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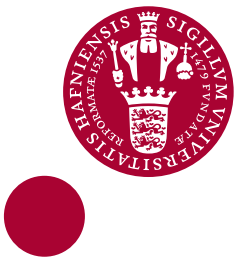

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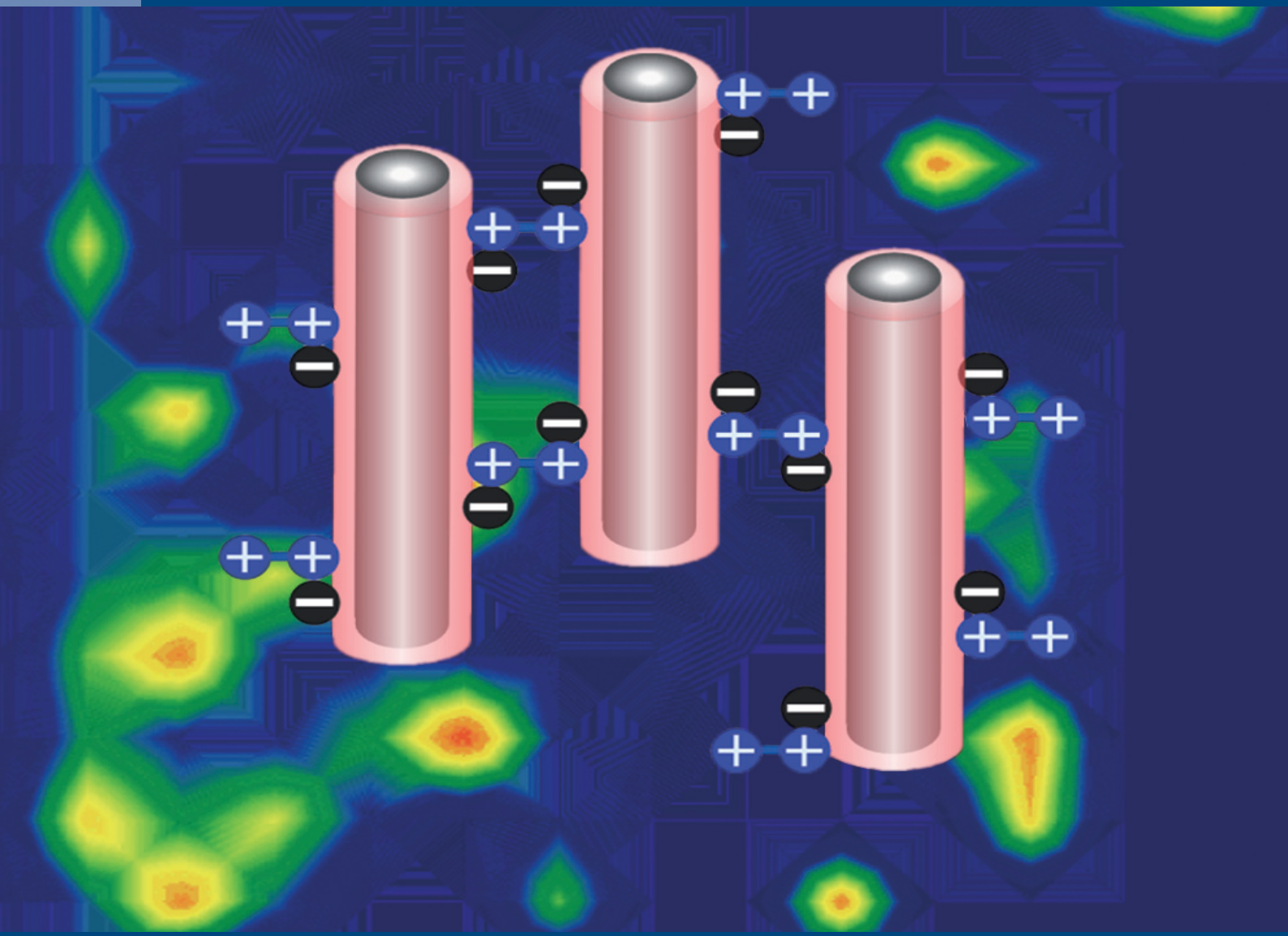

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RESEARCH ARTICLE

Influence of acid-base dissociation equilibria during electromembrane extraction

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

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

1 | INTRODUCTION

Electromembrane extraction (EME) was introduced in 2006 and offers rapid and selective extraction properties with the addition of an electrical field to a three-phase microextraction system [1]. Conventional EME comprises an aqueous sample solution, which is separated from another clean aqueous solution (acceptor) by a porous membrane coated with an organic solvent. This membrane is termed supported liquid membrane (SLM) and acts as a barrier between the two aqueous solutions. The electrical field is applied by inserting an electrode to each aqueous solution and subsequently connecting them to a power supply. The versatility of EME is increased by the

