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## Review

# Electroextraction and electromembrane extraction: Advances in hyphenation to analytical techniques

Electroextraction (EE) and electromembrane extraction (EME) are sample preparation techniques that both require an electric field that is applied over a liquid-liquid system, which enables the migration of charged analytes. Furthermore, both techniques are often used to pre-concentrate analytes prior to analysis. In this review an overview is provided of the body of literature spanning April 2012–November 2015 concerning EE and EME, focused on hyphenation to analytical techniques. First, the theoretical aspects of concentration enhancement in EE and EME are discussed to explain extraction recovery and enrichment factor. Next, overviews are provided of the techniques based on their hyphenation to LC, GC, CE, and direct detection. These overviews cover the compounds and matrices, experimental aspects (i.e. donor volume, acceptor volume, extraction time, extraction voltage, and separation time) and the analytical aspects (i.e. limit of detection, enrichment factor, and extraction recovery). Techniques that were either hyphenated online to analytical techniques or show high potential with respect to online hyphenation are highlighted. Finally, the potential future directions of EE and EME are discussed.

### Keywords:

Bioanalytical applications / Electroextraction / Electromembrane extraction / Hyphenation / Sample preparation  
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## 1 Introduction

In the field of analytical chemistry, sample pretreatment is often needed in order to enable the selective and sensitive analysis of compounds in complex samples. Commonly applied sample pretreatment techniques are solid-phase extraction (SPE), liquid-liquid extraction (LLE), and protein precipitation [1]. Recently, various miniaturized electromigration-based extraction techniques have been introduced that offer low solvent consumption, simple extraction procedures, and the ability to deal with small sample volumes [2]. Analyte extraction is not based on passive diffusion, as in conventional extraction techniques, but on active electric field-enhanced transport. This increases the extraction speed and allows exhaustive extraction to take place, thereby making electric field-assisted extraction techniques promising for high-throughput analysis and for the analysis of low-abundant analytes.

Electromembrane extraction (EME) and electroextraction (EE) are based on immiscible liquid-liquid systems comprising organic and aqueous phases. Analytes are electroextracted and concentrated from a donor phase, often via a filter phase, into an acceptor phase. Due to the fact that the electrophoretic

velocity of the ions is proportional to the electric field, extraction speed is enhanced in the organic (filter or donor) phase a high electric field strength is present there. High analyte concentration factors can be achieved, because of the fact that the acceptor phase is small in volume when compared to the donor phase.

In EME, the liquid-liquid system typically comprises donor and acceptor phases that are both aqueous and are separated by a membrane of which the pores contain an organic filter phase, i.e. a supported liquid membrane (SLM). EME was first demonstrated in 2006 by Pedersen-Bjergaard *et al.* [3] and has so far primarily been used for drug analysis studies [4, 5].

In EE, no membranes are used and the donor phase is typically comprised of an organic solvent and the acceptor phase is comprised of an aqueous solution. EE was first described by Stichlmair *et al.* as a modified liquid-liquid extraction technology in an industrial setting [6]. It was applied for analytical purposes in 1994 by Van der Vlis *et al.* [7] and for bioanalysis by Lindenburg *et al.* in 2010 [8]. Until now, EE has been mainly applied to analysis of peptides and metabolites.

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This paper is dedicated to our late colleague Shanna Shi (1978-2012)

**Colour Online:** See the article online to view Figs. 1, 4 and 5 in colour.

In 2013, Raterink *et al.* [9] and shortly thereafter Kubáň *et al.* [10, 11] reported on a system comprised of three liquid phases, i.e. aqueous donor and acceptor phase separated by a layer of organic solvent acting as a filter. Raterink *et al.* denoted this system as ‘three-phase EE’ and Kubáň *et al.* ‘EME across free liquid membranes’, but in essence both systems utilize the same extraction principle.

A PubMed – NCBI query to with either ‘electromembrane extraction’ or ‘electroextraction’ in the title that appeared between April 2012 and November 2015 resulted in 95 papers, underlining that research in this area is quite active.

A variety of instrumental setups for electrophoretic sample pretreatment have been developed, which were reviewed by Lindenburg *et al.* These setups were deemed highly promising for metabolomics and peptidomics. However, the authors noted that commercial electrophoretic sample pretreatment setups were required in order for electrophoretic sample pretreatment to become routinely used [2]. Krishna Marothu *et al.* discussed the setups and experimental procedures, innovations, and applications of EME [5]. Seip *et al.* explored the optimal experimental EME conditions for various bioanalytical applications and discussed recent advances in the use of EME for bioanalysis. The review also discussed challenges with EME, such as matrix interference during EME, difficulties with the extraction of acidic and polar compounds and the lack of commercial setups [4]. Seip *et al.* mathematically described mass transport in EME by creating a theoretical model of the process [12]. Huang *et al.* summarized the recent efforts that were made to gain understanding of mass transport taking place during EME [13].

Until now, aspects such as (online) hyphenation of EE and EME to analytical separation and/or detection techniques have not been considered in detail. Therefore, in this review, the hyphenation of EE and EME to three major separation techniques is discussed, i.e. LC, GC, and CE, as well as the hyphenation to direct detection. Special emphasis is placed on online hyphenation of EE and EME to the aforementioned separation and detection techniques. In this review, an overview is presented of the EE and EME systems that permit concentration of analytes, which can only be achieved when the acceptor phase volume is smaller than the donor phase volume. In order to provide the reader with a proper comparison, only studies that report LOD values are included. In this context, the presence of information on parameters, such as extraction recovery and enrichment factor, was also used as inclusion criteria. In Section 2, theoretical aspects, more details are provided concerning the relevant analytical parameters, i.e. extraction recovery and enrichment factor. These parameters are not always combined, but can provide useful insights in the process efficiency of EE and EME, as is discussed in the Section 3 regarding hyphenation. The review is concluded with a critical discussion on the challenges and requirements of online hyphenation of EME and EE to analytical separation and detection techniques.

## 2 Theoretical aspects

Theoretical aspects concerning the concentrative ability of EME have often been reported in literature. We have included these aspects in order to provide a framework for the next sections.

The obtained enrichment factor (EF) of analyte *i* ( $EF_i$ ) is based on the ratio of the analyte concentrations in the acceptor and donor phase, i.e.

$$EF_i = \frac{C_{acceptor_i}}{C_{donor_i}} \quad (1)$$

When the measured concentration is within the linear response of the detector, the detector signal can be used to calculate  $EF_i$ .

The maximum attainable EF ( $EF_{max}$ ), i.e. when all analyte molecules are extracted from the donor into the acceptor phase, is based on the volume ratio of the donor and acceptor phase:

$$EF_{max} = \frac{V_{donor}}{V_{acceptor}} \quad (2)$$

From this equation, it can be derived that in order to be able to concentrate analytes, an EME or EE system should encompass a larger donor phase volume than acceptor phase volume. For example, EME across free liquid membrane as reported by Kubáň & Bocek [10, 11, 14] utilizes equal volumes of donor and acceptor phase and thus has no concentrating power. A similar technique, three-phase EE, utilizes a much smaller acceptor phase volume in comparison to the donor phase and thus was able to concentrate analytes [9].

The extraction recovery (ER) of analyte *i* ( $ER_i$ ) is based on the ratio between  $EF_i$  and the maximum attainable EF ( $EF_{max}$ ), expressed as a percentage:

$$ER_i = \frac{EF_i}{EF_{max}} \times 100\% \quad (3)$$

When Equation 1–3 are combined, the extraction recovery of analyte *i* can be written as:

$$ER_i = \frac{C_{acceptor_i} \times V_{acceptor}}{C_{donor_i} \times V_{donor}} \times 100\% \quad (4)$$

and thus, combining Equation 1 and 4

$$ER_i = EF_i \times \frac{V_{acceptor}}{V_{donor}} \times 100\% \quad (5)$$

It is important to have information about both  $ER_i$  and  $EF_i$ . When a very large donor volume is used, a large  $EF_i$  can be achieved even though  $ER_i$  is low. Thus, in order to be able to assess the extraction performance correctly, either  $ER_i$  or  $EF_i$  in combination with  $V_{donor}$  and  $V_{acceptor}$  should be reported in the paper. In the case that  $ER_i$  or  $EF_i$  is missing, it can easily be calculated using Eq. 5.

