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Reviews

Electromembrane Extraction from Biological Fluids

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Electro-assisted extraction of ionic drugs from biological fluids through a supported liquid membrane (SLM) and into an aqueous acceptor solution was recently introduced as a new sample preparation technique termed electromembrane extraction (EME). The applied electrical potential across the SLM has typically been in the range of 1 – 300 V. Successful extractions have been demonstrated even with common batteries (9 V) instead of a power supply. The chemical composition of the SLM has been crucial for the selectivity and for the recoveries of the extraction. Compared to other liquid-phase microextraction techniques (LPME), extraction times have been reduced by a factor of 6 – 17, and successful extractions have been obtained at extraction times of 1 – 5 min, and even down to a few seconds with online microfluidic EME devices. The technique has provided very efficient sample clean-up and has been found well suited for the extraction of sample sizes in the low μL range. Extractions have been performed with both rod-shaped hydrophobic porous fibers and with flat hydrophobic porous sheets as SLM support. The technique has been successfully downscaled into the micro-chip format. The nature of the SLM has been tuned for extraction of drugs with different polarity allowing extractions to be tailored for specific applications depending on the analyte of interest. The technique has been found to be compatible with a wide range of biological fluids and extraction of drugs directly from untreated human plasma and whole blood has been demonstrated. EME selectively extracts the compounds from the complex biological sample matrix as well as allowing concentration of the drugs. With home-built equipment fully acceptable validation results have been obtained.

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1 Introduction

New extraction techniques that are emerging as a result of the recent developments of rapid and low volume separation methods combined with sensitive detection techniques in mass spectrometry (MS), offer the opportunity to approach sample preparation from an entirely different angle. The improved separation speed and the improved detectability with MS instruments demand sample preparation methods that are faster, cheaper and greener than current methods and which are based on small sample sizes that are scaled down as a result of the highly sensitive and selective detection. As a consequence the last decade has seen a rapid development of new sample preparation techniques based on miniaturization of traditional methods such as liquid–liquid extraction (LLE) and solid-phase extraction (SPE). Liquid-phase-microextraction (LPME) is a miniaturization of LLE, and solid-phase microextraction (SPME) is a miniaturization of SPE. In LPME, analytes are extracted from an aqueous sample into a small amount of a water immiscible organic solvent. It can be divided into single-drop microextraction (SDME), dispersive liquid–liquid extraction (DLLME) and hollow-fiber microextraction (HF-LPME).[1] HF-LPME is based on a supported liquid membrane (SLM) comprising an organic solvent held by capillary forces in the pores of the wall of a hydrophobic porous hollow fiber. Extractions can be carried out either as two-phase systems or as three-phase systems. Sample preparation prior to gas chromatography (GC) is based on two-phase systems. The analytes are extracted from the aqueous sample solution into the organic solvent (acceptor phase) placed inside the lumen of the hollow fiber. After extraction the acceptor phase is injected directly into the GC. Sample preparation prior to high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) is based on three-phase systems. In the three-phase system, the analytes are extracted in a continuous process from the aqueous sample solution, through the SLM, and into an aqueous acceptor phase placed inside the lumen of the hollow fiber. The latter can subsequently be injected directly into HPLC or CE.

The mass transfer of an analyte across the SLM in a three-phase system is based on passive diffusion, and the extraction recovery depends on the extraction time, and the distribution constant of the analytes into the SLM at the sample-SLM interface and out of the SLM at the acceptor solution. For extraction of ionic analytes high extraction recoveries are obtained by sustaining a high pH-gradient across the SLM. Thus for extraction of basic analytes, the pH in the sample solution is adjusted to an alkaline pH to suppress ionization which promote their distribution into the SLM. The pH in the aqueous acceptor phase is adjusted to an acidic pH that ionizes the analytes at the acceptor side of the membrane and promotes their distribution into the acceptor phase. HF-LPME has found widespread use particularly in environmental analysis, but also in food analysis and in the analysis of drugs in biological fluids.[2] Numerous reports of high enrichment factors, excellent sample clean-up, a great reduction in the consumption of organic solvents, reduction of sample volumes and the possibility of automation have contributed to the success of HF-LPME.

