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Exhaustive and stable electromembrane extraction of acidic drugs from human plasma

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ABSTRACT

The first part of the current work systematically described the screening of different types of organic solvents as the supported liquid membrane (SLM) for electromembrane extraction (EME) of acidic drugs, including different alcohols, ketones, and ethers. Seven acidic drugs with a wide log *P* range (1.01–4.39) were selected as model substances. For the first time, the EME recovery of acidic drugs and system-current across the SLM with each organic solvent as SLM were investigated and correlated to relevant solvent properties such as viscosity and Kamlet and Taft solvatochromic parameters. Solvents with high hydrogen bonding acidity (α) and dipolarity–polarizability (π^*) were found to be successful SLMs, and 1-heptanol was the most efficient candidate, which provided EME recovery in the range of 94–110%. Both hydrogen bonding interactions, dipole–dipole interactions, and hydrophobic interactions were involved in stabilizing the deprotonated acidic analytes (with high hydrogen bonding basicity and high dipole moment) during mass transfer across the SLM. The efficiency of the extraction normally decreased with increasing hydrocarbon chain length of the SLM, which was mainly due to increasing viscosity and decreasing α and π^* values. The system-current during EME was found to be dependent on the type and the volume of the SLM. In contact with human plasma, an SLM of pure 1-heptanol was unstable, and to improve stability, 1-heptanol was mixed with 2-nitrophenyl octyl ether (NPOE). With this SLM, exhaustive EME was performed from diluted human plasma, and the recoveries of five out of seven analytes were over 91% after 10 min EME. This approach was evaluated using HPLC-UV, and the evaluation data were found to be satisfactory.

1. Introduction

Supported liquid membranes (SLM) have been used for analytical sample preparation since 1986 [1]. Especially for biological samples, SLM extractions have been of interest to minimize interferences from the sample matrix and to maximize enrichment of target analytes [2,3]. With modern analytical instrumentation, the sample preparation procedure normally becomes the most time consuming step of an analytical procedure; it often takes 50–75% of the total analysis time [4], and therefore simpler and faster sample preparation techniques are highly attractive [2,5].

Electromembrane extraction (EME) was presented for extraction of hydrophobic basic drugs (log *P* > 2.5) for the first time in 2006, using an external potential over the SLM in a hollow fiber liquid-phase microextraction system (HF-LPME) [6]. In EME, the analytes are charged in the sample, and the charged analytes migrate from the sample, through the SLM, and finally into the acceptor solution under the influence of an external electric field. EME provides efficient sample clean-up and rapid extraction due to the electrokinetic nature of the mass transfer [7,8]. In addition, EME can be performed with inexpensive equipment, using only a few μL of solvent per sample. Up to date, EME has been applied for the extraction of basic drugs [9,10], organic pollutants [11], acidic drugs [12,13], small peptides [14], amino acids [15], and metal ions [16] from different sample matrices.

Several articles have reported on EME of acidic drugs using traditional EME, pulsed EME (PEME), voltage-step PEME (VS-PEME), and EME with a cylindrical cathode [12,13,17–20]. The studied acidic

