries versus extraction time from whole blood diluted 1:1 with 100 mM phosphoric acid. Sample 50  $\mu\text{L}$  of 5  $\mu\text{g/mL}$  pethidine, haloperidol, nortriptyline, methadone and loperamide in 1:1 diluted whole blood, acceptor solution: 50  $\mu\text{L}$  10 mM phosphate buffer (pH 2.7), SLM: 3  $\mu\text{L}$  NPOE, voltage: 125 V n = 3.



**Fig. 8.** 1) UV-chromatogram after 60 min EME from 50  $\mu\text{L}$  of 5  $\mu\text{g}/\text{mL}$  pethidine, haloperidol, nortriptyline, methadone and loperamide in 1:1 diluted whole blood, 2) UV-chromatogram after 60 min EME from 50  $\mu\text{L}$  of 5  $\mu\text{g}/\text{mL}$  pethidine, haloperidol, nortriptyline, methadone and loperamide in 10 mM phosphate buffer (pH 2.7). SLM: 3  $\mu\text{L}$  NPOE. Voltage 1) 125 V, 2) 75 V.



**Fig. 9.** Peak signal to methadone concentration in sample after 60 min EME of diluted whole blood with error bars (SD). Sample: 50  $\mu\text{L}$  of 1:1 diluted whole blood spiked to different concentration of methadone (50, 100, 200, 400, 1500 ng/mL), acceptor solution: 50  $\mu\text{L}$  0.1% formic acid, voltage: 125 V, and SLM: 3  $\mu\text{L}$  NPOE.  $n = 5$ .

a higher degree of unbound analyte. Still, a certain degree of protein binding is assumed, since the same compound-to-compound variability demonstrated with the blood samples with pH 6.8, was observed with the acidified blood samples.

Extraction times of 60 min are not commonly reported and might limit the throughput of stagnant EME. However, considering that the sample needs no further sample preparation, demands only minimal sample handling, and the potential for multiple extractions at the same time, stagnant EME can be comparable to other sample preparation techniques such as protein precipitation, which have lower time consumption but demands numerous sample handling steps as well as equipment.

The HPLC-UV chromatograms in Fig. 8 illustrates the selectivity of stagnant EME. The organic SLM and the electrical field discriminated most matrix compounds in whole blood, and the acceptor solution represented a highly purified extract of the sample based on UV-absorbance at 214 nm.

### 3.4. Evaluation of whole blood extraction

Stagnant EME was evaluated briefly in combination with LC-MS/MS for the determination of methadone in spiked whole blood samples. Methadone was selected as model analyte due to its relevance in drug monitoring both to control patient concordance and to make adequate dose adjustments. The MS/MS transitions were in accordance with what has been described previously [27]. Methadone was spiked into whole blood samples ( $n = 5$ ) below, within, and above the therapeutic window (i.e. 80–700 ng/mL) [28]. The calibration curve (Fig. 9) was linear in this range with a correlation of  $R^2 = 0.99$ . RSD values were 13%, 6%, 10%, 7% and 20% for 50, 100, 200, 500 and 1500 ng/mL, respectively. LOD and LOQ were calculated to be 0.4 ng/mL and 1.3 ng/mL using a signal to noise ratio (S/N) of 3:1 and 10:1 respectively. No methadone peaks with the respective ion transitions were observed in the analysis of blank whole blood samples ( $n = 3$ ).

#### 4. Conclusion

The present work has improved the understanding and operation of EME under stagnant conditions. The development of a closed extraction setup opens up the possibility for prolonged extraction times without evaporation of sample and acceptor solution. The diffusion distance (i.e. lower sample volume) in the sample proved to be the essential design parameter. Different electrode geometries, ion balances, and voltages provided only small variances in extraction recoveries. Exhaustive extraction was obtained from pure buffer solutions by extending the extraction time to 60 min. Extraction from spiked whole blood yielded lower recoveries, due to higher viscosity and protein binding. Evaluation of stagnant EME combined with LC-MS/MS for determination of methadone in whole blood yielded acceptable linearity and repeatability within the therapeutic window. This work has demonstrated that simplifying the EME setup (i.e. by removing the agitation device) provided a portable extraction system. This system has future potential for in-field operations and will be explored in upcoming research in combination with portable analytical devices and smartphones (not trace-level applications and LC-MS/MS).

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### CRediT authorship contribution statement

**Magnus Saed Restan:** Conceptualization, Formal analysis, Writing - review & editing. **Øystein Skjærvø:** Conceptualization, Formal analysis, Writing - review & editing. **Ørjan G. Martinsen:** Conceptualization, Formal analysis, Writing - review & editing. **Stig Pedersen-Bjerggaard:** Conceptualization, Formal analysis, Writing - review & editing.

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## Electromembrane extraction of sodium dodecyl sulfate from highly concentrated solutions

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This fundamental work investigated the removal of sodium dodecyl sulfate (SDS) from highly concentrated samples by electromembrane extraction (EME). SDS concentrations were in the range of 0.1–1.0% w/v, covering both sub- and super-critical micellar concentrations (CMC). Under optimal conditions, we extracted SDS from 100  $\mu\text{L}$  aqueous sample, through 3  $\mu\text{L}$  supported liquid membrane (SLM) and into 200  $\mu\text{L}$  10 mM NaOH in water as waste solution. The SLM comprised 1.0% w/w Aliquat 336 in 1-nonanol, and extraction voltage was 5 V. From 0.1% SDS samples, EME removed 100% during 30 minutes operation (100% clearance). SDS concentration above the critical micellar concentration (CMC) challenged the capacity of the system. Thus, to reach 100% clearance from 0.5% samples, we extracted for 120 minutes and replenished the SLM after 60 minutes. Increasing the membrane area of the SLM from 28  $\text{mm}^2$  to 43  $\text{mm}^2$  provided 100% clearance from 0.5% samples after 30 min EME. Complete clearance of 1.0% SDS samples was not achieved under the tested conditions, and maximal clearance was 60%. Mass balance experiments demonstrated that most of the removed SDS is trapped in the SLM, rather than transferring to the waste solution. For super-CMC samples, aggregation of SDS in the SLM exceeded the SLM capacity and impeded further mass transfer.

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### 1. Introduction

Electromembrane extraction (EME) was introduced in 2006<sup>1</sup> and provided a new approach to microextraction utilizing an electrical field as the main driving force. EME can be considered as a hybrid between liquid-phase microextraction (LPME)<sup>2</sup> and electrophoresis and is performed in a three-phase system with an aqueous sample (donor) solution, an aqueous acceptor solution, and an organic solvent immobilized inside the pores of a porous membrane sandwiched in between. The latter is termed a supported liquid membrane (SLM) and acts as a barrier between the two aqueous phases (sample and acceptor). Electrodes are placed in the sample and acceptor, and the electrical field is applied using an external power supply. Charged substances of interest (target substances) thus transfer from the sample, through the SLM, and into the acceptor by electrokinetic migration. In order to promote electrokinetic migration, the sample and acceptor are neutral or acidic for EME of basic substances, and the cathode

is located in the acceptor. For EME of acidic substances, the sample and acceptor are neutral or alkaline, and the anode is in the acceptor. The electrical field improves extraction kinetics, and the operator controls the selectivity by the polarity and magnitude of the electrical field, by the chemical composition of the SLM, and by pH. EME is compatible with complex real samples and can provide highly purified extracts.

To date, EME has been used for the extraction of hydrophilic drugs,<sup>3</sup> hydrophobic drugs,<sup>4</sup> acidic and basic drugs,<sup>5,6</sup> metals,<sup>7,8</sup> peptides,<sup>9</sup> inorganic ions,<sup>10</sup> contaminants in environmental samples<sup>11</sup> and wastewaters,<sup>12</sup> and drugs and drugs of abuse in whole blood,<sup>13</sup> plasma<sup>14</sup> and saliva.<sup>15</sup> In general, target substances have been at the trace level, corresponding to  $\text{ng ml}^{-1}$  (ref. 5 and 16–21) or low  $\mu\text{g ml}^{-1}$  concentrations.<sup>22–25</sup> Under such conditions, the individual phases of the EME system are far from saturation.