One major drawback of HF-LPME and other microextraction techniques is however the long extraction times. Passive diffusion is a relatively slow process and extraction times in the range of 20 – 60 min are common. Particularly for the analysis of drugs in biological fluids, rapid extractions which contribute to high sample throughput are essential. Therefore, to increase the applicability of the technique, further research into solvent based microextraction systems for analysis of drugs in biological fluids was aimed at reducing extraction times. The reason for investigating electro-assisted extraction systems in recent years was based on the hypothesis that charged molecules can be transferred faster across a thin SLM by the force of an electrical potential rather than by passive diffusion.

Prior to year 2006, no reports had shown electro-driven extraction (EE) of analytes from an aqueous sample solution through a SLM and into an aqueous acceptor phase that was compatible with HPLC and CE. Van der Greef *et al*. reported early attempts of analytical EE in the period 1994 – 1996.[3–5] Three research papers demonstrated EE of charged substances from an organic solvent (ethyl acetate) and into aqueous solutions for subsequent analysis by CE or HPLC. Thus, conventional LLE was performed prior to EE when the technique was used for aqueous samples such as biological fluids. By application of an electrical field of typically 15 kV across the interface between the organic solvent and the aqueous buffer, mass transfer was induced across the liquid–liquid interface. Cationic analytes were electro extracted from the organic solvent and into an aqueous buffer at pH 5. In one setup, the extraction was accomplished with a CE instrument, where the power supply of the CE was used both for EE and for the final separation. However, this system has not been further investigated. In 2005, Arrigan *et al*. proposed another approach to LLE driven by an electrical potential with the development of electrochemically-modulated LLE of ions.[6,7] In a hydrodynamic flow-injection system, target analytes were extracted from a flowing aqueous phase and into a stationary organo-gel phase. The system was similar to earlier electrochemistry systems studying the interface between two immiscible electrolyte solutions (ITIES). The driving force for the extraction was the electrical potential sustained over the phase boundary, and the

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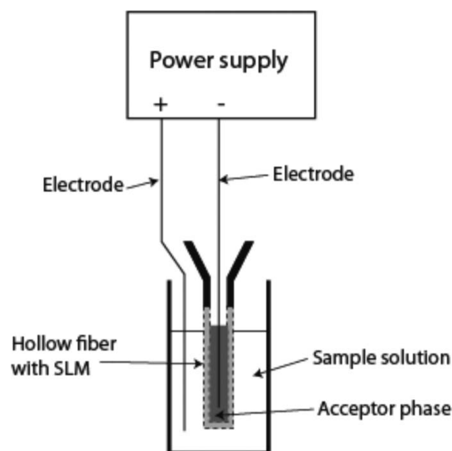

Fig. 1 Schematic illustration of EME device.

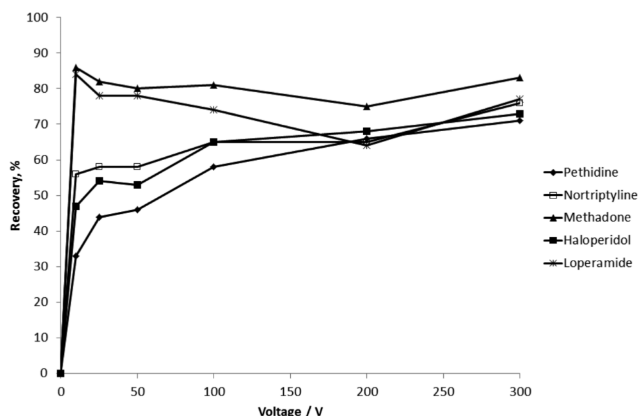

Fig. 2 Extraction recovery *versus* applied voltage. SLM, NPOE; extraction time, 5 min; acceptor, 30 µl 10 mM HCl; sample, 300 µl 10 mM HCl containing 1 µg/ml pethidine, nortriptyline, methadone, haloperidol, and loperamide.

analytes were determined electrochemically by plotting the ion-transfer current in hydrodynamic voltammograms. Both anionic and cationic substances were successfully extracted with an electrical potential typically in the range of –1 to +1 V. Several applications of ITIES extraction have been reported recently, including extraction of drugs from biomimetic fluids and additives from beverages.[8,9]

The first work on EE of drugs across a SLM was reported in 2006.[10] As a SLM separated the aqueous sample solution and the aqueous acceptor solution, the technique was termed electromembrane extraction (EME). EME has recently attracted substantial interest and has been discussed in several reviews.[1,11-15] This current review focuses on the basics of EME, on EME in different formats, on recent applications of EME, and on the future potential of EME.