In a few examples, no acceptor phase volume could be reported because the acceptor phase was solid [15, 16]. In a few other examples, the acceptor phase [17–19] was nonstagnant. However, the technologies reported here were clearly

concentrating analytes and were considered as relevant for this review due to their innovative character.

### 3 Hyphenation of EME and EE: an overview

Thus far, the majority of the reported EME and EE work was coupled offline to separation and/or detection techniques. In these cases, the extracts are collected after the experiment, transferred to a sample vial and introduced into an analytical system. However, there are several examples where EME and EE are fully integrated into the analytical system and sample extraction takes place in conjunction with consecutive analysis. In the next sections, we discuss progress of online hyphenation and steps taken towards online hyphenation using LC, GC, CE, and direct detection, respectively.

#### 3.1 Hyphenation to LC

Table 1 provides a detailed overview of the work in which EME and EE have been combined with LC. A total of 29 studies have been included of which two were coupled online to LC, which are highlighted in this section. In the majority of the EE-LC and EME-LC work optical detection techniques were used, such as UV (17) or fluorescence (2) detection. Furthermore, several instances of MS detection (12) were reported. Certain applications reported combinations and have not been assigned separately. In order to make a comparison between the different techniques, the reported detection limits were converted to nM, which was also done for the articles discussed in the remainder of the review. Analyses performed with LC-MS and both LC-UV both typically yielded sub-nM to  $\times 10^2$  nM detection limits, which is surprising, as MS is generally considered more sensitive than UV detection. One EME-LC-UV method [20] managed to achieve remarkably low sub-nM detection limits using UV. The majority of the EME and EE work was applied to drug compounds or relatively hydrophobic compounds, such as aromatic amino acids [21], azo red dyes [22], herbicides [23], and volatile organic compound metabolites [24, 25]. Notable exceptions are the EME of inorganic anions [26], pulsed mode EME of histidine, phenylalanine, tryptophan [21], and asparagine and glutamine [27]. Not all papers included in the table reported both ER and EF. EFs and ERs listed in *italics* were calculated using Eq. 5. The highest EFs were achieved using EE, which was applied to extract peptides from plasma [28] and EE, which was applied to extract acylcarnitines from urine [29]. These high EFs were achieved by having nearly exhaustive extraction for selected target compounds combined with the fact that the donor volume was 1000 times larger than the acceptor volume. The EE flow cell developed by Schoonen *et al.* [30] showed demonstrated near exhaustive extraction for target analytes except for lauroylcarnitine, which is likely caused by solubility issues. The largest achievable EF in the cited works was in studies that made use of a 24 mL donor phase [31–33] in combination with

acceptor phases of 10–25  $\mu$ L. According to Eq. 2, very high EFs of up to 2400 can theoretically be achieved with this system. However, since the extractions were not exhaustive, with ERs typically below 15%, moderate EFs were obtained as follows from Eq. 5. Recently, it was demonstrated that pH changes due to electrolysis affecting the acceptor phase during EME play an important role in the ability of an extraction to be exhaustive. Furthermore, by choosing an acceptor phase with a stronger buffering capacity, the ERs that can be achieved are strongly enhanced [34]. This can be explained by the fact that the analytes maintain their charge in the acceptor phase for a longer duration due to reduced effects of electrolysis of the acceptor phase. A potential downside of this approach is that the sample might not be suitable for injection into an analytical system due to the high/low pH value or the salt content becomes too high when the sample is neutralized prior to injection. The extraction times for all techniques were in the range of 5 min to 20 min, with exception of the chip-based EME setups, where the optimized extraction times were >33 min [35] and 25 min [23]. It is important to note that the flow rate and the donor volume play a critical role in the extraction times of chip-based setups. A chip-based setup for the EME of chlorinated phenoxyacetic acids [23] was faster, despite having a donor volume that was five times higher than the EME chip for basic drugs [35]. The parallel EME setup [36] had the highest throughput, despite taking 8 min for the EME procedure. The achieved high throughput is a consequence of the extractions taking place in parallel. The system with the fastest total analysis time reported the extraction of drugs from whole blood [37], taking 5 min for EME and 5.5 min for separation. The range of voltages applied span several orders of magnitude, from 1.5 V [38] to 300 V [39] for EME and 300 V [30] to 15 kV [28, 29] for EE. The 1.5 V for EME [38] was chosen due to the fact that the extraction took place over an ionic liquid-based membrane, which typically has a lower resistance than commonly used SLM solvents. This results in higher currents and necessitates the use of a low extraction voltage in order to avoid effects of electrolysis and Joule heating. EE usually takes place from a thick organic phase, often ethyl acetate, into an aqueous phase. Because of this, higher voltages can be applied during EE than in EME, where the organic phase, which is located inside the SLMs, is typically thin. When hyphenating EE and EME to LC, care should be taken that the acceptor phase is suited for injection onto the LC column. In all the LC-related work that is within the scope of this review, the acceptor phase is of aqueous nature, which explains the fact that reversed phase LC is almost exclusively used in combination with EE and EME, one exception being ion chromatography for the analysis of inorganic anions [26]. Furthermore, this might also explain the fact that the application area of EME-LC currently mainly covers medium-apolar to apolar compounds, such as peptides and drugs.

An important aspect that should be taken into account when EE and EME are hyphenated online to LC is that the setup should either be resistant to high pressure or decoupled from this pressure. Moreover, current leakage from the EE or

Table 1. Overview of EE and EME hyphenated to LC. EF and ER values are calculated based on data provided in the paper

Ref.	Tech.	Hyph.	Det.	Matrix	Compounds*	LOD (nM)	EF	ER (%)	V <sub>donor</sub> (mL)	V <sub>acceptor</sub> (μL)	ET (min)	ST (min)	EV (V)
[29]	EE	online	MS	Urine	stearyl carnitine, hexanoyl carnitine, acetyl carnitine	1.4–380	175–970	17.5–97.0	0.1	0.1	6	30	15000
[28]	EE	online	MS	Plasma	angiotensin 2, angiotensin 1, angiotensin 2 (3-8)	10–50	±570–990	57.0–99.0	0.1	0.1	6	40	15000
[39]	EE	offline	MS	Urine	lauroyl carnitine, octanoyl carnitine, hexanoyl carnitine	0.3–2	34–80	46–108	0.5	13.4	10	31	300
[60]	EME	offline	UV	Environmental samples	Cr(IV) and Cr(III)	54–100	21.8–33	31–47	2.1	30	9	15	30
[22]	EME	offline	UV	Academic	Azo red dye 46, 51 and 18	0.75–1.8	96–162	48–81	6	30	20	12	60
[30]	EME	offline	UV	Urine	naltrexone, nalmefene	2.9	100–199	4.2–8.3	24	10	20	12	100
[21]	EME	offline	UV	Academic	naltrexone, nalmefene histidine, phenylalanine, tryptophan	2.9 32–146	130–140 11.8–36	5.4–5.8 7.1–21.6	2.5	15	20	10	100
[61]	EME	offline	UV	Academic	amitriptyline, nortriptyline diclofenac, mefenamic acid	1.8–3.8 6.8–8.3	148–160 73–83	89–96 44–53	2.5	15	14.5 10	n.r.	30–250
[27]	EME	offline	UV	Gelatin	terbutaline, salbutamol	4.4–10	110–138	66–83	2.5	15	20	20	137
[31]	EME	offline	UV	Urine	asparagine, glutamine	189–342	29–53	43–79	24	10 (×2)	20	12	40
[32]	EME	offline	UV	Urine	nalmefene, diclofenac	5.9–14	300–350	12.5–14.6	24	25	14	10	100
[37]	EME	offline	UV	Plasma	betaxolol, atenolol	33–38	86–245	9.0–25.5	3	10	10	13	1.5
[62]	EME	offline	UV	Urine	betaxolol, atenolol	6.5–7.5	69–236	7.1–24.6	7	25	20	15	200
[63]	EME	offline	UV	Academic	strychnine, brucine	6.9–21	114–122	38.0–41.7	6.5	20	15	10	90
				Urine	verapamil, clomipramine, trimipramine	7.2–15	n.r.	n.r.					
				Wastewater		129–256	115–259	42.6–84.2					
				Milk	melamine	38	85	59					
				Powder milk		46	82	92					
				Water		16	192	85					