Recent work reported on EME of propranolol and probenecid templates used in the synthesis of molecularly imprinted polymers (MIPs), at the 1–1000  $\mu\text{g mL}^{-1}$  level.<sup>26</sup> In these experiments, the flux of target ions ( $\mu\text{g cm}^{-2} \text{min}^{-1}$ ) through the SLM was less than expected, based on the flux at lower concentrations and the area of the SLM. Lower mass transfer capacity was due to saturation of the SLM and to excessive boundary layer conditions in the donor/SLM interface. Another research reported on EME of selected basic drugs (haloperidol, amitriptyline, fluoxetine, and sertraline) at the

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0.5–200  $\mu\text{g mL}^{-1}$  level.<sup>27</sup> Again, mass transfer apparently decreased with increasing concentration levels. A third paper reported on EME of sodium chloride, with concentrations up to 500 mM NaCl (29 mg  $\text{mL}^{-1}$ ).<sup>28</sup>

Conceptually, EME research focuses on the extraction of target analytes from real samples and into pure buffer solution, followed by an instrumental analysis of the latter. This mode of EME is termed *extraction mode*. Potentially, EME can be used in a different way, namely to remove abundant matrix components from real samples prior to instrumental analysis of the latter. In this approach, termed *removal mode*, the acceptor serves as waste solution. EME in removal mode is not common, but the literature describes the removal of salt from micro-liter volumes of biological fluids,<sup>28</sup> removal of phospholipids from plasma samples,<sup>29</sup> and removal of fluorescein isothiocyanate (FITC) from tagged protein solutions.<sup>25</sup> Potentially, there are many similar applications where EME in removal mode may play a future role, including removal of surfactants from protein solutions prior to analysis by chromatography and mass spectrometry. However, the development of such applications requires additional fundamental knowledge. In particular, experiences above 1 mg  $\text{mL}^{-1}$ , where the system is close to saturation, are important to understand.

The purpose of the current fundamental paper was to investigate EME at 1–10 mg  $\text{mL}^{-1}$  concentrations. Attention was on mass transfer as a function of high concentration, to develop a better understanding of concentration limits related to EME in removal mode. Sodium dodecyl sulfate (SDS) was chosen as a target substance. SDS is an anionic detergent, and at the 0.02% level it has been reported to enhance mass transfer in EME.<sup>30</sup> However, much more commonly, SDS is used with biological cell samples during cell lysis to solubilize membrane and intracellular proteins.<sup>31</sup> In order to promote lysis, the concentration of SDS added to biological cell samples is 0.5–1.0% (5–10 mg  $\text{mL}^{-1}$ ).<sup>32</sup> This high concentration is undesirable in the final analysis due to its detrimental effects on chromatographic columns and contribution to ion suppression in mass spectrometry.<sup>33</sup>

## 2. Experimental

### 2.1. Chemicals and solutions

Stains-All (1-ethyl-2-[3-(1-ethylnaphtho[1,2-*d*]thiazolin-2-ylidene)-2-methylpropenyl]-naphtha-[1,2-*d*]thiazolium bromide, 3,3'-diethyl-9-methyl-4,5,4',5'-dibenzothiacarbocyanine), SDS, 2-nitrophenyl octyl ether (NPOE), 1-octanol, 1-nonanol, 1-decanol, and 2-decanone were purchased from Sigma Aldrich (St Louis, MO, USA). *N,N*-Dimethyl-formamide was purchased from Merck (Kenilworth, New Jersey, USA), Aliquat 366 was from Cognis Corporation (Cincinnati, Ohio, USA), NaOH was from VWR International (Radnor, PA, USA), and deionized water was obtained from a Milli-Q water purification system (Molsheim, France).

1.0 g of SDS was dissolved in 10 mM NaOH for the preparation of 1.0% w/v stock solution. All stock solutions were

stored at room temperature and protected from light. Working solutions were freshly prepared before experiments by dilution of the stock solutions with the same solvent. Preparation of Stains-All working solution was a 1 : 64 dilution of a stock solution, containing 2 mg  $\text{mL}^{-1}$  Stain-All in *N,N*-dimethyl-formamide, with deionized water. New stock solutions were prepared monthly and stored in darkness at 4 °C while working solutions were prepared daily right before measurements.

### 2.2. UV-spectrophotometry

Quantification of SDS was conducted on a Beckman 530 UV/Vis Spectrophotometer (Beckman Coulter, Fullerton, CA, USA), using a 10 × 10 mm quartz cuvette (Hellma Analytics, Mulheim, Germany). Calibration and verification of the instrument were according to the European Pharmacopeia. Quantification of SDS involved pipetting 1  $\mu\text{L}$  solution into a quartz cuvette, preloaded with 3 mL Stains-All working solution, and measuring the absorbance at 438 nm.<sup>34,35</sup>

### 2.3. Electromembrane extraction (EME)

Equipment used for EME has been described previously (Fig. 1).<sup>25</sup> In brief, a laboratory built stainless steel plate with 0.5 mL wells served as a compartment for the waste solutions and as an electrode (96-well waste reservoir plate). A 96-well MultiScreen-IP filter plate with 100  $\mu\text{m}$  thick polyvinylidene fluoride (PVDF) filter membranes of 0.45  $\mu\text{m}$  pore size (Merck Millipore Ltd, Carrigtwohill, Ireland) served as a compartment for the samples (96-well filter plate) and SLMs. A laboratory built aluminum plate with 96 rods tailor-made for the wells of the filter plate served as the second electrode (96-electrode plate). A model ES 0300-0.45 (Delta Elektronika BV, Zierikzee, The Netherlands) was used as a power supply, and a Vibramax 100 Heidolph shaking board (Kellheim, Germany) was used to agitate the entire extraction system.

EME involved the following procedure for each sample: (a) pipetting 200  $\mu\text{L}$  of 10 mM NaOH into the 96-well waste reservoir plate, (b) pipetting 3  $\mu\text{L}$  organic solvent onto the filter membrane of the 96-well filter plate, and (c) pipetting 100  $\mu\text{L}$  sample into the 96-well filter plate. Subsequently, we clamped the 96-well waste reservoir plate and filter plate and placed the 96-electrode plate on top. The rod electrodes of the electrode plate were then in contact with the samples. Finally, we connected the power supply to the waste reservoir plate (anode) and the electrode plate (cathode), and we conducted EME by simultaneous application of voltage and agitation.

### 2.4. Electromembrane extraction (EME) with an extended SLM area

EME with an extended SLM area was according to previous work.<sup>36</sup> In brief, a 100  $\mu\text{m}$  thick porous Accurel PP 1E (R/P) polypropylene membrane (Membrana, Wuppertal, Germany) was fastened to the wide end of a 10–1000  $\mu\text{L}$  pipette tip and sealed with a soldering iron. The total surface area of the membrane was measured as 43  $\text{mm}^2$ . A 0.5 mm hole was drilled in the bottom of a 2 mL Eppendorf tube, and a silver electrode (0.5 mm diameter) was inserted and fastened with

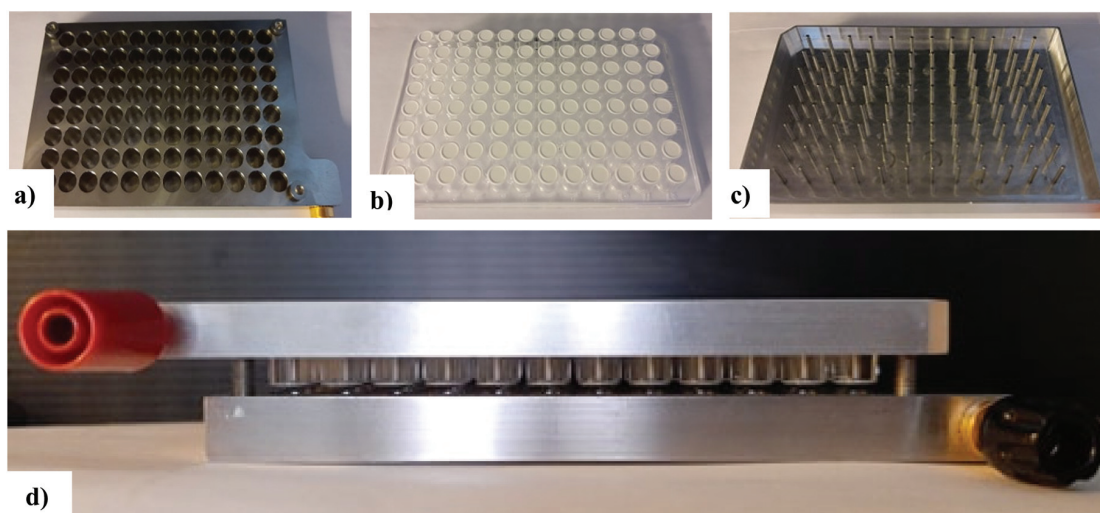


Fig. 1 96-Well EME setup. (a) 96-well waste reservoir plate, (b) 96-well filter plate, (c) 96-electrode plate, and (d) all plates clamped.

superglue. 500  $\mu\text{L}$  10 mM NaOH was pipetted into the Eppendorf tube and served as waste solution. Subsequently, the membrane was inserted into the Eppendorf tube, with a 3 mm clearance to the waste solution. 100  $\mu\text{L}$  sample solution was pipetted on top of the membrane, and a silver electrode was placed in contact with the sample solution. The electrodes were connected to a power supply and the extraction setup was agitated during extraction.