2 Initial Studies of EME

In the first EME publication the device (shown in Fig. 1) was similar to the device used for HF-LPME except that one platinum electrode was placed in the sample solution and another platinum electrode was placed in the acceptor phase inside the lumen of the hollow fiber.[10] The porous hollow fiber made of polypropylene had a wall thickness of 200 µm, a pore size of 0.2 µm, and the internal diameter of 1.2 mm. The fiber was cut to a length of 25 mm and was mechanically closed in the bottom by a brief deformation with a pincer. The upper end was sealed to the end of a pipette tip as a guiding tube by heating. The SLM was formed by dipping the fiber into 2-nitrophenyl octyl ether (NPOE) for 5 s allowing approximately 15 µl of solvent to be immobilized in the pores of the hollow fiber. The volume of the sample solution was between 150 and 500 µl. The acceptor phase (30 µl), which had a pH that kept the analytes charged, was placed in the lumen of the hollow fiber. When the electrodes were connected to a power supply, an electrical field was created across the SLM where the SLM functioned as a resistor. For extraction of cations, the cathode was located in the acceptor phase and the anode was located in the sample solution, whereas the polarity was reversed for extraction of anions. Thus, charged analytes were promoted to migrate from the sample solution through the SLM and towards the electrode of opposite charge placed in the acceptor phase. To speed up the extraction, the device was shaken on a platform shaker to reduce the stagnant layer at the interface between the sample solution and SLM and to promote convection of the analytes in the sample solution.

In the first publication, the sample solution contained the basic drugs, pethidine, nortriptyline, methadone, haloperidol, and loperamide as model analytes. These drugs are non-polar with log *P* values above 1.7. In the initial experiments a voltage of 300 V was applied for 5 min. After EME, the acceptor phase was collected with a micro-syringe for analysis by CE.

One major challenge in the development of the new technique was the choice of solvent for the SLM. The solvent should be water immiscible, be immobilized in the pores of the hollow fiber, have a high boiling point to avoid evaporation, and provide the necessary solubility for charged analytes to allow their transport through the SLM. Of a wide range of solvents that were initially tested, the nitro-substituted solvent NPOE gave extraction recoveries of 70% or more for all the model analytes. As an alternative green solvent peppermint oil also showed promising results, but since the amount of organic solvent used for the SLM is less than 20 µl the impact of chemical waste on the environment is of minor influence.

When a suitable membrane liquid was found, the next step was to study the extraction recovery as a function of the applied voltage. These results are demonstrated in Fig. 2. Particularly for methadone and loperamide, high recoveries were found with only 10 V as potential difference across the SLM. For most of the model analytes the recoveries increased up to the upper limit of 300 V for the power supply. These results demonstrated the potential of selective extraction at low voltages and the potential of extracting a wide range of substances at higher voltages.

The effect of the chemical composition of the acceptor phase was studied in another experiment. The pH of the acceptor phase was the most important. To keep the basic analytes charged, acids such as hydrochloric acid and formic acid were used for the acceptor solution. Poor recoveries were found with buffers in the pH range 6 – 8 for the acceptor. When the pH was increased in the acceptor solution, the electro-kinetic migration into the acceptor phase was reduced due to partial deprotonation of the analytes and back-diffusion based on passive diffusion from the acceptor solution into the liquid membrane. When the system was tested on biological samples, very clean extracts were obtained from plasma and urine as illustrated in Fig. 3. Linear calibration curves and fully acceptable relative standard deviations were obtained.[10]

