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Salt effects in electromembrane extraction

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ABSTRACT

Electromembrane extraction (EME) was performed on samples containing substantial amounts of NaCl to investigate how the presence of salts affected the recovery, repeatability, and membrane current in the extraction system. A group of 17 non-polar basic drugs with various physical chemical properties were used as model analytes. When EME was performed in a hollow fiber setup with a supported liquid membrane (SLM) comprised of 2-nitrophenyl octyl ether (NPOE), a substantial reduction in recovery was seen for eight of the substances when 2.5% (w/v) NaCl was present. No correlation between this loss and the physical chemical properties of these substances was seen. The recovery loss was hypothesized to be caused by ion pairing in the SLM, and a mathematical model for the extraction recovery in the presence of salts was made according to the experimental observations. Some variations to the EME system reduced this recovery loss, such as changing the SLM solvent from NPOE to 6-undecanone, or by using a different EME setup with more favorable volume ratios. This was in line with the ion pairing hypothesis and the mathematical model. This thorough investigation of how salts affect EME improves the theoretical understanding of the extraction process, and can contribute to the future development and optimization of the technique.

1. Introduction

Biological samples contain substantial amounts of salts. Extracellular fluids and interstitial fluids have an osmolarity that corresponds to a solution of 0.9% (w/v) NaCl, which is used for medical purposes, and the salt content of urine samples can be even higher because of the active salt secretion in the kidneys. Environmental water samples can also contain various amounts of salts from natural sources. The presence of salts in biological and environmental samples may sacrifice the quality of chromatographic measurements, and in some cases removal of salts during the sample preparation step is needed. In other cases, salts are added to samples as an important way to increase extraction recovery from sample preparation procedures such as liquid–liquid extraction (LLE) through the salting out effect [1]. Thus, from a sample preparation point of view, salt contents play an important role for different reasons.

One way to remove much of the salt content in a sample is hollow fiber liquid-phase microextraction (HF-LPME), which has emerged as an interesting alternative to classical sample preparation techniques in recent years. HF-LPME is a supported liquid membrane (SLM) based extraction technique that was introduced in 1999 [2]. The principle is based on extraction of analytes through an SLM comprised of an organic solvent impregnated in the pores of a hollow fiber, and into a small volume of aqueous acceptor solution loaded into the lumen of the hollow fiber. This results in a clean and highly enriched extract. HF-LPME is based on low-price and disposable equipment and each extraction requires only a small amount of organic solvent. Unfortunately, extraction times are typically in the range of 15–60 min, and the extractions are normally non-exhaustive [3,4].

To overcome the latter drawbacks, electromembrane extraction (EME) was introduced as an alternative technique in 2006 [5]. The general principle is similar to that of HF-LPME, but utilizes an electric field across the SLM as a way to improve the mass transfer. Several applications using EME have been published, showing the potential for fast and efficient sample cleanup and good recovery from a variety of matrices, including analysis of drug substances or peptides from various biological fluids such as undiluted whole blood, plasma, urine, breast milk, and oral fluids [6–13]. In addition

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heavy metals and organic micro pollutants have been extracted by EME from environmental water samples [10,14–16].

Even though several EME applications have been published, only a few of these have investigated how the salt content can influence the extractions [11,17–21]. These discussions have mainly been connected to how the salt content can affect the ion balance in the extraction system according to a model describing the flux of analytes in EME [4,22].

A recent review on EME emphasized the need for more thorough investigations on how salts can affect the extraction process [23]. The following article answers this request, and presents a thorough and fundamental approach toward a better understanding of how salts affect the extraction recovery, repeatability, and membrane current in EME. Several experiments on a wide range of analytes have been performed with and without substantial amounts of salt in the sample solution. The results are described theoretically, and a mathematical model for the observed effects have been presented and related to a recently described model for the time dependent distribution of analytes in EME [24]. This is the first time the effect of salts in the sample solution has been thoroughly described for EME and the results can serve as an important contribution toward a better understanding of the extraction process.

2. Experimental

2.1. Chemicals

2.1.1. Model analytes

Amitriptyline hydrochloride, citalopram hydrobromide, clemastine fumarate, clomipramine hydrochloride, fenfluramine hydrochloride, haloperidol hydrochloride, loperamide hydrochloride, methadone hydrochloride, nortriptyline hydrochloride, papaverine hydrochloride, perphenazine, pethidine hydrochloride, promethazine hydrochloride, prochlorperazine dimaleate, pyrilamine maleate, reserpine, and verapamil hydrochloride were all obtained from Sigma–Aldrich (Steinheim, Switzerland).