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drugs with log *P* values, sample matrix, EME operation conditions, and recovery (*R* %) are summarized in the Supporting information (Table S1). In a first paper, EME of acidic drugs (log *P* > 2.0) was performed from 300 μL spiked aqueous alkaline samples (pH 12) and into 30 μL acceptor solutions (pH 12) with an SLM of 1-heptanol and a voltage of 50 V. The corresponding recoveries were in the range of 8–100% after 5 min of EME [12]. Later, six non-steroidal anti-inflammatory drugs (NSAIDs) with log *P* > 2.0 were extracted from 10 mL waste water samples (pH 12) and into 50 μL acceptor solution (pH 12) with an SLM of 1-octanol and a voltage of 10 V. The corresponding pre-concentration was in the range of 28–49 times after 10 min of EME [13]. Recently, five NSAIDs (log *P* > 2.0) were extracted from 10 mL spiked alkaline samples of pure water (pH 12) and into 50 μL acceptor solution (pH 12) with an SLM of dihexyl ether (DHE) [17]. Extraction of acidic drugs from human plasma has only been studied in a few cases. In one paper, diclofenac (DIC, log *P* 4.06)) and mefenamic acid (MEF, log *P* 5.33) were extracted by PEME from diluted human plasma and urine with an SLM of 1-octanol, and the recoveries were in the range of 23–33% [18]. In another paper, DIC and MEF have been extracted from 10 mL diluted human plasma and urine into 10 μL using 20 V using a cylindrical cathode, and recoveries in the range 5–36% was obtained after 15 min [20]. NSAIDs with log *P* > 2 were extracted by EME with the assistance of a surfactant (Triton X-100) in the sample [21], and carbon nanotubes in the SLM, which involved the principles of EME and solid-phase microextraction to enhance the EME efficiency [22]. Although several papers have been published related to EME of acidic drugs, most work has been performed with 1-heptanol or 1-octanol (higher alcohols) as SLM. Other solvents have also been tested but they have been far less efficient. The solvent properties required for efficient mass transfer of deprotonated acidic analytes, and the interactions involved between the analyte molecules and the solvent, are still not fully clarified and discussed. For future EME, however, this type of knowledge is mandatory. Additionally, very little data has been reported on EME of acidic drugs from human plasma [18,20], and exhaustive extraction of acidic drugs from plasma has not been reported yet. Finally information on EME of acidic drugs with log *P* < 2, and how to optimize recoveries from plasma, how to stabilize the SLMs based on higher alcohols in contact with human plasma, and how to avoid excessive system-current in such systems are lacking.

Therefore, in this paper, exhaustive EME of acidic drugs from human plasma was investigated and reported for the first time. Unexpectedly, based on existing knowledge from the literature, exhaustive extraction was highly challenging. Clearly more fundamental experiments were required. Thus, attention was focused on (i) the fundamental understanding of the link between the SLM properties and the EME efficiency, (ii) on high extraction recoveries (exhaustive extraction) from human plasma, and (iii) on the stability of the EME system with respect to SLM integrity and system-current. EME with different SLM solvents were studied to understand the chemical interactions involved and the solvent properties for highly efficient EME of acidic drugs. Also, the effect of the SLM composition on the stability of the EME system was investigated, and the SLMs were modified with 2-nitrophenyl octyl ether (NPOE) to enhance the stability. This is mandatory in order to obtain reliable data in the future for acidic drugs when using EME.

2. Experimental

2.1. Chemicals and materials

Flurbiprofen (FLU), gemfibrozil (GEM), ibuprofen (IBU), ketorolac (KEC), ketoprofen (KET), probenecid (PRO), quinaldic acid (QUI), 1-heptanol, 1-octanol, 1-nonanol, 1-undecanol, 2-octanone, 2-decanone, 2-undecanone, and dihexyl ether (DHE) were all purchased from Sigma–Aldrich (St. Louis, MO, USA). 2-Nitrophenyl octyl ether (NPOE) and 1-dodecanol were from Fluka (Buchs, Switzerland). Deionized water was purified with a Milli-Q water purification system (Molsheim, France). Formic acid, phosphoric acid, sodium dihydrogen phosphate monohydrate, disodium hydrogen phosphate dodecahydrate, trisodium hydrogen phosphate dodecahydrate and methanol were all supplied by Merck (Darmstadt, Germany).

Accurel polypropylene PP 1E (R/P) flat membrane (a thickness of 100 μm) was from Membrana (Wuppertal, Germany). Standard 10–1000 μL Biohit tips and Eppendorf safe-lock 2.0 mL PP tubes were supplied by, Sartorius Biohit Liquid Handling Oy (Helsinki, Finland) and Eppendorf AG (Hamburg, Germany), respectively. The platinum wires (0.5 mm in diameter) were from K. A. Rasmussen (Hamar, Norway).