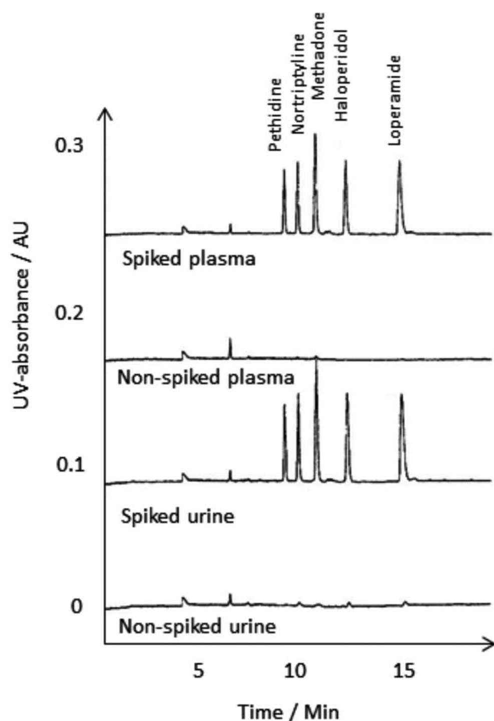

Fig. 3 EME extracts of basic drugs in human plasma and urine analyzed with CE with UV-detection (200 nm). Peak identification: (1) pethidine, (2) nortriptyline, (3) methadone, (4) haloperidol, (5) loperamide. All drugs were spiked to an concentration of 1 μg/ml in either plasma or urine. Samples were extracted for 5 min using NPOE as the SLM. Extraction voltage, 300 V; acceptor, 30 μl 10 mM HCl; sample, 100 μl plasma or urine mixed with 200 μl 15 mM HCl.

3 EME of Different Basic Drugs

The same experimental set-up as discussed above was also used to extract more polar basic drugs with log *P* values below 1.7.[16] In this case the initial extraction failed because the very polar analytes were unable to penetrate the NPOE membrane. By mixing 25% (w/w) of di-(2-ethylhexyl) phosphate (DEHP) into the NPOE membrane the problem was solved. DEHP is an ion-pairing reagent that ion-paired with the analytes at the interface between the sample and the SLM, and facilitated their transport through the SLM. With the modified SLM, recoveries up to 83% were obtained with different polar basic drugs with log *P* values in the range of –1.3 to 1.0. Interestingly, the modified SLM was inefficient for the more non-polar drugs, which were trapped inside the SLM. This experiment demonstrated that selectivity can be changed dramatically by simple modification of the composition of the SLM.

Many basic drugs are protonated under physiological conditions, and this was utilized in another EME publication where pethidine, nortriptyline, tramadol, methadone, haloperidol, and loperamide were extracted directly from untreated human plasma and whole blood.[17] Even with high protein binding, the electrical potential in EME was efficiently extracting the drugs alone, probably by constantly removing the free drug fraction by the extraction and thereby constantly removing the equilibrium. Recoveries in the range 25 – 65% were obtained after 10 min extraction. EME from human plasma under physiological conditions was also shown in very recent publications for amitriptyline, citalopram, fluoxetine, and fluvoxamine,[18] and for naltrexone and nalmefene.[19] This opportunity may be very interesting for the future, for extraction of labile analytes prone to degradation under non-physiological conditions, and in other cases where disturbance of the biological sample is undesired.

Several other publications have also addressed EME of different basic drugs, including amlodipine enantiomers,[20] mebendazole,[21] amphetamine,[22] methamphetamine,[22] 3,4-methylenedioxymethamphetamine,[22] 3,4-methylene-dioxyethamphetamine,[22] methylbenzodioxolybutanamine,[22] levamisole,[23] lithium,[24] naltrexone,[19] and nalmefene.[19] In these applications either NPOE,[20,21] 1-octanol,[24] NPOE mixed with DEHP,[19] or NPOE mixed with tris-(2-ethylhexyl) phosphate (TEHP)[22,23] were used as SLM, and extractions were accomplished with voltages in the range 75 – 250 V from acidic sample to acidic acceptor solution.

Both NPOE and 1-octanol have low conductance and voltages in the range 50 – 300 V had been used to maintain a high electrical field strength across the SLM to ensure acceptable recoveries in a short time. By replacing NPOE with 1-isopropyl-4-nitrobenzene (IPNB) as SLM, pethidine, nortriptyline, methadone, haloperidol, and loperamide were extracted with high recoveries with potentials in the range of 1 – 10 V.[25] In this case, the use of common batteries as power supply was demonstrated. EME at low voltages may be very interesting for the future, especially in cases where the analytes are prone to electro-chemical degradation.

4 EME of Acidic Drugs and Peptides

Extraction of acidic drugs was first demonstrated in 2007.[26] Again, exploring different solvents as membrane liquids was challenging because of lack of literature data and earlier experience. Of the solvents tested, 1-octanol worked properly. To ensure full ionization of the analytes, both the sample solution and the acceptor phase were made alkaline by the addition of 10 mM NaOH. In this case extracting the de-protonated acidic drugs, the anode was placed in the acceptor solution, and the direction of the electrical field was reversed as compared to EME of basic drugs. For a range of anti-inflammatory, antirheumatic, analgesic, hypnotic, anticoagulant, and uricosuric drugs, the recoveries were in the range of 8 – 100%.