Continued

Table 1. Continued

Ref.	Tech.	Hyph.	Det.	Matrix	Compounds*	LOD (nM)	EF	ER (%)	V <sub>donor</sub> (mL)	V <sub>acceptor</sub> (μL)	ET (min)	ST (min)	EV (V)
[64]	EME	offline	UV	Academic	diclofenac	5.1	59	84.3	2.1	30	5	n.r.	20
[34]	EME	offline	UV	Urine	nortriptyline, amitriptyline	11-14	17-18	34-36	1	20	33.3	15	40
[65]	EME	offline	UV	Urine	nortriptyline, amitriptyline	1.9-3.6	89-94	11.6-12.5	30	40	20	12	100
[20]	EME	offline		Plasma	amitriptyline, nortriptyline	1.8-1.9	83-92	11.1-12.3	10	30	15	15	50
[66]	EME	offline	UV	Academic	fluoroquinolones**	0.039-0.22	40-85	12-26					
			FLD	Academic	fluoroquinolones***	0.014-0.10	40-85	12-26	10	50	7	12	10
			UV	Academic	basic drugs#	0.17-6.6	51-86	26-43					
			FLD		naproxen, salicylic acid, ibuprofen	0.0026-3.5							
[26]	EME	offline	CD	Academic	chloride, nitrate, fluoride, nitrite, bromide, sulfate, phosphate	17-79	67-117	40-70	5	30	10	14	15
[38]	EME + LPME	offline	UV/MS	Plasma	ketoprofen, ibuprofen, citraprolam, sertraline	0.79-6.2	3.8-5.7	63-95	0.6	100	15	6.1 (UV)	300
[36]	EME	offline	MS	Whole blood	basic drugs##	0.16-15	0.7-2.3	9-28	0.08	10	5	5.5	15
[67]	EME	offline	MS	Dried bloodspots	methadone, nortriptyline, pethidine	0.3-3.6	44-50	83-95	1	19	25	26	100
[57]	EME	offline	MS	Dried bloodspots	citaprolam, methadone, loperamide, sertraline	1.2-17	6.3-16	44-115	0.3	21	10	24	100
[68]	EME	offline	MS	Saliva	basic drugs###	2.3-46	1.2-7.8	10-65	0.3	25	5	20	15
[35]	EME	offline	MS	Academic standards	quetiapine, amitriptyline, fluoxetine, sertraline	0.052-0.91	0.51-1.13	15-33	0.24	70	8	18	200

Continued

Table 1. Continued

Ref.	Tech.	Hyph.	Det.	Matrix	Compounds*	LOD (nM)	EF	ER (%)	V <sub>donor</sub> (mL)	V <sub>acceptor</sub> (μL)	ET (min)	ST (min)	EV (V)
[24]	EME	offline	MS	Dried urine spots	Volatile organic compound metabolites ***	5.0-388	13.2-27.6	33.0-69.1	1	25	20	5	200
[25]	EME	offline	MS	Urine	Volatile organic compound metabolites ####	1.3-33.3	11 - 16	52.8-70.7	0.8	40	15	4	300
[23]	EME	online	MS	River water	Chlorinated phenoxyacetic acids ###	0.12-0.43	201-235	80-94	5	20	25	28	200

\*matrix is only mentioned when extraction performance is tested therein.

\*\*in order of increasing LOD.

\*\*danofloxacin, enrofloxacin, marbofloxacin, ciprofloxacin, grepafloxacin, gatifloxacin, gatifloxacin.

\*\*\*danofloxacin, enrofloxacin, grepafloxacin, ciprofloxacin, norfloxacin, gatifloxacin, marbofloxacin.

\*\*\*\*N-acetyl-s-(phenyl)-l-cysteine, N-acetyl-s-benzyl-l-cysteine, N-acetyl-S-(2,2dichlorovinyl)-l-cysteine, N-acetyl-S-(1,2dichlorovinyl)-L-cysteine, 3-methylhippuric acid, 4-methylhippuric acid, 2-methylhippuric acid, phenylglyoxylic acid, hippuric acid, mandelic acid.

# naproxen, ketoprofen, ketorolac, salicylic acid, diclofenac, ibuprofen.

##Ketamine, 3,4-methylenedioxy-metamphetamine, metamphetamine, cathinone, 2,5-dimethoxy-4-iodoamphetamine, 3,4-methylenedioxy-amphetamine.

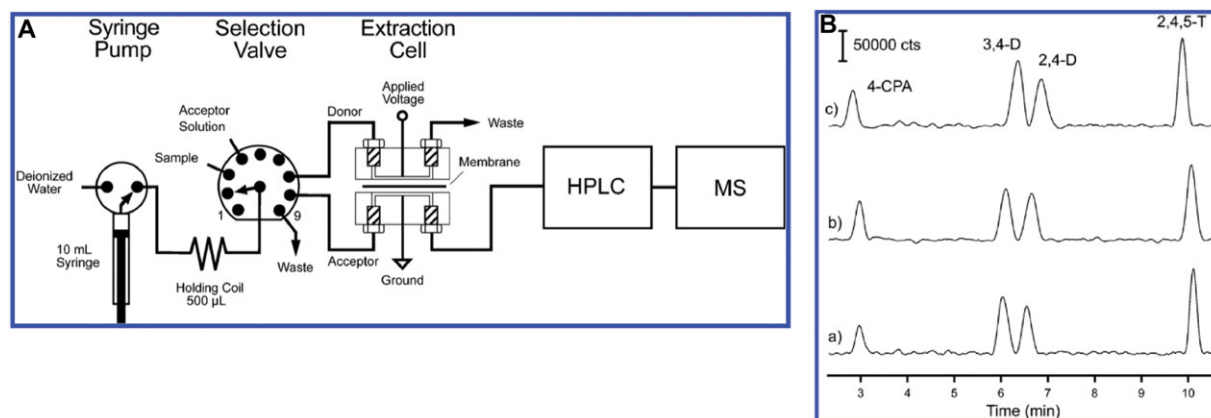
###methadone, buprenorphine, para-methoxyamphetamine, methamphetamine, para-methoxymetamphetamine.

####trichlorophenoxyacetic acid, 3,4-dichlorophenoxyacetic acid, 2,4-dichlorophenoxyacetic acid, chlorophenoxyacetic acid.

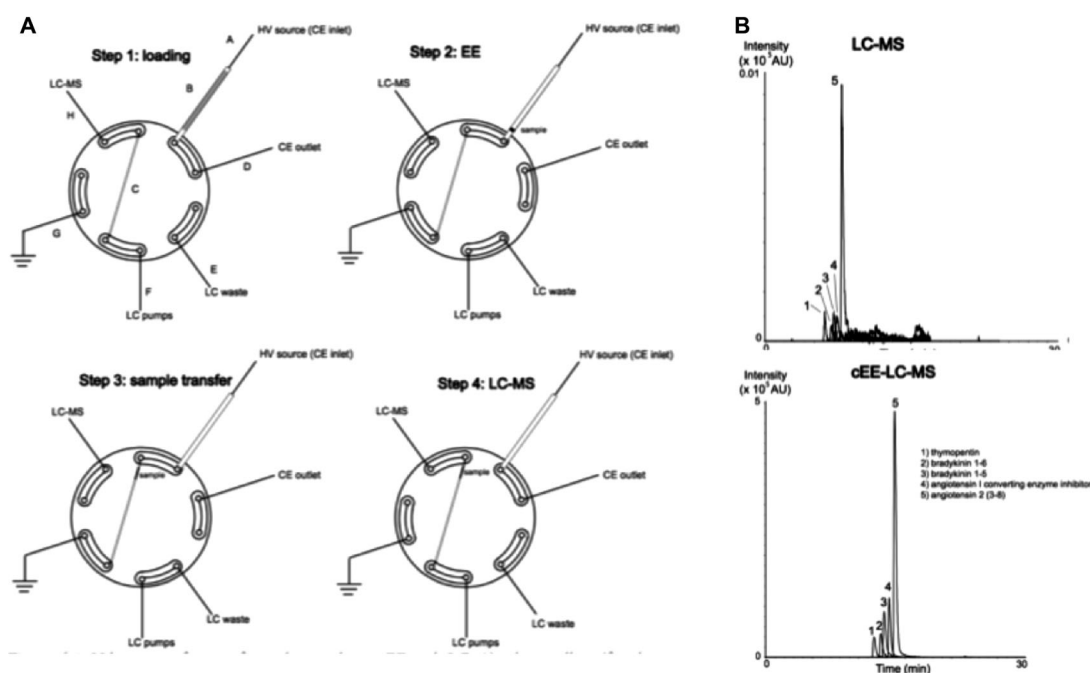
#####N-acetyl-s-(phenyl)-l-cysteine, 2-methylhippuric acid, 3-methylhippuric acid, 4-methylhippuric acid, hippuric acid, phenylglyoxylic acid.

n.r., not reported; n.a., not applicable; Ref., reference, Tech., technique, Hyph., hyphenation, Det., detection, EF, enrichment factor; ER, extraction recovery; ET, extraction time; ST, separation time; EV, extraction voltage.