### 2.5. Calculations

For the calculation of SDS clearance, we used the following equation:

$$\text{Clearance} = \left( \frac{-n_{\text{S,Original}} - n_{\text{S,Final}}}{n_{\text{S,Original}}} \right) \times 100\% \quad (1)$$

Here,  $n_{\text{S,Original}}$  was the number of moles of SDS in the original sample prior to EME, and  $n_{\text{S,Final}}$  was the number of moles of SDS left in the sample after EME.

## 3. Results and discussion

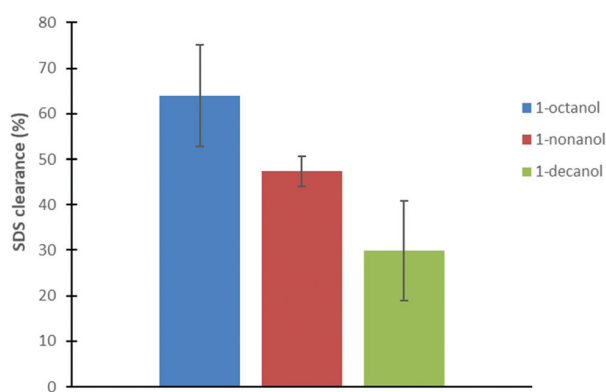
### 3.1. Operational principle

Fig. 1 illustrates the electromembrane extraction (EME) system used in this work. The complete setup consisted of a metallic 96-well waste reservoir plate, a 96-well filter plate, a 96-electrode plate, an agitator, and a power supply. The waste solution and electrode plates were laboratory-made, while the other items were commercially available. First, we pipetted 3  $\mu\text{L}$  of organic solvent to a filter in the 96-well filter plate. The organic solvent immobilized in the pores due to capillary forces and served as a supported liquid membrane (SLM). Second, we pipetted 100  $\mu\text{L}$  aqueous sample containing SDS into the 96-well filter plate above the SLM. Third, we pipetted 200  $\mu\text{L}$  of waste solution (water or 10 mM NaOH) to a well at a corresponding position in the 96-well waste solution plate.

Finally, we clamped together the three plates like a sandwich and placed them on the agitator. We connected the power supply with the waste solution plate and the electrode plate, and we applied voltage and agitation simultaneously to initiate extraction. For the extraction of SDS (negatively charged), the waste solution plate served as the anode (+) and the electrode plate served as the cathode (-).

### 3.2. Extraction under sub-critical micellar concentrations

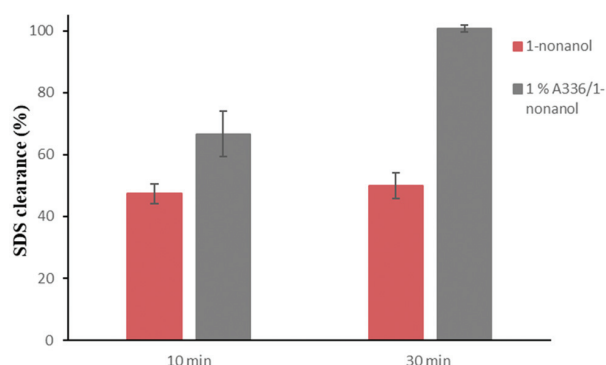
SDS forms micelles in water at concentrations higher than 0.237%.<sup>37</sup> In a first set of experiments, we performed EME from samples under sub-critical micellar conditions with 0.1% SDS (1 mg mL<sup>-1</sup>) in pure water. The concentration level of SDS was between 10<sup>3</sup> and 10<sup>6</sup> times higher than typical concentrations of target substances in published EME articles.<sup>5,22</sup> From previous EME experiments, alcohols are ideal SLM solvents for the extraction of negatively charged analytes.<sup>38</sup> This is mainly due to the hydrogen bond donor properties of alcohols, which promote partition of negatively charged analytes (hydrogen bond acceptors) into the SLM. Since the SLM acts as a barrier between the aqueous sample and the waste solution, the solvent should be immiscible with water in order to avoid leakage. On the other hand, log  $P$  should not exceed 5.5 in order to facilitate partition of charged compounds into the SLM.<sup>39</sup> Considering these factors, we tested 1-octanol, 1-nonanol, and 1-decanol for the extraction of SDS (Fig. 2). EME with 1-octanol as the SLM gave an SDS clearance of 64% after 10 minutes with 25 V. The higher alcohols 1-nonanol and 1-decanol were less efficient and provided 47% and 29% clearance, respectively. These differences were most likely due to differences in viscosity and polarity of the solvents, which in turn affected SDS diffusion and partition. We tested NPOE and 2-decanone, which are common EME solvents, under similar extraction conditions, but they were as expected less efficient than the alcohols (data not shown). Although we observed the



**Fig. 2** SDS clearance with different SLMs. Sample solution: 100  $\mu\text{L}$  0.1% SDS in 10 mM NaOH, waste solution: 200  $\mu\text{L}$  10 mM NaOH, SLM: 3  $\mu\text{L}$  organic solvent, extraction time: 10 min. Error bars represent standard deviation (SD,  $n = 3$ ).

highest SDS clearance with 1-octanol, we continued with 1-nonanol as the SLM due to lower solubility in water. This improved the stability of the EME system as evidenced by stable extraction current as monitored during EME (current profile).

In the following experiment, we extended the extraction time from 10 to 30 minutes with 1-nonanol as the SLM. However, this did not improve the SDS clearance and the system removed no more than 50% of total SDS from the sample. Based on previous experience, the phase transfer catalyst Aliquat 336 (A336) was added to the SLM.<sup>25</sup> A336 is a lipophilic quaternary ammonium chloride salt that facilitates partition of negatively charged compounds through ionic interactions. This involves exchange of chloride ions with SDS during transfer across the SLM. With the addition of 1.0% (w/w) A336 to 1-nonanol, the SDS clearance reached 100% after 30 minutes of extraction (Fig. 3). Because the electrical resistance of the SLM decreased due to A336, the voltage was lowered to 5 V to avoid excessive electrolysis and potential



**Fig. 3** SDS clearance without and with Aliquat 336 in the SLM. Sample solution: 100  $\mu\text{L}$  0.1% SDS in 10 mM NaOH, waste solution: 200  $\mu\text{L}$  10 mM NaOH, SLM: 3  $\mu\text{L}$  1-nonanol or 1.0% A336/1-nonanol, extraction time: 10 or 30 min. Error bars represent standard deviation (SD,  $n = 3$ ).

Joule heating.<sup>40</sup> The average current profile for each extraction well was 40  $\mu\text{A}$  after initial voltage application, followed by a slow decrease and stabilizing at approximately 30  $\mu\text{A}$ . As a rule of thumb, current levels of 50  $\mu\text{A}$  per extraction well should be avoided.<sup>41</sup>

### 3.3. Extraction under super-critical micellar concentrations

In the next series of experiments, we conducted EME from 0.5 and 1.0% solutions of SDS. Now SDS was above the critical micellar concentration (CMC). EME was conducted for 30 minutes at 5 V, with 1.0% A336 in 1-nonanol as the SLM. Surprisingly, even though the SLM was in contact with a soap-type sample solution, the SLM remained intact. Clearly, capillary forces strongly immobilized the organic solvent in the porous structure of the PVDF filters. This was evident from the stable current profile throughout the extraction and visual inspection. The EME performance was lower above the CMC, and SDS clearance was 55% and 25% for 0.5 and 1.0% SDS, respectively.

In the following experiments, we tested different approaches to increase SDS clearance from super-critical micellar concentrations. First, we increased the A336 concentration from 1.0 and up to 10% (w/w). The resistance decreased with increasing concentration of A336, and therefore voltage was decreased to 1–2 V. Similar current profiles measured with 1.0% A336 in the SLM were achieved. Interestingly, clearance was almost unaffected by the increased level of A336. Thus, while mass transfer increased significantly up to 1.0% of A336, there was no major gain above this level. Second, we replaced the waste solution after 15 minutes, but SDS clearance remained unaffected. Third, we tested replenishment of the SLM after 15 minutes, and this gave some improvement of SDS clearance. Finally, we increased the extraction time to 120 minutes with replenishment of the SLM after 60 minutes, and this provided 100% SDS clearance from 0.5% samples. When the same conditions were applied to 1.0% SDS, the clearance was 60%. With 0.5 and 1.0% samples, the appearance of the SLM changed from transparent to cloudy white during the extraction. This cloudiness was more prevalent at higher concentrations of A336 in the SLM, and it was absent when we used pure 1-nonanol as the SLM.