In a series of recent publications, also peptides have been extracted by EME.[27–30] In the first paper, small model peptides were extracted as net-cationic species from acidic sample (pH 3), through a SLM of 1-octanol and 15% DEHP (w/w), and into 25 μl of 100 mM HCl as acceptor phase using 50 V. DEHP in the SLM was found to be crucial for the transfer of peptides into the SLM. During 5 min of operation, the peptides were enriched by a factor of up to 11 by EME.[27] In a subsequent paper, angiotensin peptides were extracted by EME from human plasma for the first time.[28] In this case, the extraction voltage was 15 V, and the SLM consisted of 1-octanol and 8% DEHP (w/w). Both the voltage and the content of DEHP were reduced as compared to the first paper, to avoid excessive current in the system which may result in electrolysis and formation of small bubbles in the aqueous compartments. In a very recent paper,[30] EME was used for the first time in combination with LC-MS/MS for fast, selective, and sensitive analysis of angiotensin 2, leuenkephalin, and endomorphin 1 in human plasma. Detection limits were in the range 24 – 60 pg/ml, acceptable linearity was found in the range 100 to 1000 pg/ml, and repeatability ranged between 15 and 24% RSD. EME of peptides is currently under

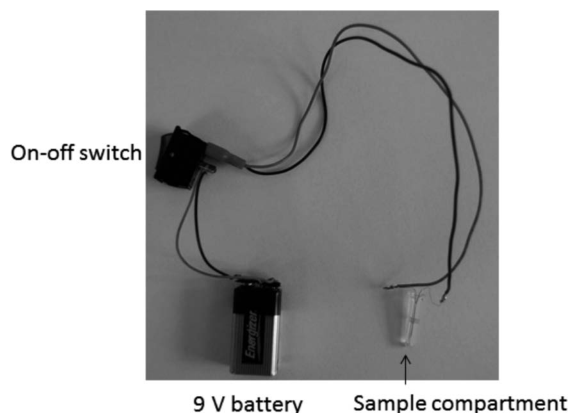

Fig. 4 Photo of stagnant EME system powered by a 9-V battery.

intensive development, and may be an interesting field in the future.

5 Technical Formats of EME

Most EME up to date has been accomplished in systems as illustrated in Fig. 1, where the sample was filled into a conventional glass vial (0.5 to 4 ml), the SLM was sustained in the wall of a hollow fiber, the acceptor phase was located inside the lumen of the hollow fiber, and where the whole assembly has been agitated to promote efficient mass transfer. However, EME has also been accomplished in a totally stagnant system, from very small volumes of sample (50 – 100 μl) where the short diffusion distance from the sample and to the SLM eliminated the need for agitation.[18] This in combination with the use of a 9-V battery as power supply resulted in a very simple extraction system as shown in Fig. 4.

EME has recently been developed into the micro-chip format.[31-33] In one configuration, urine samples containing different basic drugs were pumped at 2.5 μl/min into the sample channel of a micro-chip.[32] This sample channel was in direct contact with a small SLM (NPOE), sustained in a small piece of a flat polypropylene membrane, and the drugs were extracted across this SLM and into a small reservoir on the other side containing 7 μl of 10 mM HCl (acceptor phase). This acceptor phase was subsequently collected by a micro-pipette, transferred to a micro-vial, and analyzed off-line by CE. At low sample flow rates (≈0.5 μl/min), this EME system provided almost exhaustive extraction, and this may be an interesting concept for extraction from small volumes of biological fluid in the future.

In a subsequent paper, chip-EME was developed further and coupled on-line to UV and MS, and this system was used for the continuous and on-line measurement of drug metabolism by rat-liver microsomes (Fig. 5).[33] Amitriptyline as model drug was mixed with a suspension of rat liver microsomes, $MgCl_2$, potassium phosphate buffer (pH 7.4), and β-nicotinamide adenine dinucleotide 2-phosphate (NADPH) as co-factor to initialize the metabolism (37°C). This reaction mixture was continuously pumped into the micro-chip (sample inlet), and amitriptyline as well as the metabolites was extracted across the SLM (NPOE) inside the chip, and into a flowing acceptor phase of 100 mM HCOOH. This acceptor phase was continuously pumped into an electrospray ionization MS interface, and the signals for amitriptyline and metabolites were measured continuously during the metabolism. This enabled both the identification of the different metabolites as well as their formation rates. The half time for the metabolism of amitriptyline was estimated and in accordance with literature.