2.2. Preparation of solutions

The stock solutions (5 μg/mL) of QUI and PRO were prepared by dissolving the drugs in methanol/water (50/50), while the stock solutions for the other model drugs were prepared by dissolving the corresponding drugs into pure methanol with a concentration of 5 mg/mL. All the stock solutions were protected from light and stored at −32 °C. Drug-free human plasma, supplied by Oslo University Hospital (Oslo, Norway), was stored at −32 °C. The sample was prepared by spiking phosphate buffer or human plasma with the stock solutions.

2.3. EME set-up and procedure

The preparation of the thin flat membrane-based EME device and the extraction procedure has been presented in our recent work [23,24]. First, 10 μL of the SLM was applied on both sides of the membrane. Two “L-shaped” platinum electrodes were connected to an ES 0300-0.45 power supply (Delta Elektronika BV, Zierikzee, Netherlands), and the cathode was placed into the sample, while the anode was placed into the acceptor solution (Fig. 1). Subsequently, the acceptor container was inserted into the sample container with a fixed gap (about 1 mm) between the sample interface and the SLM. The EME process was initiated by applying a voltage between the electrodes, and turning on the agitation with a Vibramax 100 (Heidolph Instruments, Kelheim, Germany) using

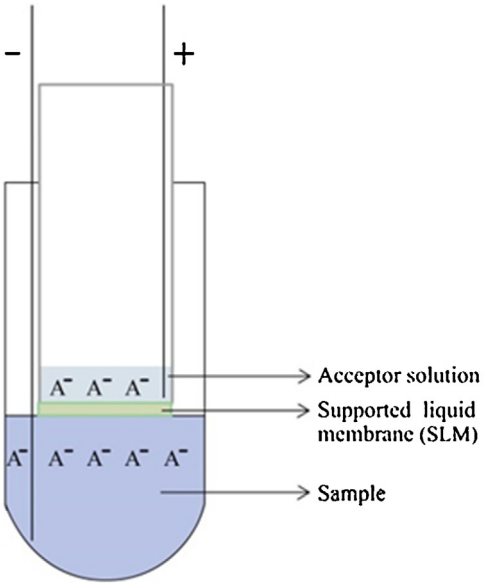

Fig. 1. Schematic illustration for EME of acidic drugs.

an agitation speed of 1200 rpm according to our previous experience [23]. A home-made current monitor coupled with a computer was connected to the power supply to monitor the time-dependent system-current with LabVIEW 8.2 software (National Instruments, Austin, TX, USA). After the desired extraction time, the power was turned off automatically by the software, and the EME was terminated by turning off the agitator. Subsequently, the acceptor solution was collected immediately and analyzed by HPLC-UV.

2.4. HPLC-UV

The chromatographic separation was carried out using a Dionex Ultimate 3000 system including an autosampler (WPS-3000SL), a degasser (SRD-3200), a pump (HPG-3200M), and a VWD-3400 UV/VIS detector (all from Dionex Corporation, Sunnyvale, CA, USA). The UV/Vis detector was operated at 214 nm, and Chromeleon software (v. 6.80 SP2 Build 2212) from Dionex Corporation was used for instrument control, data collection and data processing. Separations were carried out on a Gemini C18 column (150 mm × 2.00 mm, 5 μm) (Phenomenex, Torrance, CA, USA) with an injection volume of 10 μL. Mobile phase A and B contained 20 mM formic acid and methanol in the ratio of 95/5 and 5/95 (v/v), respectively. Mobile phase B was increased from 30 to 80% in 9 min, and further increased to 85% in 3 min at a flow rate of 0.4 mL/min. At the end of the gradient, mobile phase B was further decreased to 30% in 0.1 min, and kept at 30% for 3 min for equilibration.

2.5. Calculations

The EME recovery ($R\%$) was defined using the following equation for each acidic drug:

$$R\% = \left[\frac{C_{Ai}(t)\cdot V_A}{C_{Di}(0)\cdot V_D}\right] \times 100\% \quad (1)$$

Here $C_{Ai}(t)$ was the time-dependent concentration of the analyte in the acceptor solution, while $C_{Di}(0)$ was the spiked concentration in the sample. V_A and V_D were the volumes of acceptor and sample solution, respectively.