### 3.4. Mass balance considerations

To understand the observations above, we performed a set of mass balance experiments. Both the sample and the waste solution were analyzed after extraction and based on mass balance the content of SDS trapped in the SLM was calculated. As shown in Table 1, we detected no SDS in 0.1% ( $\approx 1$  mg  $\text{mL}^{-1}$ ) samples after 30 minutes of EME. This confirmed 100% clearance as discussed above. Surprisingly, we detected no SDS in the waste solution. Thus, the total amount of SDS (= 0.1 mg) was trapped in the SLM. Since the volume of the SLM was 3  $\mu\text{L}$ , the final SDS concentration in the SLM was 33 mg  $\text{mL}^{-1}$ .

In the following experiment, we conducted EME from 0.5% samples ( $\approx 5$  mg  $\text{mL}^{-1}$ ). With this experiment, we exceeded the capacity of the system, and SDS clearance decreased to 55%.

**Table 1** Distribution of SDS after 3-phase EME

SDS concentration	With voltage			Without voltage		
	0.1%	0.5%	1.0%	0.1%	0.5%	1.0%
Sample	≤0.01%	45%	71%	50%	73%	81%
SLM	99.99%	47%	23%	50%	27%	19%
Waste solution	≤0.01%	8%	5%	≤0.01%	≤0.01%	≤0.01%

SDS content in the SLM is calculated by measurements performed on donor and waste solution after EME. SLM: 100% – donor concentration (%) – acceptor concentration (%). Sample: 100 μL SDS in 10 mM NaOH, SLM: 3 μL 1% A336/1-nonanol, waste solution: 200 μL 10 mM NaOH, and extraction time: 30 min ( $n = 3$ ).

Consequently, 45% of total SDS (total SDS = 0.5 mg) remained in the sample after extraction. With 0.5% samples, we detected 8% of the total SDS in the waste solution. Based on mass balance calculation, the SLM trapped 47% of total SDS. From 1.0% samples (total SDS = 1.0 mg), 71% of total SDS was left in the sample, 5% was detected in the waste solution, and 24% was trapped in the SLM. With both 0.5 and 1.0% samples, the SDS concentration in the SLM increased up to 80 mg mL<sup>-1</sup>, and SDS concentration was about 0.5 mg mL<sup>-1</sup> in the waste solution. The data with 0.5 and 1.0% samples were thus consistent.

The solubility of SDS in aqueous solution is more than 200 mg mL<sup>-1</sup>,<sup>42</sup> and therefore we initially considered aqueous 10 mM NaOH as an appropriate waste solution. However, we performed a simple liquid-liquid extraction experiment, with SDS in water as the aqueous phase and 1-nonanol with 1.0% A336 as the organic phase, and this demonstrated very high partition into the organic phase (partition coefficient higher than 1000). This is in accordance with the literature.<sup>42</sup> Thus, due to strong partition into the SLM, SDS transferred rapidly into the SLM, but for the same reason, transfer into the waste solution was strongly limited.

Due to the strong partition into the SLM, we repeated the mass balance experiment above without voltage (0 V). With all SDS concentrations, the SDS clearance was lower without voltage. Interestingly, without voltage, we were unable to detect SDS in the waste solution. This confirmed that the electrical field influenced the SDS partition.

### 3.5. Experiences with two-phase EME

Because transfer from the SLM to waste solution limited clearance capacity, we investigated a two-phase EME system in a separate set of experiments. We conducted EME from 1.0% and 0.5% SDS samples for 30 minutes, with 1-nonanol + 1.0% A336 serving both as the SLM and as the waste solution. The total volume of the organic phase was 275 μL. The electrical resistance was higher in the two-phase system, and therefore we increased the voltage to 30 V. This provided a current level of 40 μA per well, which was similar to that reported by experiments discussed in previous sections. Table 2 summarizes the experimental results. From 0.5% samples, SDS clearance was 75%, while the value decreased to 10% for 1.0% SDS samples. Conducting the same experiments with a closed circuit and 0 V, to evaluate contribution from pure diffusion, provided

**Table 2** Distribution of SDS after 2-phase EME

SDS concentration	With voltage		Without voltage	
	0.5%	1.0%	0.5%	1.0%
Sample	25%	90%	51%	75%
SLM/waste solution	75%	10%	49%	25%

SDS content in the SLM is calculated by measurements performed on donor and waste solution after EME. SLM: 100% – donor concentration (%) – acceptor concentration (%). Sample: 100 μL SDS in 10 mM NaOH, SLM: 3 μL % A336/1-nonanol, waste solution: 275 μL 1% A336/1-nonanol, and extraction time: 30 min ( $n = 3$ ).

slightly better results for the 1.0% SDS sample (25% clearance) while worse for the 0.5% sample (49% clearance).

Considering the high solubility of SDS in 1.0% A336/1-nonanol, the poor clearance both with and without voltage was surprising. However, due to the relatively large volume of organic solvents in the system, the electrical field (V cm<sup>-1</sup>) in the organic phase was less than that in the 3-phase system. This probably reduced the electrokinetic transport of SDS into the organic phase. Furthermore, the fraction of organic solvents immobilized in the filter was stagnant during extraction and this seriously limited mass transfer. Thus, the mass transfer capacity of the SLM was the main bottleneck when the system was applied at high concentrations of SDS.

### 3.6. Experiences with an extended SLM area

In order to evaluate the SDS clearance as a function of SLM surface area, we conducted a new set of experiments with a modified EME setup. This provided an SLM area of 43 cm<sup>2</sup>, as compared to 28 cm<sup>2</sup> in the previous experiments. The membrane was made of polypropylene, which together with polyvinylidene difluoride are the most common membranes in EME. The polypropylene membrane was attached to the head of a pipette tip and functioned as a SLM and reservoir for the sample solution as described in the Experimental section. With the increase in the surface area, 10 μL organic solvent was immobilized within the pores of the SLM. As expected, with the increased SLM area, the SDS clearance increased. With 10 μL 10% A336/1-nonanol as the SLM, a voltage of 1 V, and 30 min extraction, no SDS was detected in the 0.5% SDS samples after EME. Thus, the system completely removed



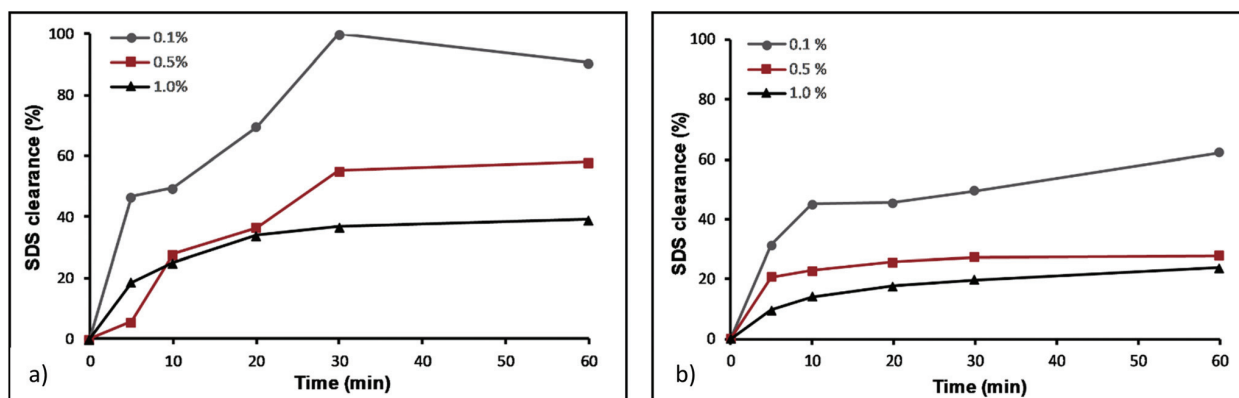


Fig. 4 SDS clearance as a function of extraction time. (a) SDS clearance with voltage and (b) SDS clearance without voltage. Sample: 100  $\mu\text{L}$  0.1, 0.5, 1.0% SDS in 10 mM NaOH, waste solution: 200  $\mu\text{L}$  NaOH, SLM: 3  $\mu\text{L}$  1.0% A336/1-nonanol, voltage: 5 V. RSD ( $n = 3$ ) < 15% for all time measurements.

0.5% SDS. With 1.0% SDS, clearance was now increased to 46%.