**Figure 1.** (A) Experimental setup of the developed EME-LC-MS system (B) EME-LC-MS results of CPAs spiked to river water (extraction voltage 200 V, donor phase flow rate 0.2 mL/min). (a) 5 mL of 0.5 ng/mL CPAs (b) 10 mL of 0.25 ng/mL CPAs and (c) 25 mL of 0.1 ng/mL CPAs. Note that for each experiment the total amount of CPAs was the same. 4-CPA = chlorophenoxyacetic acid; 3,4-D = 3,4-dichlorophenoxyacetic acid; 2,4-D = 2,4-dichlorophenoxyacetic acid; 2,4,5-T = trichlorophenoxyacetic acid. Adapted from [23].



**Figure 2.** (A) Valve setup for interfacing large volume cEE with LC. (a) inlet capillary, (b) EE capillary, (c) sample loop, (d) outlet capillary, (e) LC waste, (f) LC pump tubing, (g) tubing to earth, (h) tubing to LC-MS. Step 1: situation after all phases have been loaded. The grey zone in the EE-capillary depicts the organic phase with analytes. Before EE starts, the valve is switched. Step 2: situation after EE is finished; the small black zone depicts the concentrated analytes. Step 3: situation after the sample has been transferred into the sample loop by applying pressure. After sample transfer, the valve is switched back. Step 4: situation where the sample zone is being injected into the LC-MS system. (B) comparison of chromatograms obtained from LC (0.1  $\mu$ L injection) and large volume cEE-LC-MS (100  $\mu$ L extraction volume). The initial peptide concentration was equal and ERs. Adapted from [28].

EME system to the LC system should be prevented in order to avoid possibly damaging the equipment and to prevent endangering the operator.

See & Hauser reported on an automated system for EME coupled online with LC-MS [23]. In this work a previously developed polymer inclusion membrane (PIM) was used [40, 41]. This PIM consisted of cellulose triacetate as base polymer, tris(2-ethylhexyl)phosphate as plasticizer and varying amounts of Aliquat 336 as cationic carrier.

The EME system (Fig. 1A) was composed of a two-way syringe pump, a nine-port channel selection valve and a miniaturized flow-through extraction cell. The donor and acceptor channels had a volume of 20  $\mu$ L. The acceptor side of the chamber was filled with acceptor solution and remained stagnant during extraction, while the donor solution was flowing. The operation of the entire system was performed using an open-source electronics prototyping platform. In one experiment the donor volume and analyte concentration

were varied while the total amount of chlorinated phenoxyacetic acid herbicides (CPAs) that were led through the extraction cell were kept constant. This led to similar chromatograms (Fig. 1B), this indicates exhaustive extraction took place regardless of the concentration. The LODs that were obtained (using 5 mL donor phase) with this system for CPAs were 0.12–0.43 nM and EFs of 201–235 were reported. Furthermore, the repeatability was good; when the CPAs were spiked to river water samples the relative standard deviations were in the range of 4.8–5.5%. The (nearly) exhaustive extraction of samples as large as 25 mL was successfully demonstrated, suggesting that very high EFs and thus very low LODs can be achieved. However, this comes at the cost of unfavorably long extraction times.

Lindenburt *et al.* coupled EE online to reversed-phase LC-MS via a 2-way 10-port switching valve for the determination of peptides spiked to plasma [28]. EE took place in a large bore (1 mm inner diameter) PEEK capillary. A schematic diagram of this setup is shown in Fig. 2A. First, the injection of the donor and acceptor phases, application of the electric field and transfer to the sample loop of the switching valve, were performed using a CE apparatus. Next, the loop was switched and the extracted analytes were injected onto the separation column using an LC-MS system. The entire system, i.e. CE instrument, switching valve and LC-MS equipment, was automated and could extract and analyze a series of samples without human interference. The organic donor phase volume was 100  $\mu$ L of ethyl acetate containing the sample ions and 1.8% (v/v) trifluoroacetic acid, the extraction voltage was 15 kV and the extraction time 6 min. LODs of 10–50 nM were obtained, and EFs of 570–990, corresponding to ERs of 57–99%. Figure 2B shows a comparison between EE-LC-MS and conventional LC-MS.

The aforementioned method was successfully applied to the analysis of acylcarnitines in urine [29], resulting in estimated LODs of 1.4–390 nM and EFs of 175–970, corresponding to ERs of 17.5–97%. The polarity of the acylcarnitines, which is mainly a result of the acyl chain, was observed to be of major influence on extraction performance (with a bias to the more apolar acylcarnitines in the test mixture).

### 3.2 Hyphenation to GC

Table 2 shows detailed information on the works that combined EME with GC. Five EME procedures were analyzed by GC-FID, while two were analyzed by GC-MS. Generally EME-GC was applied to tricyclic anti-depressants [16, 42–44], once to opioids [45] and once to pyridine derivatives [46]. Detection limits in general were in the low nM range. The extraction times ranged between 14 and 20 min, with voltages ranging from 50 to 240 V and separation times between 5 and 25 min.

The number of reported EME-GC applications is lower than the combination of EME and LC. This is due to the fact that EME typically takes place from an aqueous donor into

an aqueous acceptor. However, when combined with GC only one EME work made use of an aqueous acceptor phase, which was subsequently directly injected into the GC system. Several precautions were taken in order to make this possible, such as keeping the injection volume low, preventing the water from condensing on the column and selecting appropriate column [44]. However, despite these complications, this system had the fastest total analysis time. It took 25 min from extraction to completion of the GC analysis. The other works made use of organic injection solvents by applying EME directly into a solid support, i.e. solid-phase microextraction (SPME) [15, 16], into an organic phase [43, 45] or by applying dispersive liquid-liquid microextraction (DLLME) after the EME procedure [42, 46]. In general, polar groups need to be derivatized prior to GC analysis in order to increase the vapor pressure of the analytes. Only one technique [15] included derivatization, which was performed after EME into a solid pencil support.

The largest EFs were achieved with EME-DLLME of drugs from urine followed by GC-MS analysis [42]. As already mentioned in the LC section, the highest EFs can be obtained where the difference between donor and acceptor volume is the largest. However, the EF cannot be deduced from EME-SPME, as the analytes adsorb to a solid sorbent. The combination of EME-SPME and EME-DLLME typically had a low reported recovery. This is due to the fact that the reported recoveries are not the recoveries of EME alone, but recoveries from the combination of EME and SPME or DLLME. Two-phase EME and regular EME show satisfactory recovery values (>70%). It is important to note that in order to properly assess the total analysis time when combining EME with GC that the duration of the SPME, DLLME and derivatization procedures need to be taken into account. These analysis times were >10 min for the EME-DLLME of pyridine derivatives [46], >3 min for the EME-DLLME of tricyclic anti-depressants [42] and >2 min for the thermal desorption and derivatization during EME-SPME [15, 16].

Until now, EME has not been coupled online to GC, but important steps have been taken towards achieving online hyphenation. For example, an EME system called ‘electromembrane surrounded solid phase microextraction’ bridges the gap between EME and GC considerably [16]. Here, the analytes were extracted from an aqueous donor phase, via an organic supported liquid membrane, into an aqueous acceptor phase in which the analytes were adsorbed to a solid sorbent, i.e. a pencil lead, that acted as the cathode. After EME, the pencil lead was inserted into a GC injector for thermal desorption of the analytes and consecutive analysis. The method was applied to analysis of doxepin and amitriptyline in urine (LOD 3.6 nM for both) and plasma (LOD 18 and 9 nM, respectively); reported ERs (covering both EME and SPME) were in the range 3–10%. In the work performed by Razazadeh *et al.* [15], EME-SPME was applied to the analysis of acidic herbicides in plant material, with LODs of 2.2–25 nM and ERs (including SPME) of 0.6–4.8%.