3. Results and discussion

3.1. Initial experiments

Seven acidic drugs were selected as model analytes as illustrated in Table 1, with log P values (octanol/water partition coefficient) in the range of 1.01–4.39. Initially, the acidic model drugs were extracted from 600 μL of spiked phosphate buffer (pH 8) and into 600 μL of 10 mM NaOH solution as the acceptor solution, and EME was performed using an SLM of 5 μL 1-octanol [18]. However, the system-current exceeded 100 μA with 50 V (Fig. 2), probably because the system-current depended on the thickness (and volume) of the SLM [24]. Such high system-current is not recommended in EME, as this can result in excessive electrolysis and bubble formation. As shown in Fig. 2, the system-current was below

Table 1
Protein binding, pK_a and log P for all model substances.[a]

Analytes	Protein binding	pK_a	log P
Quinaldic acid (QUI)	Not available	1.07	1.01
Ketorolac (KEC)	99%	3.84	2.28
Ketoprofen (KET)	99%	3.88	3.29
Probenecid (PRO)	75–95%	3.53	1.52
Flurbiprofen (FLU)	>99%	4.42	3.57
Ibuprofen (IBU)	90–99%	4.85	3.84
Gemfibrozil (GEM)	95%	4.42	4.39

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

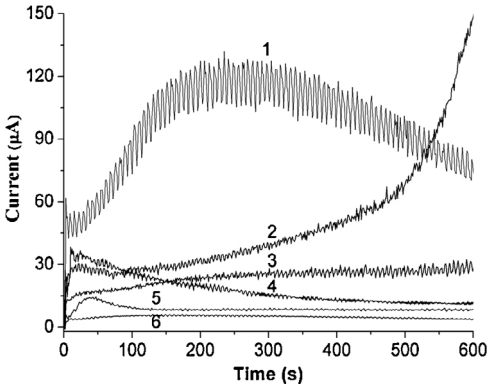

Fig. 2. Recorded system-current profiles during EME using different SLMs including (1) 5 μL of 1-octanol, and 10 μL of (3) 1-octanol, (4) 1-heptanol, (5) 2-octanone and (6) 1-undecanol from water samples using 50 V as the extraction voltage, and (2) 10 μL of 1-heptanol from 6 times diluted human plasma using 5 V as the extraction voltage.

50 μA when the volume of 1-octanol was increased to 10 μL. We consider system-current up to 50 μA as acceptable, and therefore 10 μL of SLM was used in all subsequent experiments in this work. However, the recoveries (after 10 min EME) of the model analytes were in the range of 2–96% (Table 2), and only the very hydrophobic substances were extracted exhaustively. In order to gain more knowledge about the suitable solvent properties for EME of acidic drugs, different types of organic solvents were screened (see Section 3.2).

3.2. Selection of SLM

In this set of experiments, different organic solvents were tested as the SLM for EME of acidic drugs. The selection of solvents was based on earlier experiences with EME of acidic drugs and all the solvents have previously been described in the literature [12,17]. In all experiments, the extraction voltage was 50 V and the extraction time was initially 10 min. The recovery data are summarized in Table 2. From this experiment, 1-heptanol (C7-OH) was found to be the most efficient SLM, and this provided exhaustive extraction of all the seven model analytes from spiked water.

As seen from Table 2, the alcohols with longer hydrocarbon chains from 1-octanol (C8-OH) and to 1-dodecanol (C12-OH) were less efficient as SLM. The principal reason for this was mainly due to their higher viscosity and lower affinity to the analytes resulting in slower mass transfer, especially to hydrophilic ones. Thus, while the recoveries at 10 min with 1-heptanol were equilibrium data, the recoveries corresponding to the alcohols with longer hydrocarbon chains were non-equilibrium data at 10 min due to slower extraction kinetics and less efficient interaction. EME with 1-nonanol (C9-OH) and 1-undecanol (C11-OH) was also performed for longer time to reach the equilibrium, and the data are summarized in Table 3. With 1-nonanol, 20 min was required to obtain equilibrium data, while longer time was required in the case of 1-undecanol. Clearly, the longer hydrocarbon chain alcohols were less efficient in terms of extraction kinetics due to relatively high viscosity and a lower affinity to acidic drugs.