### 3.7. Impact of the electrical field

To assess the impact of the electrical field on the SDS clearance, we conducted a final set of time experiments with and without voltage. The extraction conditions were similar to the ones used in section 3.4. The results are presented in Fig. 4. Both with and without voltage, SDS mass transfer was fast the first 5–10 minutes, and SDS clearance increased until 30 min and then leveled off. Thus, extending the extraction time to 60 min gave only negligible gain in SDS clearance. A comparison of data showed that kinetics was more or less unaffected by the electrical field. This is consistent with the hypothesis above that the limit of the system is the SLM capacity. However, with voltage, the clearance was significantly higher than that without voltage. This supports the fact that partition of SDS into the SLM is favored by the electrical field. Clearly, even at very high concentrations, where the extraction system was close to saturation, there are still important benefits from application of voltage across the SLM.

## 4. Conclusion

The present work has for the first time demonstrated removal of sodium dodecylsulfate (SDS) using electromembrane extraction (EME). Highly concentrated samples of 0.1–1.0% SDS represented both sub- and super-critical micellar concentrations. Complete SDS removal was achieved for 0.1% SDS samples using 1.0% of the ionic phase transfer catalyst Aliquat 336 in 1-nonanol as the supported liquid membrane (SLM). Complete removal of SDS from 0.5 and 1.0% samples proved more challenging, and mass transfer suffered from accumulation of SDS within the SLM. Replenishment of the SLM or increasing the surface area circumvented some of these issues and complete removal was achieved for 0.5% SDS samples.

Based on the experiences from this work, EME removal of SDS shows potential. However, due to complexity, and to obtain a full understanding of fundamentals, we currently conducted experiments with pure solutions of SDS only. Therefore, forthcoming research should test the concept with real protein samples (biological samples), to evaluate performance under the influence of a complex biological matrix, and to study the behavior of target proteins. The experiences from this work have also improved upon the understanding of EME at very high concentration levels. Under such conditions, the benefit of the electrical field and electro-kinetic migration is less, compared with similar systems operated purely by diffusion. Similar observations are reported for a few other model compounds in the literature, and may be inherently connected with EME.

## Conflicts of interest

The authors declare no conflict of interest.

## Acknowledgements

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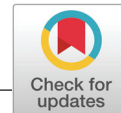
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## RESEARCH ARTICLE

# Influence of acid-base dissociation equilibria during electromembrane extraction

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Electromembrane extraction is affected by acid–base equilibria of the extracted substances as well as coupled equilibria associated with the partitioning of neutral substances to the supported liquid membrane. A theoretical model for this was developed and verified experimentally in the current work using pure 2-nitrophenyl octyl ether as supported liquid membrane. From this model, extraction efficiency as a function of pH can be predicted. Substances with  $\log P < 0-2$  are generally extracted with low efficiency. Substances with  $\log P > 2$  are generally extracted with high efficiency when acceptor  $\text{pH} < \text{p}K_{\text{aH}} - \log P$ . Twelve basic drug substances ( $2.07 < \log P < 6.57$  and  $6.03 < \text{p}K_{\text{aH}} < 10.47$ ) were extracted under different pH conditions with 2-nitrophenyl octyl ether as supported liquid membrane and fitted to the model. Seven of the drug substances behaved according to the model, while those with  $\log P$  close to 2.0 deviated from prediction. The deviation was most probably caused by deprotonation and ion pairing within the supporting liquid membrane. Measured partition coefficients ( $\log P$ ) between 2-nitrophenyl octyl ether and water, were similar to traditional  $\log P$  values between *n*-octanol and water. Thus, the latter have potential for  $\text{p}K_{\text{aH}} - \log P$  predictions.

**KEYWORDS**

acid–base equilibria, electromembrane extraction, microextraction, sample preparation

## 1 | INTRODUCTION

Electromembrane extraction (EME) was introduced in 2006 and offers rapid and selective extraction properties with the addition of an electrical field to a three-phase

microextraction system [1]. Conventional EME comprises an aqueous sample solution, which is separated from another clean aqueous solution (acceptor) by a porous membrane coated with an organic solvent. This membrane is termed supported liquid membrane (SLM) and acts as a barrier between the two aqueous solutions. The electrical field is applied by inserting an electrode to each aqueous solution and subsequently connecting them to a power supply. The versatility of EME is increased by the

**Article Related Abbreviations:** EME, electromembrane extraction; FA, formic acid; NPOE, 2-nitrophenyl octyl ether; SLM, supported liquid membrane

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possibility to adjust the magnitude (voltage) and direction (polarity) of the electrical field. For compounds to migrate from the sample solution, through the SLM, and to the acceptor solution, they need to be ionized. This is achieved by adjusting the pH of both sample and acceptor. For basic compounds, pH is neutral or acidic, while pH is neutral or basic for acidic compounds. Finally, in order to promote efficient extraction, the EME system needs to be agitated.

The number of potential applications is vast. Up to date, EME has been utilized, among others, for the extraction of polar drugs [2,3], nonpolar drugs [1,4] basic drugs [5,6], acidic drugs [7,8], metal ions [9], and peptides [10,11] from different matrices such as wastewater [12–14], urine [15–17], plasma [4,18,19], and whole blood [20–22]. Due to the effect of the electrical field, extraction kinetics are fast and exhaustive extraction can be achieved after only 5–10 min [1].

The main selectivity parameters of the EME system are the organic solvent in the SLM, pH and the electrical field. To ensure stable and selective extractions properties the choice of organic solvent for the SLM is crucial. The solvent needs to have a certain hydrophobicity to be immiscible with the surrounding aqueous solutions [23]. A hydrophobic SLM acts as a barrier against hydrophilic matrix compounds and provides efficient sample cleanup. However, to promote partition of ionized analytes into the SLM, the solvent should not be too hydrophobic. 2-Nitrophenyl octyl ether (NPOE) satisfies these criteria and has shown excellent extraction performance for moderately hydrophobic basic analytes ( $2 \leq \log P \leq 5$ ), with high recoveries and high selectivity [19]. For more hydrophilic analytes ( $\log P < 2$ ), ionic carriers are added to the SLM to facilitate partition [2]. Sample and acceptor pH are another selectivity parameters, controlling the degree of ionization based on analyte  $pK_a$  values. As a rule of thumb, the pH of the sample and acceptor should be adjusted to at least 2–3 pH units below the  $pK_a$  value for a given basic analyte [24].

From the discussion above, analyte extractability is typically linked to the  $\log P$  value, while pH conditions are linked to  $pK_a$  values. However, EME is affected by acid–base equilibria of the extracted species as well as coupled equilibria associated with the partitioning of neutral species into the SLM. The magnitude and direction of these effects are dependent on whether a base or an acid is extracted, and whether the neutral form is hydrophobic or hydrophilic. In the present work, we developed a model for predicting extraction recovery and pH conditions in EME based on the coupled relationship between  $pK_a$  and  $\log P$  of a given analyte. From this model, sample pH should generally be acidic for basic analytes, while pH in the acceptor should be used actively for controlling selectivity. Twelve hydrophobic basic model analytes, with  $\log P$  in the range

2.07–6.57 and  $pK_a$  in the range 6.03–10.47, were extracted and evaluated according to the theoretical model.

## 2 | MATERIALS AND METHODS

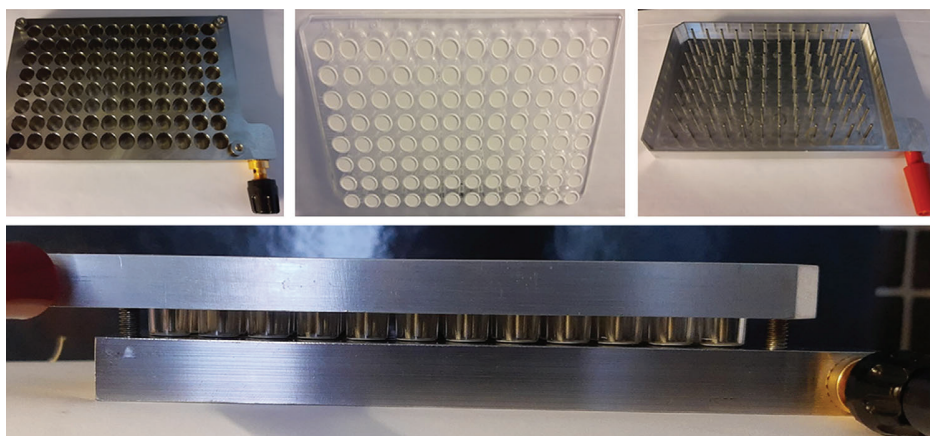
### 2.1 | Chemicals and solutions

Haloperidol and hydrochloride salts of pethidine, nor-triptyline, loperamide, lidocaine, promethazine, prochlorperazine, mianserin, and papaverine were purchased from Sigma–Aldrich (St Louis, MO, USA). Hydrochloride salts of cocaine, methadone, and methamphetamine were obtained from NMD (Oslo, Norway). Phosphoric acid, sodium dihydrogen phosphate, disodium hydrogen phosphate, sodium acetate, acetic acid, sodium formate, formic acid, hydrochloric acid, sodium carbonate, sodium bicarbonate, NPOE, 1-octanol, and methanol were purchased from Merck (Darmstadt, Germany). Ethanol was obtained from Arcus (Oslo, Norway) and water (18 M $\Omega$  cm) was obtained from a Milli-Q water purification system (Molsheim, France).