Two reports of an EME setup where the acceptor phase and SLM phase were comprised of the same organic solvent



Table 2. Overview of EME hyphenated to GC. EF and ER values indicated in *italic* are calculated based on data provided in the paper

Ref.	Tech.	Hyph.	Det.	Matrix	Compounds*	LOD (nM)	EF	ER (%)	V <sub>donor</sub> (ML)	V <sub>acceptor</sub> (μL)	ET (min)	ST (min)	EV (V)
[42]	EME	offline	FID	Plasma Urine	imipramine, clopramine	1.7-2.5 1.4-2.4	215-250 240-265	72 81	2.1	7	20	5	200
[45]	EME-DLLME	offline	FID	Urine	quinoline, 2,4-lutidine, 3-methylpyridine, 4-dimethylaminopyridine	1.9-16	40-202	1.7-8.4	24	10	20	10	50
[15]	EME-SPME	offline	FID	Plant tissue	2,4-dichlorophenoxyacetic acid, 2-methyl-4- chlorophenoxyacetic acid	2.3-25	n.a.	0.6-4.8	24	n.a.	20	12	50
[16]	EME-SPME	offline	FID	Urine	doxepin, amitriptyline	3.6	n.a.	4.0-10.4	24	n.a.	20	8	120
[44]	Two-phase EME	offline	FID	Plasma Academic	amitriptyline, doxepin methadone, alfentanil, sufentanil	9.0-18 1.9-3.6	n.a. 353-476	5.9-3.1 70.5-95.2	3	6	20	12.5	80
[16]	EME-SPME	offline	FID	Urine	doxepin, amitriptyline	3.6	n.a.	4.0-10.4	24	n.a.	20	8	120
[43]	Two-phase EME	offline	MS	Plasma	amitriptyline, doxepin imipramine, citaprolam, sertraline, desipramine	9.0-18	n.a.	5.9-3.1	1.2	6	15	25	60
[46]	EME-DLLME	offline	MS	Academic Urine	doxepin, amitriptyline, trimipramine	0.36-0.75 n.r.	140-175 145	70-87 73	24	10	14	10	240
				Urine	doxepin, amitriptyline, trimipramine	11-51	753-781	31.3-32.5	24	10	14	10	240
				Plasma	trimipramine, doxepin, amitriptyline	10-54	383-397	16.0-16.5					

<sup>1</sup>matrix is only mentioned when extraction performance is tested therein.

\*in order of increasing LOD

were made, i.e. two-phase EME [43, 45]. This allowed for direct injection of the extracted analytes into a GC system. In both cases the EME setup consisted of a sample vial containing the donor phase in which one electrode is immersed and a hollow SLM fiber containing the acceptor phase in which the other electrode is immersed. Using a magnetic stirrer, the donor solvent was agitated during EME to enhance extraction. After extraction, the acceptor solution was collected and injected directly into a GC system. FID was used in case of optimization experiments and MS in case of analytical characterization.

The authors tested several organic solvents as acceptor solvent and found that 1-heptanol yielded the highest ERs for tricyclic anti-depressants [43]. A requisite of the solvent is that it has some electric conductance, which draws parallels to EE. The pH of the donor solution, extraction voltage, stirring speed and extraction time were optimized and a comparison with conventional (three-phase) EME was made. Two-phase EME was shown to have an interday repeatability in the range of 6.2–10.8% while intraday repeatability was in the range of 6.0–11.8%, indicating robust analytical performance. Two-phase EME was found to be faster than three-phase EME (15 versus 20 min extraction time), while the EF and LOD were somewhat lower, i.e. 145 versus 280 and 0.1 ng/mL versus 0.8 ng/mL respectively. However, this comparison does not take into account differences in volume ratios of donor and acceptor phase that affect both EF and ER (Eq. 5). There is an important difference in the electric field distribution in conventional (three-phase) EME and two-phase EME, which could account for the shorter extraction time in two-phase EME. In the two-phase EME setup, the majority of the electric field is applied over the acceptor phase, which has the highest resistance, which could indicate slow extraction and low recovery. However, the ER of some selected test compounds (imipramine, desipramine, sertraline and citalopram) after 15 min of EME with 1-heptanol as organic phase was surprisingly high:  $\pm 70$ –87%. Interesting to note is that further improvements have been made by performing two-phase EME in pulsed mode and in combination with a surfactant to enhance mass transport across the SLM [45]. Here the acceptor phase consisted of 1-octanol instead of 1-heptanol and the donor phase contained 0.02% (w/v) sodium dodecyl sulfate. By performing the EME in pulsed mode with the aforementioned surfactant, the recoveries increased compared to conventional two-phase EME from 48.3–68.5% to 70.5–95.2%.

In our opinion, the fact that analytes were successfully extracted from an aqueous donor phase into an organic donor phase, which is opposite to all other EE and EME work, is important. This is because it not only opens up possibilities for online hyphenation of EME to GC, but also to normal phase LC and HILIC. As the donor and acceptor solvent are immiscible and present in sufficient volumes, the membrane could perhaps be abandoned, which might result in a simpler EE system.

### 3.3 Hyphenation to CE

Since CE, EE, and EME are all based on electromigration, combinations of CE with one of the two is a logical and attractive option. However, no reports of EE coupled with CE have been reported within the timeframe of this review. Table 3 shows that the combination of EME with CE has been reported in the literature various times. In all cases, a combination of EME and capillary zone electrophoresis was reported. However, in the aforementioned EE of acylcarnitines in urine [29], the primary employed separation technique was LC, but the offline combination with CE was briefly explored and found to be promising: extracts from urine obtained by EE were analyzed with a CE-MS system and it was shown that metabolites from various important classes, such as amino acids and acylcarnitines could be measured in these extracts.

As can be observed in Table 3, UV detection has been reported nine times in combination with EME-CE. MS detection in combination with EME-CE has not been reported, despite the sensitivity and selectivity it offers. Capacitively coupled contactless conductivity detection ( $C^4D$ ) is reported three times.  $C^4D$  has two advantages for usage in combination with EME, (i) integration into a CE system is relatively simple and (ii) detection of all charged species is possible, including those that cannot be detected with optical detection methods [47]. As CE is more suited than reversed phase LC for separation of hydrophilic and charged analytes, some EME applications to amino acids [48], polyamines [49] and polar herbicides [40] were combined with CE. The reported LODs were generally in the low nM range, regardless of the use of  $C^4D$  or UV as detection method. It is interesting to note that some applications had low ERs, but still had favorable, low nM to  $\mu$ M LODs, such as EME performed at constant current [48] and nano-EME [50]. The low LODs with nano-EME that were obtained, despite the ERs <1%, were achieved due to the fact that the potential EF would be 25 000 times on the basis of exhaustive extraction. Nearly exhaustive extractions were obtained when using carbon nanotubes in SLMs [51, 52], with ERs of >90% and EFs of >180. Extraction times ranged from 2 to 32 min and separation times between 4 and 25 min. The fastest EME and separation procedure using CE takes 9 min to complete. Voltages ranged between 4 and 1500 V. In case of the 1500 V potential, as was applied in a polymer inclusion membrane (PIM) EME flow cell [40], electrolysis issues were resolved by placing the electrode in the donor channel at the outlet, which caused bubbles to be flushed away and prevented them from intervening with the extraction process.