As shown in Table 2, different ketones also worked as SLMs for EME of the acidic drugs. Difference in recoveries obtained from 1-nonanol and 2-nonanone (and between the other alcohols and ketones with the same hydrocarbon chain length), illustrated that the alcohols were more efficient as SLM than the corresponding ketones. This observation supported that hydrogen bonding interaction was important for the mass transfer of the acidic substances. The alcohols all have high hydrogen bonding acidity, while the ketones only exhibit hydrogen bonding basicity. Consequently, the

Table 2
Recoveries (*R* %) of the model substances using different organic solvents as the SLM for EME of acidic drugs ($n = 3$, RSD values presented in the parentheses).[a]

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

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

deprotonated acidic drugs (with high hydrogen bonding basicity and high dipole moment) were solubilized and transferred into the alcohol SLM based on both hydrogen bonding interactions and dipole–dipole interactions, while only dipole–dipole interactions were involved with the ketones. Due to lack of hydrogen bonding acidity, the ketones, DHE, and NPOE were less efficient as SLM. Among the carbonyl, ether, and nitro functional groups, only the carbonyl group appeared to provide sufficiently strong dipole–dipole interactions for partial mass transfer of hydrophobic acidic analytes.

Two additional trends can be derived from Table 2. First, the more non-polar analytes were generally extracted with higher efficiency than the more polar ones. This trend was observed with almost all solvents tested and indicated that also hydrophobic interactions were involved to some extent during transfer of the analytes into the SLM. Thus, both hydrogen bonding interactions (strong), dipole–dipole interactions (intermediate), and hydrophobic interactions (weak) were involved in the extraction. Second, the efficiency of the SLM generally increased with decreasing hydrocarbon chain length. This is consistent with their Kamlet and Taft solvatochromic parameters for hydrogen bonding acidity (α) and dipolarity–polarizability (π^*) [25,26], which are decreasing with increasing hydrocarbon chain length.

Based primarily on differences in hydrogen bonding acidity and viscosity, the tested SLM solvents differed significantly in terms of extraction efficiency as discussed above. As shown in Fig. 2, the system-current was dependent on the volume of the SLM, and it was also affected by the type of solvent. With an SLM volume of 10 μL and an extraction voltage of 50 V, most of the alcohols and ketones provided system-current in the range 10–50 μA.

3.3. Stabilization of SLMs containing 1-heptanol

Initial extraction from plasma samples using 10 μL of pure 1-heptanol as SLM caused a dramatic increase in the current as illustrated in Fig. 2. Thus, with 5 V, the current exceeded 50 μA after 5–7 min, and increased to 150 μA after 10 min. This indicated an unstable EME system where the SLM solvent gradually leaked into the plasma sample. In order to stabilize the SLM, the mixtures of 1-heptanol and other organic solvents with lower water solubility including DHE, NPOE, 2-undecanone, and 1-dodecanol (80:20, v/v) were tested. The recovery data from spiked water samples are summarized in Table 4. As seen from this set of data, the SLM containing 20% (v/v) NPOE was the most efficient one. Therefore, NPOE was used as SLM modifier during the rest of this study. The most polar analytes were relatively sensitive to the SLM modifications, whereas the less polar analytes were still extracted with high efficiency with the modified SLMs. This is in accordance with the discussion above related to molecular interactions between the deprotonated analytes and the SLM solvent. In addition, the recorded system-current during EME for all combinations was below 50 μA and similar to that of 1-heptanol from spiked water samples. Subsequently, the optimization of acceptor solution volume was carried out from spiked water samples using 1-heptanol containing 20% NPOE as the SLM, and similar recoveries were observed with different acceptor solution volumes from 60 to 600 μL, which is summarized in the Supporting information (Table S2). However, 60 μL of acceptor solution was used for the rest of this study because this provided the highest enrichment factors (Table S2).