Stock solutions of 1 mg/mL were prepared for each analyte and all analytes combined. For each analyte, 10 mg was transferred to a vial, dissolved in ethanol (96%), and diluted to 10 mL with the same solvent. The stock solution with all analytes was prepared in the same manner, but with 10 mg of each analyte transferred to the same vial. The stock solutions were stored in darkness at 4°C. Sample solutions and HPLC-standards of 5  $\mu$ g/mL were prepared weekly by diluting 50  $\mu$ L of the stock solution to 10 mL with 10 mM HCl, 10 mM NaOH, diluted phosphoric acid, or the appropriate buffer.

### 2.2 | Equipment for EME

Equipment used for EME (Figure 1) has been described previously [25]. In brief, a laboratory built stainless steel plate with 96, 0.5 mL wells served as compartment for the sample solutions and as the anode (96-well sample plate). A 96-well MultiScreen-IP filter plate with polyvinylidene fluoride (PVDF) filter membranes of 0.45  $\mu$ m pore size (Merck Millipore, Carrigtwohill, Ireland) served as SLM and as compartment for the acceptor solutions (96-well filter plate). A laboratory built aluminum plate with 96 rods tailor-made for the wells of the MultiScreen plate, served as the cathode (96-electrode plate). A model ES 0300-0.45 (Delta Elektronika BV, Zierikzee, the Netherlands) was used as power supply, and a Vibramax 100 Heidolph shaking board (Kellheim, Germany) was used to agitate the entire extraction system.



**FIGURE 1** Electromembrane extraction setup. Left: 96-well sample plate, middle: 96-well filter plate, right: 96-electrode plate, bottom: all plates clamped

### 2.3 | EME procedure

EME involved the following procedure for each sample; 200  $\mu\text{L}$  sample solution was pipetted into the 96-well sample plate, followed by pipetting 3  $\mu\text{L}$  organic solvent (with 1–10  $\mu\text{L}$  micro-pipette) on to the filter membrane of the 96-well filter plate. The organic solvent was allowed to immobilize within the pores of the membrane before 100  $\mu\text{L}$  of acceptor solution was pipetted into the 96-well filter plate. Subsequently, the equipment was assembled by clamping the 96-well sample plate, the 96-well filter plate, and the 96-electrode plate together. The rod electrode of the 96-electrode plate was then in contact with the acceptor solution. Finally, the plates were fastened to an agitator and the power supply was connected to the sample plate (anode) and the electrode plate (cathode). EME was conducted by simultaneous application of 100 V and 900 rpm agitation. After extraction, the acceptor solutions were transferred to the HPLC–UV instrument for analysis. In the current fundamental work, enrichment was limited to a factor of 2. This may be increased by reducing the volume of the acceptor solution.

### 2.4 | Measurement of log P

Measurements of  $\log P_{\text{NPOE}}$  and  $\log P_{\text{OCTANOL}}$  were conducted with the same equipment presented in Section 2.3. Solutions of each basic analyte were made by diluting the stock solution with 10 mM NaOH (pH 12) to a concentration of 5  $\mu\text{g}/\text{mL}$ . This basic pH solution ensured that all analytes were neutral. Three microliters of either NPOE or 1-octanol were pipetted onto the filter of the 96-well filter plate. Subsequently, 200  $\mu\text{L}$  of sample solution was pipetted in the sample compartment of the 96-well filter plate. The filter plate was covered with a plastic lid to avoid evap-

oration. The complete setup was placed on an agitator and agitated for 180 min, with a speed of 900 rpm. After extraction, the sample solutions were transferred to the HPLC–UV instrument for analysis.

### 2.5 | High-performance liquid chromatography-ultraviolet

Quantification was performed using a Dionex UltiMate 3000 RS UHPLC system equipped with a UV-detector. The chromatographic separation was accomplished using an Acquity UPLC<sup>®</sup> HSS T3 column (100  $\times$  2.1 mm ID, 1.8  $\mu\text{m}$ ) from Waters (Wexford, Ireland). The mobile phase was kept at 0.4 mL/min, with mobile phase A consisting of 95:5 v/v 20 mM formic acid (FA) and methanol, and mobile phase B consisting of 5:95 v/v 20 mM FA and methanol. The separation was performed over 20 min with a linear gradient from 10% to 80% mobile phase B. The mobile phase composition was kept constant for 5 min to flush the system. Finally, the gradient was reestablished at 10% mobile phase B before the next sample injection. Injection volume was set to 20  $\mu\text{L}$ , detection wavelength to 214 nm, and column temperature to 60°C

### 2.6 | Calculations

The extraction recovery ( $R$  (%)) was calculated using the following equation:

$$R(\%) = \frac{C_{ap}V_{ap}}{C_{dp}V_{dp}} \times 100 \quad (1)$$

Here,  $C_{ap}$  is the final concentration of the analyte in the acceptor,  $C_{dp}$  is the initial analyte concentration in the sample,  $V_{ap}$  is the volume of the acceptor, and  $V_{dp}$  is the volume of the sample.

Calculation of  $\log P_{\text{NPOE}}$  and  $\log P_{\text{OCTANOL}}$  were according to the following equation:

$$\text{Log} \frac{[\text{analyte}]_{\text{org}}}{[\text{analyte}]_{\text{aq}}} = \text{Log} \frac{1 - 66.7 \times C_w}{C_w} \quad (2)$$

Here,  $C_w$  is the final concentration ( $\mu\text{g/mL}$ ) in the aqueous phase measured according to Section 2.4.

### 3 | RESULTS AND DISCUSSION

#### 3.1 | Theoretical discussion, recovery versus $\text{p}K_a - \log P$

EME is affected by acid–base equilibria of the extracted species as well as coupled equilibria associated with the partitioning of neutral species to the SLM. The magnitude and direction of these effects are dependent on whether a base or an acid is extracted, and whether the neutral form is hydrophobic or hydrophilic.

We first consider hydrophobic monobasic analytes, defined as substances with an SLM water partition coefficient ( $P_{\text{SLM}}$ ) higher than 1. We assume activity coefficients are 1. Initially, the phase ratio is not taken into account, but it may significantly affect recovery in situations where the material is trapped in the membrane. The base dissociation equilibrium in the sample and acceptor (both aqueous), and the partition equilibrium between sample/acceptor and SLM are:



$\text{BH}^+$ ,  $\text{B}_w$ , and  $\text{B}_o$  represent the protonated based (cationic acid), the free base in sample/acceptor, and the free base in SLM, respectively. Corresponding equilibrium equations are:

$$K_{\text{aH}} = \frac{[\text{B}]_w \cdot [\text{H}^+]}{[\text{BH}^+]} \quad (5)$$

$$P_{\text{SLM}} = \frac{[\text{B}]_o}{[\text{B}]_w} \quad (6)$$

The two equilibria above are coupled at the SLM interfaces. The net process and the corresponding equilibrium equation are:



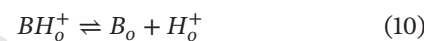
$$K = \frac{[\text{B}]_o \cdot [\text{H}^+]}{[\text{BH}^+]} = \frac{P_{\text{SLM}} \cdot [\text{B}]_w \cdot [\text{H}^+]}{[\text{BH}^+]} = P_{\text{SLM}} \cdot K_{\text{aH}} \quad (8)$$

In the presence of an extraction potential,  $\text{BH}^+$  is extracted across the SLM according to the following equilibrium:



$\text{BH}_w^+$  and  $\text{BH}_o^+$  represent the protonated base in the sample/acceptor and SLM, respectively. The equilibrium is governed by the extraction potential.