2.1.2. Other chemicals

Acetonitrile (HPLC-grade) was obtained from VWR International (Leuven, Belgium). Formic acid (>98%), 2-nitrophenyl octyl ether (≥99%), potassium chloride (≥99.9%), sodium chloride (≥99.5%), sodium sulfate (≥99%), imidazole (≥99%), and 6-undecanone (97%) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Sodium hydroxide (99%) was obtained from VWR (Leuven, The Netherlands). Hydrochloric acid (37%), monosodium dihydrogen phosphate monohydrate (analysis grade), ortho-phosphoric acid (85%), and potassium sulfate (≥99%) were all purchased from Merck (Darmstadt, Germany). A Milli-Q integral 3 water purification system (Millipore, Billerica, MA, USA) supplied deionized water for all experiments.

2.2. Samples

Sample solutions were prepared daily by diluting stock solutions containing 1 mg mL^{-1} of each model drug in methanol; stored at 4 °C and protected from light. Dilutions were performed with 10 mM HCl, to achieve the desired concentration of 1 μg mL^{-1} or 100 ng mL^{-1} of each drug substance before extraction.

2.3. Electromembrane extraction

2.3.1. Equipment and setup

Most of the extractions were performed using a hollow fiber setup as depicted previously [7]. The device consisted of three parts: a sample vial containing the sample solution, a porous hollow fiber containing a supported liquid membrane in the pores of the fiber walls, and an acceptor solution located inside the lumen of the hollow fiber. The sample compartment was a glass vial with a volume of 2 mL of the type 2-SV with screw cap (Chromacol, Welwyn Garden City, UK). A 2.4 cm piece of porous hollow fiber of the type PP Q3/2 polypropylene hollow fiber, with a wall thickness of 200 μm, internal diameter of 1.2 mm, and a pore size of 0.2 μm (Membrana, Wuppertal, Germany) was mechanically sealed in the lower end and attached by heat to the 2.1 cm end-piece of a pipette tip (Finntip 200 Ext, Thermo Electron, Vantaa, Finland) in the upper end. Before extraction, this piece of hollow fiber was impregnated by an organic liquid, comprising the SLM in the extraction setup. The lumen of the hollow fiber was filled with an aqueous acceptor solution, making a three phase extraction system when the hollow fiber was placed in the sample solution through a perforated hole in the screw cap of the sample reservoir. Platinum wires with a diameter of 0.5 mm were used as electrodes (K.A. Rasmussen, Hamar, Norway), and placed in the sample solution, through the lid of the sample compartment, and into the acceptor solution in the lumen of the hollow fiber.

The other setup contained a thinner membrane and was used for a few experiments where the membrane thickness had to be reduced, leading to a comparatively low SLM-to-acceptor solution volume ratio. In this previously published extraction setup [25], the extraction system is vertically aligned, with the sample solution in a lower compartment and acceptor solution in an upper compartment, separated by an SLM between them. The sample solution was kept in a 2.0 mL Eppendorf safe-lock PP tube (Eppendorf AG, Hamburg, Germany), and the membrane was of the type Accurel PP 1E (R/P) with a thickness of 100 μm (Membrana, Wuppertal, Germany), sealed by heat on the top of the wide end of a 10–1000 μL pipette tip (Sartorius Biohit Liquid Handling Oy, Helsinki, Finland) by use of a Cotech soldering iron station (Clas Ohlson AB, Insjon, Sweden). The SLM was made by impregnating this membrane with an organic solvent and the acceptor solution reservoir was made up by the remaining volume of the pipette tip. Electrodes of the same type as used in the hollow fiber setup were positioned in the sample and acceptor solution. The piece of pipette tip containing the SLM and the acceptor solution were placed on top of the sample solution in the sample compartment.

In both systems, a power supply of the model ES 0300-0.45 from Delta Power Supplies (Delta Electronika, Zierikzee, The Netherlands), with programmable voltage in the range 0–300 V and current output from 0 to 450 mA, was used to create an electric field between the electrodes. The systems were agitated during the extractions by an Eppendorff thermomixer comfort (Eppendorff, Hamburg, Germany), and the SLM current was measured using a custom-built device for measuring micro-currents. This device was controlled by a computer with LabVIEW 8.2 software (National Instruments, Austin, TX, USA), which resulted in a plot of SLM current over time for each extraction.

2.3.2. Extraction procedure

All extractions were performed according to previously optimized conditions for similar drug substances [26]. A precalculated amount of a standard drug mix, containing the chosen model analytes, was diluted with 10 mM HCl with or without 2.5% (w/v) amounts of either sodium chloride or potassium sulfate. A final concentration of 1 μg mL^{-1} in the sample solution for each model analyte was typically used.

In the hollow fiber setup, the fiber was immersed in an organic liquid (either 2-nitrophenyl octyl ether (NPOE) or 6-undecanone) for approximately five seconds to make the SLM. Any excess solvent was wiped off by a medical wipe. A volume of 25 μL acceptor solution, comprising 10 mM HCl, was then filled into the lumen of the hollow fiber by the use of a microsyringe. The hollow fiber containing the SLM and acceptor solution was then inserted, through