In a next experiment, the SLM comprising 80% (v/v) 1-heptanol and 20% (v/v) NPOE was tested with six times diluted human plasma samples with phosphate buffer pH 8. As seen from Fig. 3a, the current was initially below 50 μA with 10 V as extraction voltage, but after 5–7 min the current increased substantially. The experiments were repeated with higher contents of NPOE in the SLM, with the purpose of keeping the current below 50 μA for 10 min. As shown in Fig. 3a, the recorded system-current was below 50 μA, when EME was accomplished with an SLM comprising 65% (v/v) 1-heptanol and 35% (v/v) NPOE. In Fig. 3b, recoveries from plasma are shown as function of NPOE content in the SLM. As expected, the extraction efficiency decreased with increasing amount of NPOE in the SLM. Based on this, it was decided to use a mixture of 65% (v/v) 1-heptanol and 35% (v/v) NPOE as SLM for all remaining experiments.

Table 3
Investigation of steady-state recovery (*R*%) in EME using 1-nonanol and 1-undecanol as SLMs, and 20–60 min as the extraction time ($n = 3$, RSD values presented in the parentheses).

Analytes	1-Nonanol, 20 min	1-Undecanol, 20 min	1-Undecanol, 30 min	1-Undecanol, 60 min
QUI	25 (30)	13 (50)	8 (32)	6 (53)
KEC	81 (12)	33 (19)	42 (17)	45 (21)
KET	91 (9)	48 (17)	57 (23)	60 (11)
PRO	98 (12)	71 (12)	75 (3)	89 (6)
FLU	92 (4)	90 (5)	99 (5)	99 (2)
IBU	91 (2)	72 (12)	88 (6)	97 (2)
GEM	110 (5)	92 (6)	103 (7)	103 (6)

Table 4
Recoveries (*R*%) of the model substances from water samples using 1-heptanol containing 20% of different organic solvents as the SLM. EME was carried out with 50 V for 10 min ($n = 3$, RSD values presented in the parentheses).

Analytes	DHE	NPOE	2-Undecanone	1-Dodecanol
QUI	39 (3)	89 (3)	51 (3)	41 (1)
KEC	83 (4)	98 (6)	75 (7)	76 (3)
KET	95 (7)	100 (3)	91 (7)	87 (1)
PRO	98 (1)	97 (2)	96 (12)	90 (4)
FLU	96 (2)	100 (4)	88 (1)	87 (1)
IBU	92 (1)	99 (4)	89 (1)	91 (10)
GEM	97 (3)	97 (6)	97 (12)	86 (1)

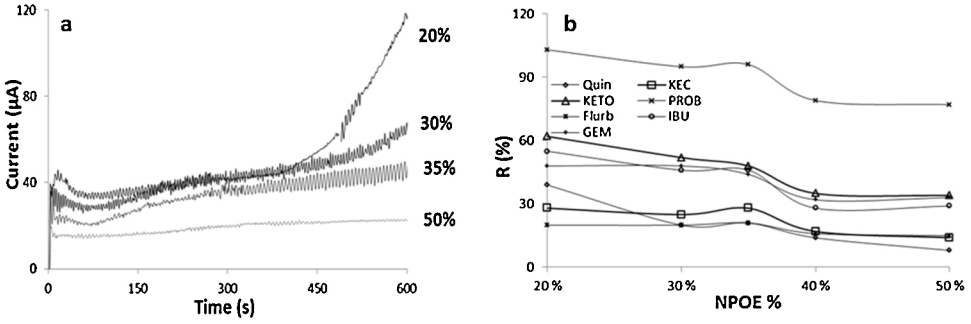

Fig. 3. (a) Recorded system-current profiles during EME from six times diluted plasma using 1-heptanol containing different amount of NPOE as the SLM and 10 V as the extraction voltage, and (b) EME recoveries of model substances from 6 times diluted human plasma after 10 min EME versus the NPOE content in the SLM (n = 3, RSD values for all were <15%).