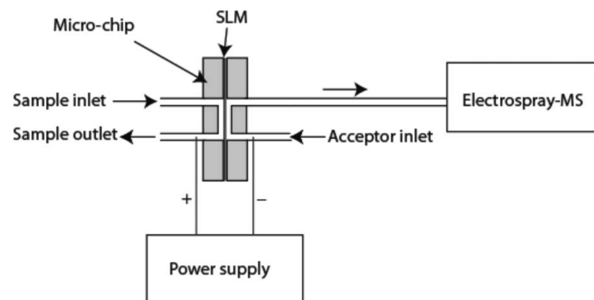

Fig. 5 Schematic illustration of chip-EME coupled on-line to MS.

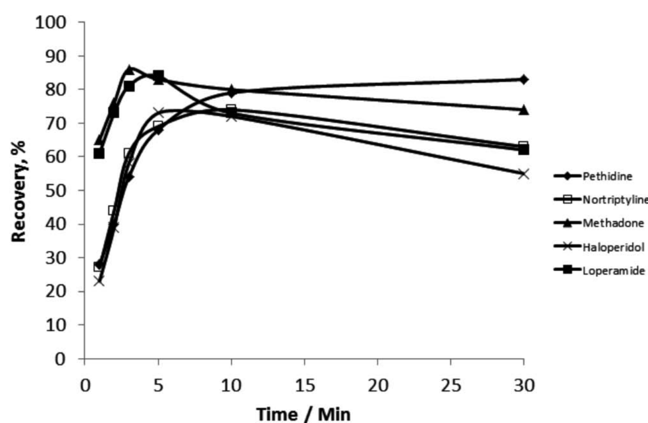

Fig. 6 Extraction recovery *versus* time. SLM, NPOE; voltage, 300 V; acceptor, 30 μl 10 mM HCl; sample, 300 μl 10 mM HCl containing 1 μg/ml pethidine, nortriptyline, methadone, haloperidol, and loperamide.

6 Performance of EME

Extraction performance of EME has been reported in several publications, and data collected from two of the papers illustrate typical performance values.[10,21] Typically, recoveries from sample volumes of 0.3 – 3 ml samples are in the range of 55 – 80%. Recoveries are dependent on the SLM and the polarity of the analyte, and analytes with log P values in the range of 2 – 4 provide highest recoveries. For more polar substances, recoveries may be below 55% because of poor extraction into the SLM. Enrichment in EME is highly dependent on the sample volume, but with 3 ml samples, enrichment factors of 108 – 140 have been reported.[21] Thus, similar to HF-LPME, EME may be used as a very efficient preconcentration technique. Another very interesting feature of EME is the rapid extraction times; typically, the EME systems come to equilibrium after 5 to 7 min, with no further gain in recovery *versus* time (Fig. 6). EME therefore provides very fast extractions as compared to HF-LPME.[20,34] The principal reason for this is that analytes are transferred by electro-kinetic migration across the SLM in EME as compared to passive diffusion in HF-LPME. The consumption of organic solvent is low, and typically only 10 to 20 μl of solvent is used per

Table 1 Figures of merit of EME and HPLC with UV-detection of naltrexone and nalmefene in human plasma and urine[19]

Sample	Analyte	Limit of detection/ ng ml^{-1}	Linearity/ ng ml^{-1}	R^2	RSD%[a]	
					Intra day	Inter day
Plasma	Naltrexone	20	40 – 1000	0.9956	5.0	10.6
	Nalmefene	20	40 – 1000	0.9946	2.0	12.4
Urine	Naltrexone	10	20 – 1000	0.9989	8.3	12.7
	Nalmefene	20	30 – 1000	0.9977	3.4	9.6

a. Intra day and inter day RSD% were obtained by five and three replicate measurements, respectively.

extraction for the SLM. Each hollow fiber and SLM is discarded after extraction to avoid carry-over from sample to sample. In the chip format the same SLM could be used for several days without carryover or loss in performance.[32,33] Finally, EME provides very efficient sample clean-up of biological samples as illustrated in Fig. 3. In this case, the EME system was tuned for basic substances with NPOE as SLM, and the cathode placed in the acceptor phase. Only non-polar cationic species passed the SLM, whereas polar, acidic, or neutral components all remained in the sample (or partly in the SLM). Sample clean-up by EME was recently compared to SPE, and was found to be comparable or even superior to this well-established technique.[18]