When hyphenating EME online with CE, care must be taken that the acceptor phase is suited for CE analysis. Thus, the acceptor phase solvent should not disturb CE separation and, especially in the case of online coupling, the acceptor phase volume should match the CE injection volume. A typical hydrodynamic injection into a CE capillary should be preferably below 2% of the capillary volume, often

**Table 3.** Overview of EME hyphenated to CE. EF and ER values indicated in *italic* are calculated based on data provided in the paper

Ref.	Tech.	Hyph.	Det.	Matrix	Compounds*	LOD (nM)	EF	ER (%)	V <sub>donor</sub> (mL)	V <sub>acceptor</sub> (μL)	ET (min)	ST (min)	EV (V)
[50]	EME	online	UV	Urine	loperamide, methadone, haloperidol, nortryptiline, pethidine	0.41-60	25-196	0.10-0.79	0.2	0.008	2	25	200
[48]	EME	offline	UV	Urine	drugs and amino acids**	2.1-2500	<i>0.35-39</i>	0.20-21.7	3.5	20	5	4	±4/±40
[69]	EME	offline	UV	Urine	S-and R-propranolol	27	132-134	66-67	4	20	32	19	40
[70]	EME	offline	UV	Plasma		39	108-110	54-55	4	20	20	7	125
[71]	EME	offline	UV	Hair	methadone, ephedrine, methamphetamine	9.8-41	116-140	56-64					
[52]	EME	offline	UV	Urine	methadone, methamphetamine, ephedrine	4.9-18	112-128	58-70					
[71]	EME	offline	UV	Urine	thebaine, ibuprofen	23-24	150-160	75-80	4	20	25	10	40
[52]	EME	offline	UV	Urine		4			4	20	10	14	5
[51]	EME	offline	UV	Academic	naproxen, ibuprofen	4.3-7.3	180-188	90-94	4	20	15	10	200
[72]	EME	offline	UV	Plasma		n.r.	<i>138-154</i>	69-77	4	20	15	20	15
				Waste water		n.r.	<i>180-184</i>	90-92					
				Breast milk		n.r.	<i>148-156</i>	74-78					
				Urine		n.r.	<i>170-176</i>	85-88					
				Urine	buprenorfine	2.1	185	92	4	20	15	10	200
				Academic	naproxen, diclofenac	6.56-8.17	176-184	88-92	4	20	15	20	15
				Urine	diclofenac, naproxen	15.3-15.8	164-176	82-88					
				Plasma	diclofenac, naproxen	19.7-20.5	158-166	79-83					
[73]	EME	offline	UV and C <sup>4</sup> D	Environmental water	chlorinated phenoxyacetic acids***	≤19-≤39	<i>6.8-11</i>	15-25	0.9	20	5	10	150
[49]	EME	offline	C <sup>4</sup> D	Saliva	spermine, spermidine, cadaverine, putrescine	8.4-79	17 - 106	1.9-12	7	8	20	22	10
[74]	EME	offline	C <sup>4</sup> D	Plasma	3,4-methylenedioxy-metamphetamine, amphetamine, metamphetamine	5.2-17	97-103	65-69	3	20	10	7	300
[40]	EME	offline	C <sup>4</sup> D	Academic	glyphosphate,	0.25-0.58	87-95	86-96	10	100	10	5.3	1500
				River water	aminomethylphosphonic acid	n.r.	85-95	85-95					

\*matrix is only mentioned when extraction performance is tested therein.

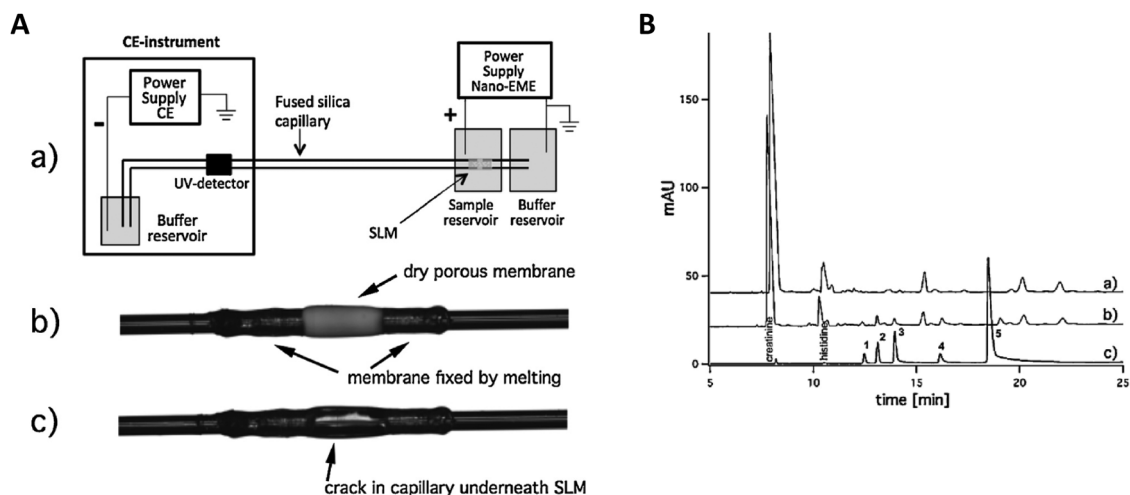
\*\*in order of increasing LOD.

\*\*\*loperamide, haloperidol, nortriptyline, tryptophan, phenylalanine, creatinine, tyrosine, histidine, arginine.

\*\*\*\*pentachlorophenol, 2,4,6-trichlorophenol, 2,3-dichlorophenol, 2,3-dichlorophenol, 2,3-dichlorophenol, 3-chlorophenol, 4-chlorophenol.

\*\*\*\*\*3,4-methylenedioxy-metamphetamine, amphetamine, methamphetamine.

#loperamide, methadone, nortriptyline, pethidine, haloperidol.



**Figure 3.** (A) Instrumentation used for nano-EME (B) urine analysis by CE-UV, comparing (a) blank urine without nano-EME, (b) spiked urine without nano-EME and (c) spiked urine with nano-EME, applying 200 V for only 15 s. Peak assignment: 1 pethidine, 2 nortriptyline, 3 methadone, 4 haloperidol, 5 loperamide. Adapted from [50].

corresponding to volumes of up to 20 to 30 nL, unless stacking procedures are used. The low loadability of CE can be considered a limiting factor in achieving low LODs; this is where online coupling of EE and EME to CE can be highly advantageous.

Ramos-Payán *et al.* [50] fully integrated EME in a CE separation capillary. This integration resulted in an EME-CE capillary that could be used as many as 200 times, before it needed to be replaced. These 200 runs took place in a period of two months in which water samples and urine samples were analyzed. This EME mode was aptly named ‘nano-EME’, as the acceptor volume consisted of a minute amount of 8 nL phosphate buffer. The nano-EME setup was realized with a cracked fused silica capillary, which was surrounded by an SLM (Fig. 3A). With this setup, pethidine, nortriptyline, methadone, haloperidol, and loperamide were extracted from urine, separated by CE and detected with UV. The sample volume was 200  $\mu$ L, the membrane solvent was nitrophenyl octyl ether, the extraction time 120 s and the extraction voltage 200 V. EFs were 25–196, but the ERs were low (<1%). This could be a benefit when the setup is applied to study reaction kinetics, as oversampling would disturb the kinetics. Importantly, the authors developed a electromigration-based SLM cleaning procedure by submerging the SLM for 2 min in 10 mM HCl while applying 200 V and simultaneously flushing the capillary with separation buffer. This cleansing extraction procedure eliminated carry-over in the system. Furthermore, the relative standard deviations were less than 15% when this cleansing extraction procedure was applied and it ensured that one EME-CE capillary could be used 200 times. The authors also show highly promising urine analysis results in which several spiked drugs were significantly enhanced by rapid 15 s extractions (Fig. 3B). No between-capillary repeatability was reported. However, the authors indicated that a more robust nano-EME design should be created.

For the analysis of low-abundant analytes in biological samples the low recovery does not pose a limitation (the obtained LODs were 0.41–60 nM). Nonetheless, online coupling of EME-CE to a more sensitive detector, such as LIF or MS seems attractive for the analysis of low-abundant compounds.