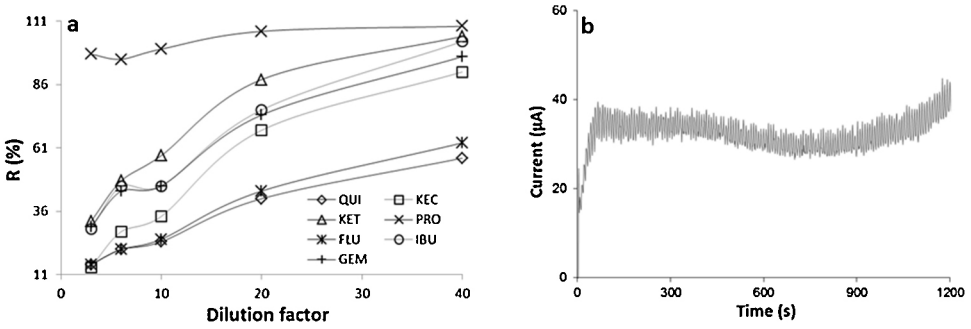

Fig. 4. (a) EME recoveries of model substances versus dilution factor of human plasma with phosphate buffer pH 8 (EME was performed using 10 V for 10 min with an SLM of 1-heptanol containing 35% NPOE, and RSD values (n = 3) for all were <15%), and (b) recorded system-current profiles during EME from 40 times diluted human plasma for 20 min using a voltage of 10 V and an SLM of 1-heptanol containing 35% NPOE.

3.4. Optimization of extraction recoveries

Although the mixed 1-heptanol/NPOE SLM provided a stable extraction system from human plasma, recoveries were limited to the range 25–55%, except for probenecid which was extracted with a 95% recovery. Therefore, in a couple of additional experiments, different approaches were tested to improve the extraction efficiency. In all experiments, the extraction potential was set to 10 V as a compromise between extraction efficiency and system-current . First, different dilutions of plasma (diluted with 10 mM phosphate buffer) were tested, and the results are illustrated in Fig. 4a. As expected, the extraction efficiency increased with increasing dilution of the plasma sample [27]. The recoveries of all analytes from 40 times diluted human plasma were over 91% except those of QUI (57%) and FLU (63%), which might be due to the fact that QUI has the lowest log P (1.01) and FLU has the highest protein binding (>99%) among all the analytes (Table 1). Lower recovery of QUI suggested that the hydrophobic interaction between the SLM and the analytes plays a role for efficient mass transfer. In addition, lower recovery of FLU indicated that strong protein binding of the model drugs was the primary reason for limited extraction recoveries, and this hindered exhaustive extraction [28]. However, by using longer extraction time (20 min), the recoveries of QUI and FLU from 40 times diluted human were increased further to 98% and 93%, respectively. Additionally, the recorded system-current was relatively stable and below 50 μA (Fig. 4b). However, longer time than 20 min (30 min) resulted in lower recovery, which was due to the pH shift caused by electrolysis [29]. The chromatograms of the standard solution (STD), the acceptor solution after 10 min EME at 10 V from 6 times diluted plasma, and the acceptor solution after 20 min EME from 40 times diluted plasma are shown in Fig. 5. Exhaustive EME of both hydrophilic and hydrophobic acidic drugs was obtained from diluted human plasma with stable SLM, and the system-current was below 50 μA.

3.5. Evaluation

Finally, EME of acidic drugs was evaluated with QUI, KET, IBU, PRO, FLU and GEM as model substances from diluted human plasma, and the acceptor solutions after EME were analyzed by HPLC-UV. KEC was excluded from the evaluation because of the lower therapeutic range (0.5–3 μg/mL) [30]. To cover the therapeutic ranges, the investigated concentrations for all selected analytes were 1–200 μg/mL, including data points at 1, 5, 10, 25, 50, 100, and 200 μg/mL, respectively [30]. The evaluation data are summarized in Table 5. The R^2 values obtained by linear fit were no less than 0.9929 within the individual linearity range for each model analyte,