In the SLM, protonated analyte ( $\text{BH}_o^+$ ) may deprotonate (forming  $\text{B}_o$ ) to some extent. In such cases, co-extracted buffer ions possibly stabilize the protons  $\text{H}_o^+$  in SLM. The base dissociation in the SLM and the corresponding equilibrium equation are:



$$K_{\text{a,o}} = \frac{[\text{B}]_o \cdot [\text{H}^+_o]}{[\text{BH}^+_o]} \quad (11)$$

Equations 3–11 explain the observations frequently reported in the literature. First, basic analytes can be extracted with sample pH above their  $\text{p}K_a$  value [26]. This is in accordance with Equation 3 as  $\text{BH}^+$  is extracted from the sample, the base dissociation equilibrium is shifted accordingly. An acidic boundary layer at the sample/SLM interface has been hypothesized [24], and this shifts the dissociation equilibrium Equation 3 further in favor of  $\text{BH}^+$ . Second, extraction times in EME are often very short to reach near-maximum recovery, but to reach steady-state conditions and maximum recovery, extractions often have to be prolonged. This is in accordance with Equation 8, as analyte deprotonation may occur to some extent, and transfer is not only by electrokinetic migration of  $\text{BH}^+$  (fast), but is also by passive diffusion of B (slow).

Equipartition, where B partition equally between the sample/acceptor and the SLM, occurs under the following conditions:

$$\begin{aligned} \frac{[\text{B}]_o}{[\text{BH}^+]_w} &= \frac{K}{[\text{H}^+]_w} = \frac{K}{10^{-\text{pH}}} = \frac{P_{\text{SLM}} \cdot K_{\text{aH}}}{10^{-\text{pH}}} \\ &= 1 \Rightarrow \text{pH} = \text{p}K_{\text{aH}} - \log P_{\text{SLM}} \quad (12) \end{aligned}$$

Thus, for sample  $\text{pH} > \text{p}K_{\text{aH}} - \log P_{\text{SLM}}$ , the basic analyte exists predominantly in the SLM as a neutral species B, and for  $\text{pH} < \text{p}K_{\text{aH}} - \log P_{\text{SLM}}$ , the basic analyte exist predominantly in the aqueous phase (sample/acceptor) as  $\text{BH}^+$ . For a given basic analyte, efficient EME principally based



on electrokinetic mass transfer can thus be envisaged in the following situations:

$$\text{pH} \ll \text{p}K_{\text{aH}} - \log P_{\text{SLM}} \quad (13)$$

Detailed knowledge on  $\text{p}K_{\text{aH}}$  and  $\log P_{\text{SLM}}$  offers a means for rational pH selection and for introducing selectivity. For  $\text{pH} \gg \text{p}K_{\text{aH}} - \log P_{\text{SLM}}$  mass transfer will primarily be (slow) passive diffusion in the SLM. In the latter case, the extraction system will function based on the principles of liquid-phase microextraction (LPME). With  $\text{pH} \sim \text{p}K_{\text{aH}} - \log P_{\text{SLM}}$ , extractions will be mixed-mode, a hybrid between EME and LPME.

A similar theory can be developed for hydrophobic monoacidic analytes HA ( $P_{\text{SLM}} > 1$ ), and equipartition occurs at  $\text{pH} = \text{p}K_{\text{a}} + \log P_{\text{SLM}}$ . Thus, EME is favored when  $\text{pH} \gg \text{p}K_{\text{a}} + \log P_{\text{SLM}}$ . Hydrophilic monobasic and hydrophilic monoacidic analytes ( $P_{\text{SLM}} < 1$ ) behave differently. In such cases, analyte present in neutral form is not entering the SLM, and there are no coupled partition equilibria. Mass transfer of charged analyte molecules will be by electrokinetic migration only, and the extraction will follow a clean EME mechanism. In such cases, however, mass transfer is often low due to poor solubility of the extracted species in the SLM. Compounds in this category often require the addition of ion-pairing reagents.

For many substances, values for  $\text{p}K_{\text{aH}}/\text{p}K_{\text{a}}$  are found in literature, and these can be inserted in Equation 13. On the other hand, values for  $\log P_{\text{SLM}}$  are not directly available. In the following, we used computer values for n-octanol/water partition ( $\log P$ ) as an approximation for  $\log P_{\text{SLM}}$ .

### 3.2 | Experimental verification, recovery versus $\text{p}K_{\text{aH}} - \log P$

In a set of practical experiments, we tested the validity of Equation 13 with the  $\log P_{\text{SLM}} = \log P$  approximation. For this purpose, we selected 12 nonpolar basic drug substances as model analytes. Their physicochemical properties are summarized in Table 1; the model analytes were within  $\log P$  2.19 to 4.89, and  $\text{p}K_{\text{aH}} - \log P$  ranged between 3.02 and 8.18. The model analytes were extracted with the same pH in sample and acceptor, and pH 2.0, 3.0, 4.0, 5.0, 6.0, and 7.0 were tested. Extraction recoveries are summarized in Table 2 (model analytes ranged in order of decreasing  $\text{p}K_{\text{aH}} - \log P$ ). Recoveries  $\geq 40\%$  were considered as efficient extraction based on previous argumentation [27]. For seven of the model analytes (cocaine, nortriptyline, methadone, mianserin, promethazine, papaverine, and prochlorperazine), the experimental data were clearly in accordance with the theoretical discussion above. Thus,

TABLE 1 Model analytes and their physicochemical properties (data from [www.scifinder.com](http://www.scifinder.com))

Analytes	$\log P$	$\text{p}K_{\text{aH}}$	$\text{p}K_{\text{aH}} - \log P$
Methamphetamine	2.20	10.38	8.18
Cocaine	2.28	8.97	6.69
Nortriptyline	3.97	10.00	6.03
Lidocaine	2.20	7.96	5.76
Pethidine	2.19	7.84	5.65
Methadone	3.93	9.05	5.12
Mianserin	3.83 <sup>a</sup>	8.26	4.43
Haloperidol	3.76	8.04	4.28
Promethazine	4.89	8.98	4.09
Loperamide	4.15	7.76	3.51
Papaverine	2.93	6.32	3.39
Prochlorperazine	4.64	7.66	3.02

<sup>a</sup>This particular value was obtained from [www.drugbank.ca](http://www.drugbank.ca)

compounds with relatively large  $\text{p}K_{\text{aH}} - \log P$  difference, were extracted efficiently at higher pH values than those with a smaller difference. In addition, for these compounds,  $\text{p}K_{\text{aH}} - \log P$  served as a valid approximation for the highest pH where recoveries exceeded 40%. Methamphetamine, lidocaine, and pethidine deviated from this pattern, and these were extracted less efficiently than predicted by theory when pH was increased (discussed in more details in Section 3.4). Haloperidol and loperamide were extracted efficiently at slightly higher pH values than predicted by  $\text{p}K_{\text{aH}} - \log P$ . Some of the data in Table 2 were influenced by the background buffer. Especially for cocaine, nortriptyline, and pethidine, mass transfer was less at pH 4.0 and 5.0 using acetate buffer than at pH 6.0 using phosphate buffer. This indicates the occurrence of additional ion-pairing equilibria.

### 3.3 | Determination of 2-nitrophenyl octyl ether/water partition coefficients

The  $\log P$  values used above were n-octanol/water partition coefficients calculated by a computer program [28]. With pure NPOE as SLM,  $\log P_{\text{SLM}}$  is the NPOE/water partition coefficient, which may differ from the calculated  $\log P$  values. Therefore, in a new set of experiments, we measured partition coefficients experimentally in NPOE/water and n-octanol/water systems, termed  $\log P_{\text{NPOE}}$  and  $\log P_{\text{OCTANOL}}$ , respectively. Data are summarized in Table 3. The aqueous phases were adjusted to pH 12 by sodium hydroxide, to measure the partition of the free base (B). Except for lidocaine, the measured  $\log P_{\text{OCTANOL}}$  values were in close agreement with the computer  $\log P$  values. This supported that partition data obtained with our

TABLE 2 Experimental recoveries as a function of pH in sample and acceptor

Analytes	log P	pK <sub>aH</sub> - log P	Recovery (%) at different pH					
			2.0 <sup>a</sup>	3.0 <sup>a</sup>	4.0 <sup>b</sup>	5.0 <sup>b</sup>	6.0 <sup>a</sup>	7.0 <sup>a</sup>
Methamphetamine	2.20	8.18	27	21	<5	<5	<5	<5
Cocaine	2.28	6.69	70	69	32	40	73	19
Nortriptyline	3.97	6.03	86	69	53	56	62	18
Lidocaine	2.20	5.76	37	28	<5	<5	15	13
Pethidine	2.19	5.65	64	49	15	19	37	21
Methadone	3.93	5.12	85	72	69	68	67	14
Mianserin	3.83 <sup>a</sup>	4.43	74	66	36	8	<5	<5
Haloperidol	3.76	4.28	66	61	57	55	47	5
Promethazine	4.89	4.09	78	40	48	22	9	<5
Loperamide	4.15	3.51	67	65	61	63	45	5
Papaverine	2.93	3.39	70	63	20	<5	<5	<5
Prochlorperazine	4.64	3.02	56	29	43	23	<5	<5