### 3.4 Hyphenation to direct detection

Table 4 shows an overview of the efforts that have been undertaken to combine EE and EME directly with detection techniques that require no separation of analytes. Most of these efforts are highly targeted approaches, due to the fact that without prior separation interfering components might affect efforts to quantify the analytes. Several efforts were aimed at the online monitoring of drug metabolism by rat liver microsomes using direct infusion MS [17–19]. These platforms investigate kinetics involved on a continuous time-scale as opposed to the discrete concentration-time profiles that are obtained via LC-MS. As these setups had flowing acceptor phases, their EFs are not determinable. Another MS-based online approach that was reported is three-phase EE [9]. Aside from drug compounds and metabolites, two EME applications focused on the detection of nuclear fuel in aqueous environmental samples using fluorescence [53] and UV detection [54]. The LODs for the various direct detection techniques ranged from sub-nM to high nM concentrations. The ERs and EFs were varying to a great degree, but the highest EFs were achieved by EME of morphine from urine, showing a 152-fold enrichment along with a satisfactory ER of 76% [55]. The voltages ranged between 2.5 and 200 V [19]. Extraction times for the monitoring of drug metabolism kinetics depend on the time the reaction is monitored as well as the volume of the phases. For example, the EME probe [17] could theoretically be used for  $\sim$ 333 min at an acceptor flow rate

Table 4. Overview of EME hyphenated to direct detection

Ref.	Tech.	Hyph.	Det.	Matrix	Compounds*	LOD (nM)	EF	ER (%)	V <sub>donor</sub> (mL)	V <sub>acceptor</sub> (μL)	ET (min)	ST (min)	EV (V)
[17]	EME	online	MS	Rat liver microsomes	amitriptyline, promethazine, imipramine***	29	n.r.	n.r.	1	flowing	variable	n.a.	2.5
[18]	EME	online	MS	Rat liver microsomes	Amitriptyline	140	n.r.	83	1	flowing	variable	n.a.	15
[9]	Three-phase EE	online	MS	Plasma	acylcarnitines**	90-330	2-7	8.0-28	0.05	2	9	n.a.	140
[19]	EME	online	MS	Rat liver microsomes	amitriptyline, promethazine, methadone***	n.r.	n.r.	n.r.	flowing	flowing	variable	n.a.	200
[55]	EME	offline	ED	Urine	Morphine	5.3	152	76	4	20	24	n.a.	90
[53]	EME	offline	FLD	Seawater	Uranium	0.42	64.1-88.1	51.8-70.5	2.5	20	14	n.a.	80
[75]	EME-SPME	offline	MS	Academic	metenamic acid, ibuprofen	0.17-0.24	n.r.	n.r.	3	n.a.	24	n.a.	80
[54]	EME	offline	UV	Waste water	Thorium	1.2	50	10	10	20	30	n.a.	90

\*matrix is only mentioned when extraction performance is tested therein.

\*\*in order of increasing LOD.

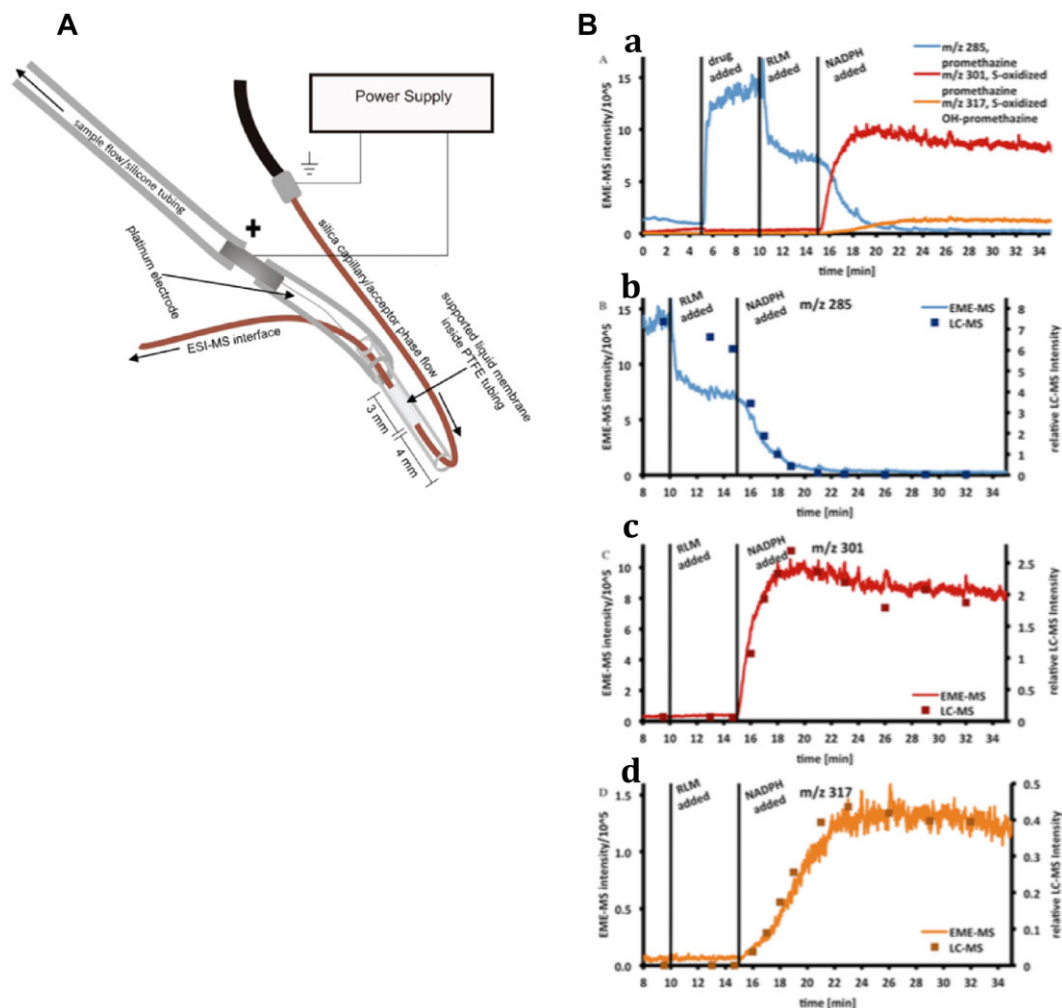
\*\*\*propionyl-, octanoyl-, butyryl-, decanoyl- and hexanoylcarnitine.

\*\*\*\*and their metabolites.

of 3 μL/min and an acceptor volume of 1 mL. Aside from the monitoring applications, the extraction times were in the range of 9 and 30 min. For the online applications this time was very close to the total analysis time.

A major complication in MS is ion suppression of analytes by co-occurring sample components, such as salts, buffer molecules and proteins [56]. EE and EME enable selective extraction of analyte molecules while leaving salts, buffer components and proteins behind, this in turn enables direct targeted MS analysis.

As mentioned, in the past few years, several EME devices have been developed that allow for direct monitoring of drug metabolism [17-19]. In these studies, the main objective was not to achieve the highest ERs and lowest LODs, but to couple EME online to MS and to apply it to study drug metabolism in real time. One such application is a microfluidic EME chip that can be coupled online to MS and allows for monitoring of metabolism of amitriptyline by rat liver microsomes in an easily accessible open reservoir [18]. The chip consisted of two polymethyl methacrylate (PMMA) plates with channels on each plate. A polypropylene SLM consisting of porous polypropylene with nitrophenyl octyl ether immobilized in its pores was located between the two plates, which separated the donor and acceptor channels. A platinum wire electrode was inserted in the sample channel, in order to close the electric circuit. Furthermore, this electrode prevented the SLM from blocking the channel upon application of negative pressure at the outlet. The EME chip was connected to a temperature-controlled reaction chamber, in which amitriptyline was metabolized by rat liver microsomes. With a syringe pump, the reaction mixture was transferred to the sample channel, where amitriptyline and its metabolites were extracted by EME. Another syringe pump transferred the acceptor solvent containing the extracted analytes towards an ion trap MS. The response time of the system, i.e. the time until the detector responded to a change in the sample, was rapid at 9 s. The EME recovery of amitriptyline from a standard solution was 83% at 5 μL/min sample flow, which was determined using a sample loop. The authors thoroughly investigated ion suppression and showed that salts, proteins and buffer components were not extracted. Dugstad *et al.* abandoned the chip-format and developed an online dip-in-probe for EM [17]. This new design was implemented in order to simplify construction and to make access to the SLM easier. The EME probe was tested on rat liver microsome mediated metabolism of amitriptyline, promethazine and imipramine. Finally, Fuchs *et al.* [19] further improved the probe to prevent sample depletion. Instead of extracting from a stagnant donor phase, as in previous online drug kinetics monitoring [17, 18], a flowing donor solution was used to perform EME. This is of importance, because depletion of the sample influences the kinetics of the reaction under study. Figure 4A shows the probe, which is located inside a tube through which the donor solution is pumped. The SLM contained nitrophenyl octyl ether. First, the dip-in flow-flow EME probe was dipped in a temperature-controlled chamber containing the reaction mixture, then the sample was slowly