Several of the developed EME methods have been validated, and have provided acceptable results from human plasma, whole blood, urine, saliva, and breast milk.[10,17-25,28,31,32] Typically, the EME methods have provided linear calibration curves over 2 decades ($r^2 > 0.99$)[22,24] and with repeatability values in the range 2 – 10% RSD.[22,24] One example of validation results are reported in Table 1 for naltrexone and nalmefene in human plasma and urine.[19] EME has also been tested for extraction of basic drugs spiked into plasma samples from different persons, and the results were found to be independent of the type of plasma.[25]

7 Theoretical Understanding and Optimization of EME

EME has also been discussed from a theoretical point of view.[34] The theoretical model, which was based on a modification of the Nernst-Planck equation, described how the potential difference across the SLM ($\Delta\phi$), the ion balance across the SLM (χ), and the absolute temperature (T) influenced the flux of a charged analyte (J_i) through the SLM by the following equation:

$$J_\mathrm{i} = \frac{-D_\mathrm{i}}{h}\left(1+\frac{\nu}{\ln\chi}\right)\left(\frac{\chi-1}{\chi-\exp(-\nu)}\right)\{c_\mathrm{i}-c_\mathrm{i0}\exp(-\nu)\} \quad (1)$$

where D_i is the diffusion coefficient for the charged analyte in the SLM, h the thickness of the SLM, ν a dimensionless driving force defined in Eq. (2), χ the ratio of the total ionic concentration (all ions) on the donor side to that on the acceptor side (ion balance), and c_i and c_{i0} the concentration of the analyte close to the membrane in respectively the sample and acceptor solution.

$$\nu = z_\mathrm{i}e\Delta\phi/kT \quad (2)$$

z_i is the charge of the analyte, e the elementary charge, and k the Boltzmann's constant.

Equation (1) predicts that the flux of analyte across the SLM is dependent on the diffusion coefficient of the analyte in the SLM. This is principally determined by the viscosity of the solvent, and solvents with a low viscosity should preferably be used. This effect was demonstrated in a paper where NPOE was replaced by 2-nitrophenyl pentyl ether (NPPE). The viscosity of NPPE is lower than for NPOE, and the former SLM provided superior extraction recoveries.[35] Also, the flux is dependent on the thickness of the membrane, and thin membranes are advantageous. The membranes used in EME until date has been 200 μm with hollow fibers[10] or 25 μm with flat membranes in the drop to drop or micro-chip systems.[31-33] Thinner membranes may be interesting to test in the future to improve performance, but the mechanical stability may be an issue for very thin membranes.

Equations (1) and (2) also predict that the flux is dependent on the voltage applied across the SLM. This has been verified experimentally in several EME papers,[10,20,23-25,27,29,31-34] where recoveries have been found to increase with increasing voltage up to a certain level. The latter point out that extraction is limited by other factors than the voltage at high voltages, and deviations from Eq. (1) occur. In some cases, a decrease in efficiency was observed at very high voltages, indicating stability problems for the EME system. From Eq. (1), the ion balance and the temperature also affect the flux of analyte in EME. This has also been verified experimentally,[34] but both parameters are not very powerful in optimization of EME.

For optimization of EME, the nature of the SLM is highly important. The composition of the SLM affects the distribution constant of each charged analyte into the SLM, and this strongly affects the extraction recovery. For non-polar basic analytes, NPOE has been found to be very efficient[10,20,21] whereas addition of carriers or SLM modifiers like DEHP or TEHP was required for extraction of more polar basic substances.[16,19,22,23] Other solvents like 1-ethyl-2-nitrobenzene (ENB), NPPE, and IPNB have also been found efficient for non-polar basic drugs.[25,35] For acidic analytes in contrast, 1-octanol has proven to be an efficient SLM,[26] whereas 1-octanol with DEHP has worked efficiently for EME of peptides.[27-30] 1-Octanol has also been used for extraction of lithium ions.[24] Selecting the organic solvent as SLM in EME is still based on trial and error and more research should be directed to fundamental understanding on how to select the SLM for certain applications.