<sup>a</sup>Phosphate buffer.<sup>b</sup>Acetate buffer.TABLE 3 Experimental NPOE/water (log P<sub>NPOE</sub>) and n-octanol/water (log P<sub>OCTANOL</sub>) partition coefficients, and theoretical log P

Analytes	log P <sub>NPOE</sub>	log P <sub>OCTANOL</sub>	vlog P <sup>b</sup>
Methamphetamine	2.05	2.23	2.20
Cocaine	- <sup>a</sup>	- <sup>a</sup>	2.28
Nortriptyline	4.38	>4.5	3.97
Lidocaine	2.21	3.00	2.20
Pethidine	2.67	2.63	2.19
Methadone	>4.5	- <sup>a</sup>	3.93
Mianserin	>4.5	- <sup>a</sup>	3.83
Haloperidol	3.95	3.93	3.76
Promethazine	>4.5	- <sup>a</sup>	4.89
Loperamide	>4.5	- <sup>a</sup>	4.15
Papaverine	3.56	3.10	2.93
Prochlorperazine	4.36	>4.5	4.64

<sup>a</sup>Not measured for this substance.<sup>b</sup>From [www.scifinder.com](http://www.scifinder.com).

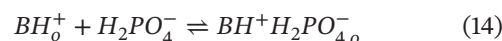
protocol were meaningful in this context. Values of log P > 4.5 were not measured due to the sensitivity of our HPLC system. Except for cocaine, the measured log P<sub>NPOE</sub> values were relatively close to the computer calculated log P values. This indicates that traditional log P data can be used for the prediction of mass transfer in EME with NPOE as SLM.

### 3.4 | The link between experiments and predictions

Methamphetamine, lidocaine, and pethidine behaved differently from the majority of model analytes and were

much more sensitive to increasing pH than predicted by the model. All three compounds have a calculated log P very close to 2.2, but the measured values for log P<sub>NPOE</sub> differed more and increased in the following order: 2.05 for methamphetamine, 2.21 for lidocaine, and 2.67 for pethidine. Extractability increased in the same order. For methamphetamine (pK<sub>aH</sub> - log P = 8.18), the extraction recovery was <30% at pH 2.0, and above pH 3.0 the compound was not detected in the acceptor (Table 2). Mass transfer was slightly more efficient for lidocaine (pK<sub>aH</sub> - log P = 5.76), and the extraction recovery was close to 40% at pH 2.0. Above pH 3, we were not able to detect lidocaine in the acceptor (Table 2). Mass transfer was even more for pethidine (pK<sub>aH</sub> - log P = 5.65), and recovery was still about 50% when pH was increased to 3.0 (Table 2). We detected pethidine in all acceptors up to pH 7.0, although recoveries were relatively low. Thus, while Eq. 13 was developed under the assumption that P<sub>SLM</sub> > 1 (log P<sub>SLM</sub> > 0), it appeared to be valid in the experimental work only for P<sub>SLM</sub> > 10<sup>2</sup>-10<sup>3</sup> (log P<sub>SLM</sub> ~ log P > 2-3).

We hypothesize the extraction of methamphetamine, lidocaine, and pethidine was influenced to some extent by deprotonation in the SLM, according to Equation 10. Most likely, ion pairing with buffer ions (phosphate and acetate) also took place in the SLM and stabilized protonated analyte molecules according to the following equations:



During 10 min of extraction, substantial amounts of methamphetamine, lidocaine, and pethidine remained in

**TABLE 4** Extraction recovery as a function of pH in the acceptor

Analytes	Recovery (%) at different pH in the acceptor				
	2.0 <sup>a</sup>	6.8 <sup>a</sup>	8.0 <sup>a</sup>	9.3 <sup>a</sup>	10.3 <sup>a</sup>
Cocaine	94	55	13	8	–
Nortriptyline	91	64	21	–	–
Pethidine	95	66	59	43	40
Methadone	101	61	11	–	–
Mianserin	95	8	–	–	–
Haloperidol	95	29	–	–	–
Promethazine	97	40	48	–	–
Loperamide	90	39	–	–	–
Papaverine	94	–	–	–	–
Prochlorperazine	56	–	–	–	–

<sup>a</sup>Sample pH: 2.0

the SLM, because deprotonated and ion-paired analyte molecules were unaffected by the electrical field.

### 3.5 | Selectivity with different pH in sample and acceptor

In a new set of experiments, we focused on selectivity and tested different buffers as acceptor, including phosphate at pH 6.8 and 8.0, and carbonate at pH 9.3 and 10.3. All buffers were 50 mM. We also included 10 mM HCl (pH 2.0) as acceptor. All extractions were from sample pH 2.0 (10 mM HCl) and were performed at 50 V for 10 min. Thus, mass transfer into the SLM was pure EME, and extraction recoveries are in Table 4. As expected, all model analytes were extracted efficiently using 10 mM HCl as acceptor, and the system was nonselective among the model analytes. Even though we used different buffers in this experiment, the pattern seen in Table 4 was in agreement with prediction based on Eq. 13. Thus, extraction of compounds with high value for  $pK_{aH} - \log P$  was efficient even with pH 6.8 acceptor, while the model analytes with low  $pK_{aH} - \log P$  values required strongly acidic conditions in the acceptor.

Pethidine deviated from the other model analytes, and even with pH 10.3 acceptor, extraction recovery was 40%. We explain this by the SLM/acceptor phase ratio. Development of Equation 13 was under the assumption that the SLM/acceptor phase ratio was 1:1, but this was not the case in the real experiments where the volumes of SLM and acceptor were 3 and 100  $\mu\text{L}$ , respectively. Due to the relatively low  $\log P$  value for pethidine, the large excess of acceptor (aqueous) changed the partition of the substance in favor of the aqueous phase. Because the  $pK_{aH} - \log P$

value is relatively large, and  $\log P$  is relatively low, this substance transferred into more alkaline acceptors than predicted by Equation 13.

In a final set of experiments, hydrochloric acid (pH 2.0), phosphate buffer (pH 6.8), carbonate buffer (pH 9.3), and sodium hydroxide (pH 12.0) were tested as sample matrix. All extractions were with acceptor pH 6.8 (phosphate buffer). We choose this high pH to induce selectivity among the model analytes. Extraction recoveries (50 V, 10 min) generally followed the principles discussed in Section 3.1. The majority of substances with low  $pK_{aH} - \log P$  were discriminated by the high acceptor pH. Extraction of substances with high  $pK_{aH} - \log P$  values was very efficient from acidic sample, based on EME mechanism. When we increased pH in the sample, extraction was less efficient, but even at pH 12.0, recoveries were still at 20–40% level. The latter was essentially a LPME system, and recoveries suffered from non-equilibrium operation.

With strongly acidic conditions on both sides of the SLM, EME provides limited selectivity among hydrophobic bases. To increase selectivity, the current data suggest that pH in the acceptor is a stronger tool for selectivity tuning than pH in the sample. With strongly acidic conditions in the sample, extraction out of sample is efficiently controlled and limited to EME, while pH in the acceptor controls selectivity according to  $pK_{aH} - \log P$ .

## 4 | CONCLUDING REMARKS

In the present work, we have discussed the impact of pH for EME of hydrophobic monobasic analytes ( $\log P > 2$ ). EME of such substances is affected by their acid–base equilibria as well as their coupled equilibria associated with the partitioning of neutral species to the SLM. From theoretical considerations, we found that EME is efficient when acceptor  $\text{pH} \ll pK_{aH} - \log P$  and this was confirmed by experimental work. We also found, by experiments, that *n*-octanol/water partition coefficients ( $\log P$ ) were very similar NPOE/water partition coefficients ( $\log P_{\text{NPOE}}$ ), and therefore extraction performance can be predicted by  $pK_{aH}$  and  $\log P$  values found in the literature. We discovered that model analytes with  $\log P$  close to 2 were prone to deprotonation and ion-pairing in the SLM, and they deviated from the model.

EME with strongly acidic conditions in both sample and acceptor provides efficient sample clean up and selective extraction of hydrophobic bases, due to the direction and magnitude of the electrical field and the discriminative nature of the nonpolar SLM. On the other hand, EME under strongly acidic conditions is relatively nonselective between different hydrophobic bases. However, in this work, we have shown that such selectivity can be



obtained by increasing pH in the acceptor, and this pH can be rationalized from  $pK_{aH}$  and  $\log P$ . This type of knowledge is highly important for the future development of EME.


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### CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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