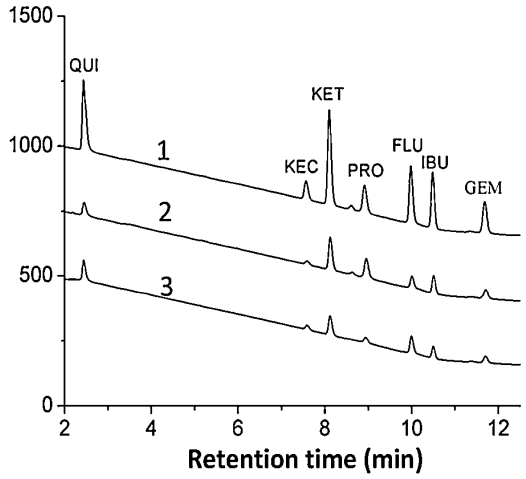

Fig. 5. Chromatograms of (1) the acceptor solution after 20 min EME from 40 times diluted plasma (2) the acceptor solution after 10 min EME from 6 times diluted plasma, (3) the standard solution (STD, 5 μg/mL).

Table 5
Evaluation results with EME–HPLC-UV from spiked human plasma.

Analyte	R^2	Therapeutic level[a] (μg/mL)	Linear range (μg/mL)	LOD (S/N = 3)	LLOQ (S/N = 5)	RSD/% (n = 6)		
						Low	Medium	High
QUI[c]	0.9964	Not available	5–100	0.7	1.2	11	9	9
KET[b]	0.9960	1–6	1–200	0.3	0.4	13	12	6
PRO[c]	0.9977	100–200	5–200	1.0	1.7	7	10	4
FLU[c]	0.9933	5–15	5–100	1.0	1.6	5	10	8
IBU[b]	0.9939	15–30	1–200	0.3	0.5	13	14	6
GEM[c]	0.9929	~ 25	5–100	2.5	4.2	14	13	12

[a] Data were adapted from Ref. [30].
[b] Low, medium and high concentrations were 1, 10, and 25 μg/mL.
[c] Low, medium and high concentrations were 5, 50, and 100 μg/mL.

and no points are significantly out of the line as shown in Fig. S1. The linearity range of KET and IBU was 1–200 μg/mL, the linearity range for PRO was 5–200 μg/mL, while the linearity range for QUI, FLU, and GEM was 5–100 μg/mL. The recoveries of all analytes from spiked six-time diluted human plasma (at 10 μg/mL) ranged from 21 to 96% (n = 3, RSD < 14%). The repeatability was tested (n = 6) at 1, 10, and 25 μg/mL for KET and IBU, and at 5, 50, and 100 μg/mL for the other analytes. The RSD-values were below 14% in all cases. The noise was obtained by analysis of the acceptor solutions after EME from six-time diluted blank human plasma, and the limits of detection (LOD, S/N = 3) and lower limit of quantification (LLOQ, S/N = 5) shown in Table 5 indicated that EME showed high potential for the determination of acidic drugs in real samples. Drug-free plasma samples were also analyzed, and no peaks were detected at the retention times for QUI, KET, IBU, PRO, FLU and GEM.

4. Conclusions

For the first time, the present work systematically studied the relation between the properties of the SLM and the EME efficiency and stability toward acidic drugs using a thin flat membrane based EME device. The EME system-current and efficiency for each solvent was investigated and linked to properties of the organic solvent such as viscosity and Kamlet and Taft solvatochromic parameters. Hydrogen bonding interactions, dipole–dipole and hydrophobic interactions were involved in EME of acidic analytes, so alcohols with high hydrogen bonding acidity (α) and dipolarity–polarizability (π^*) were found to be the most successful SLMs. In addition, the efficiency of the SLM generally decreased with increasing hydrocarbon chain length, which was mainly due to increasing viscosity and decreasing α and π^* values. The system-current during EME was found to be affected by the type of SLM and also the volume of the SLM. With an SLM comprising 1-heptanol mixed with 2-nitrophenyl octyl ether (NPOE), exhaustive extraction was obtained for most of the model analytes from diluted human plasma after 10 min EME.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2015.11.052.

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