Control of pH is also important in EME. Basically, the analyte should be charged both in the sample and in the acceptor phase for electro-kinetic migration, and pH conditions should be selected to ensure this. Thus, basic substances (and peptides) have been extracted in acidic environment, typically using HCl as background electrolyte in both the sample and in the acceptor phase,[10,17,20-23,25,27] but also HCOOH has been used as a MS friendly acceptor phase.[18,33] Acidic substances on the other hand have been extracted under alkaline conditions using NaOH as background electrolyte.[26] Several other electrolytes than HCl have been tested for EME of peptides, and the type of background electrolyte was not found to strongly affect extraction recoveries.[29]

Finally, the extraction time is important in EME. Normally, recoveries increase rapidly with time during the first couple of minutes of extraction, and maximum recoveries are obtained after 5 – 10 min.[10,17,18,20,23,25-27,36] At this stage, there is normally no further gain in recovery *versus* extraction time. In several cases, prolonged extraction has resulted in reduced recoveries, and this is probably due to instability problems of the EME system.[10,18,23,25]

8 Other Recent EME Papers

In addition to EME from biological fluids, a few other EME applications have been published recently. In one paper, nerve agent degradation products were extracted from river water samples, using 1-octanol as SLM, 300 V as extraction voltage, 30 min extraction time, and using pure water as acceptor phase.[37] From the same group, Pb ions were extracted from various matrices like amniotic fluid, blood serum, lip stick and urine samples.[38] In this case, toluene was used as SLM, and EME was conducted for 15 min using 300 V and with aqueous buffer pH 8.1 as acceptor phase. Combined with CE, Pb was detected down to a concentration of 1.9×10^{-2} mg/l. Another paper from the same group focused on EME of chlorophenols from seawater.[39] In the latter work, EME was conducted with 1-octanol as SLM, for 10 min, utilizing a 10-V driving force, and with NaOH pH 12 as acceptor phase. Finally, this group also demonstrated simultaneous extraction of acidic and basic drugs at neutral pH utilizing a compartmentalized membrane envelope.[40]

In addition to the work mentioned above on Pb,[38] the heavy metals cations: Ni, Mn, Cd, Cu, Co, and Zn (and Pb) were extracted by EME recently.[41] For this application, 1-octanol containing 0.5% DEHP (w/w) was used as SLM, and EME was conducted at 75 V for 5 min with 100 mM acetic acid as acceptor phase. Detection of the heavy metal ions in the acceptor phase was conducted with CE coupled with contactless conductivity detection.

EME was recently also used for the extraction of non-steroidal anti-inflammatory drugs (NSAIDs) in waste water samples.[42] In this set-up, 1-octanol was used as SLM, and extractions were performed at 10 V for 10 min using NaOH pH 12 as acceptor solution. Enrichment factors of 28 – 49 were reported in this paper from 10 ml sample volumes.

All publications and applications discussed so far in this review have been EME in a 3-phase configuration, where target analytes have been extracted from an aqueous sample, through an organic SLM, and into an aqueous acceptor phase, and where both electrodes have been placed in aqueous solutions. EME has, however as mentioned in the introduction, also been described in a 2-phase configuration, where 1-octanol was used both as SLM and as acceptor solution.[43] In this system, nitrobenzene, aniline, and phenol were extracted from aqueous solution and into 1-octanol during 4 min of operation at 60 V, where after the acceptor phase was injected directly into GC.

9 Conclusions and Future Trends

This review has discussed the very recent development of electromembrane extraction (EME), which is a liquid-phase microextraction technique amenable for preparation of biological samples for chromatography, electrophoresis, and mass spectrometry. EME is a further development of hollow-fiber liquid-phase microextraction (HF-LPME), which has received substantial attention in recent years, and EME offers several advantages over HF-LPME. First, extraction times are reduced significantly in EME as compared to HF-LPME, because EME relies on electro-kinetic transport of the analytes across the SLM, whereas HF-LPME is based on passive diffusion. Secondly, the driving force in EME, namely the electrical potential across the SLM, is easily adjustable in terms of both magnitude and direction by simple manipulation of the power supply connected to the extraction device. This is not possible in HF-LPME. Additionally, EME is highly interesting for the future because the technique enables very efficient analyte enrichment and very efficient sample clean-up, and because the consumption of organic solvent per sample is reduced to 10 – 20 μl or even less than 0.5 μl in the microchip format.[32,33]

The initial research on EME reviewed in this paper has demonstrated several interesting features of the technique which recall on further development in the future. Development of the technique into integrated micro total analytical systems (μ-TAS) is definitely an interesting direction, and EME of peptides and metal ions may be very important applications in the future. The flexibility of the technique, where the driving force in the system is easily tuned by an external power supply, may also be an important anchor for further investigations. Finally, development of tailor-made membranes may be an interesting future direction for highly selective extractions in specialized analytical systems.

10 References

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