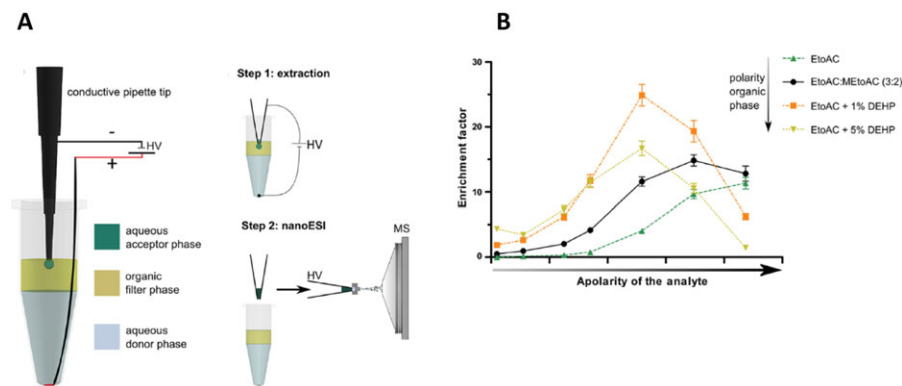


**Figure 4.** (A) Schematic drawing of the dip-in flow-flow EME probe. (B) comparison of metabolic profiles of promethazine obtained with EME-MS and LC-MS. (a) overlay of extracted ion chromatograms of promethazine (b) and its two main metabolites, S-oxide promethazine (c) and S-oxide OH-promethazine (d). NADPH was added to the reaction mixture to initiate metabolism. RLM = rat liver microsomes. Adapted from [19].

(10  $\mu\text{L}/\text{min}$ ) flushed through the chamber while EME took place (extraction voltage 200 V). Finally, the acceptor phase of EME, which was flowing as well during the extraction (10  $\mu\text{L}/\text{min}$ ), was flushed to a triple quadrupole MS to detect the analytes in selected ion monitoring mode. The setup was used to study metabolism kinetics of amitriptyline, promethazine and methadone. Figure 4B shows extracted ion chromatograms of the products of promethazine metabolism by rat liver microsomes into its two major metabolites S-oxide promethazine and S-oxide OH-promethazine. It would be interesting to know the associated LODs of this innovative approach, but these are not reported. The performance of EME-MS was shown to be consistent with LC-MS. Thus EME could provide a valuable alternative to LC, offering analysis speed and efficient removal of ion suppressing compounds, especially when the analysis of a limited number of analytes is to be measured.

Raterink *et al.* developed three-phase EE and coupled it online to nano-ESI-MS [9]. In this setup, EE took place from a 50  $\mu\text{L}$  donor solution on the bottom of a vial, via an immiscible layer of organic solvent, which acted as a filter, into a 2  $\mu\text{L}$  acceptor solvent droplet, which was hanging from a conductive pipette tip (Fig. 5A). The hanging droplet was aspirated into the pipette tip after EE. Finally, the extract was ionized via nano-ESI and analyzed on an LTQ-Orbitrap MS. The volumes used in this approach limited the maximum EF to 25. A set of acylcarnitines was used to demonstrate this approach. The optimal extraction time and voltage were 3 min and 140 V, respectively. The influence of the polarity of the organic filter phase on the enrichment of analytes was studied (Fig. 5B) and it was shown that the system became more favorable for the extraction of more hydrophilic acylcarnitines when the polarity of the organic filter phase decreased. These results indicate that extraction selectivity





**Figure 5.** (A) setup of three-phase EE (left) and its coupling to nanoESI-DI-MS (right). (B) influence of the organic filter composition on analyte enrichment factor. EtOAc = ethyl acetate, MeToAc = methyl acetate, DEHP = bis(2-ethylhexyl)phosphate. Adapted from [9].

can be tuned by altering the organic filter phase composition. Furthermore, the authors demonstrated analysis of acylcarnitines spiked to protein rich samples and plasma with relative standard deviations below 15%. In plasma, endogenous acylcarnitines were detected and LODs were 90–330 nM. Clean mass spectra were obtained, implying effective protein removal and a multitude of endogenous metabolites were detected in plasma. Lastly, the ability of automating the approach using an automated nano-ESI robot and a modified 96-wells plate was explored. As being capable of automatically performing EE and nano-ESI would make the approach highly suitable for high-throughput metabolomics studies.

#### 4 Concluding remarks and future perspectives

In this review, we presented a detailed overview of concentration enrichment studies performed by EE and EME in the period between April 2012 and November 2015. Specifically, we made a comparison between various approaches and the combination with separation techniques and direct detection. In this comparison, the importance of looking at the combination of EF and ER was emphasized, as EF alone does not yield sufficient information on the extraction efficiency. Not all papers reported on all crucial parameters, i.e. ER, EF, acceptor and/or donor phase volume and LOD, which made it difficult to put the techniques into perspective and to compare the various approaches. Therefore, we propose that at minimum the aforementioned crucial parameters are reported in EE and EME research, in order to provide results that can be compared to other methodologies.

Several papers were highlighted that demonstrated the online hyphenation of EE and EME to analytical separation and/or detection techniques or in which important steps were taken towards achieving online hyphenation. MS is often the detection method of choice, as it offers unmatched selectivity and high sensitivity. However, due to the concentration power of EME sub-nM LODs could also be obtained with UV detection. Online hyphenation of EME with LC was achieved

by coupling an EME flow cell to a channel selection valve. The advantage of this flow cell based setup is its ability to extract large donor phase volumes without compromising the high ER. The other EE and EME flow cell designs covered in this review also have a similarly high potential for online hyphenation to LC. The nano-EME technique was also coupled online to CE and has an advantage over conventional hydrodynamic injection, as the analytes are concentrated directly within the minute volume of the separation capillary and thereby the loadability of CE is enhanced. Though far from exhaustive, this type of extraction can still be of added value for monitoring reaction kinetics. Most online monitoring of reaction kinetics using EME took place via direct detection, often MS was the detector of choice. Whether via flow cell designs or probes, EME was able to extract drug metabolites generated by rat liver microsomes. The focus here was not so much on the concentration enrichment, but on the removal of components that could result in ion suppression. The EME probe setup was modified to make use of both flowing donor and acceptor phases, which prevented perturbations in the reaction kinetics. Another technique, three-phase EE, was briefly evaluated for online hyphenation to MS analysis and showed promising results for high throughput metabolomics.

Last, the two-phase EME method shows a high potential for automated online coupling to GC, as the acceptor solvents are suited for direct injection into a GC column and the manual removal of the acceptor phase by the injection syringe could be automated. The two-phase extraction without the use of an SLM, as it is identical to the acceptor phase solvent, might warrant investigation. The two-phase EME technique could also be used in conjunction with normal phase LC and HILIC due to the nature of its (organic) acceptor phase solvents.

EME will continue to move from academic research towards real-life applications, as demonstrated in forensics [37] and clinical applications (e.g. dried-bloodspot analysis) [57]. In the near future we foresee that EE and EME can become important in lab-on-a-chip (LOC) systems. The high EFs that EE and EME offer can provide LOC systems with much demanded sensitivity [58, 59], as is already shown in the works involving the metabolism of drugs via rat liver microsomes. Furthermore, as highlighted in this review, several groups

successfully developed chip-based EE and EME approaches which show excellent potential for incorporation into LOC systems, such as the EE and EME flow cells [23, 30, 35]. These setups make use of low voltages and low flows, which matches the scales that are used on LOC systems. Another EME system that demonstrates scalability with LOC systems is nano-EME, which uses low volumes and voltages. Furthermore, using nano-EME very short extraction times can be sufficient to concentrate analytes [50], which implies that it is promising for high-throughput screening of drug compounds on in vitro cell cultures in microfluidic LOC systems.

The current focus of EME is often on hydrophobic drug compounds. There are promising examples of EE and EME of hydrophilic compounds, but their number is limited. Improved knowledge of EME processes have enhanced the extraction process of these hydrophilic compounds as described recently [4], but the extractions are often not exhaustive. Further research into expanding the target range of EE and EME analysis to more hydrophilic compounds makes the extraction of many endogenous compounds, such as metabolites, feasible.

Overall, we conclude that EME and EE are highly promising electromigration-based sample pretreatment techniques with excellent prospects for online hyphenation to analytical separation and detection techniques. In the resulting systems efficient sample cleanup and analyte enrichment are integrated with high performance separation and selective, sensitive detection.

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