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

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

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

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

From the discussion above, analyte extractability is typically linked to the log P value, while pH conditions are linked to pK_a values. However, EME is affected by acid–base equilibria of the extracted species as well as coupled equilibria associated with the partitioning of neutral species into the SLM. The magnitude and direction of these effects are dependent on whether a base or an acid is extracted, and whether the neutral form is hydrophobic or hydrophilic. In the present work, we developed a model for predicting extraction recovery and pH conditions in EME based on the coupled relationship between pK_a and log P of a given analyte. From this model, sample pH should generally be acidic for basic analytes, while pH in the acceptor should be used actively for controlling selectivity. Twelve hydrophobic basic model analytes, with log P in the range 2.07–6.57 and pK_a in the range 6.03–10.47, were extracted and evaluated according to the theoretical model.

2 | MATERIALS AND METHODS

2.1 | Chemicals and solutions

Haloperidol and hydrochloride salts of pethidine, nortriptyline, loperamide, lidocaine, promethazine, prochlorperazine, mianserin, and papaverine were purchased from Sigma–Aldrich (St Louis, MO, USA). Hydrochloride salts of cocaine, methadone, and methamphetamine were obtained from NMD (Oslo, Norway). Phosphoric acid, sodium dihydrogen phosphate, disodium hydrogen phosphate, sodium acetate, acetic acid, sodium formate, formic acid, hydrochloric acid, sodium carbonate, sodium bicarbonate, NPOE, 1-octanol, and methanol were purchased from Merck (Darmstadt, Germany). Ethanol was obtained from Arcus (Oslo, Norway) and water (18 MΩ cm) was obtained from a Milli-Q water purification system (Molsheim, France).

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

2.2 | Equipment for EME

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

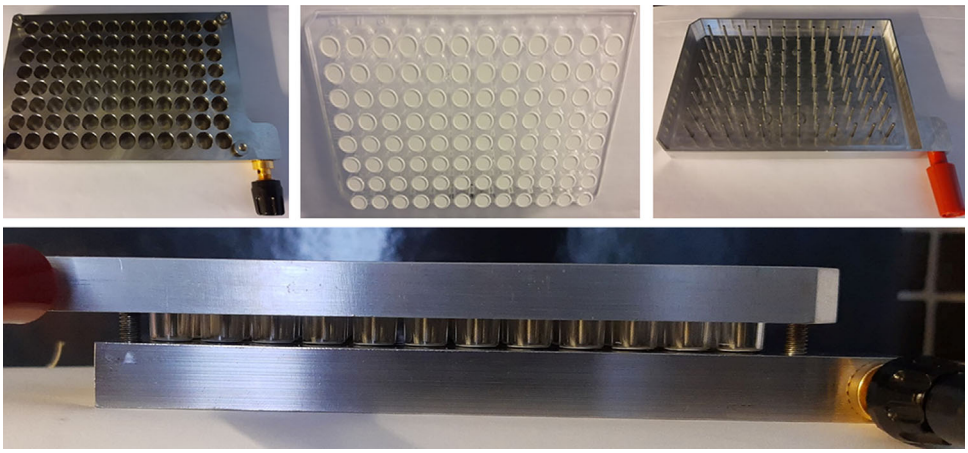

FIGURE 1 Electromembrane extraction setup. Left: 96-well sample plate, middle: 96-well filter plate, right: 96-electrode plate, bottom: all plates clamped

2.3 | EME procedure

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

2.4 | Measurement of log P

Measurements of log P_{NPOE} and log $P_{OCTANOL}$ were conducted with the same equipment presented in Section 2.3. Solutions of each basic analyte were made by diluting the stock solution with 10 mM NaOH (pH 12) to a concentration of 5 µg/mL. This basic pH solution ensured that all analytes were neutral. Three microliters of either NPOE or 1-octanol was pipetted onto the filter of the 96-well filter plate. Subsequently, 200 µL of sample solution was pipetted in the sample compartment of the 96-well filter plate. The filter plate was covered with a plastic lid to avoid evaporation. The complete setup was placed on an agitator and agitated for 180 min, with a speed of 900 rpm. After extraction, the sample solutions were transferred to the HPLC–UV instrument for analysis.

2.5 | High-performance liquid chromatography-ultraviolet

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

2.6 | Calculations

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

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

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

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

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

Here, C_w is the final concentration (μg/mL) in the aqueous phase measured according to Section 2.4.

3 | RESULTS AND DISCUSSION

3.1 | Theoretical discussion, recovery versus pK_a – log P

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

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

$$BH^+ \rightleftharpoons B_w + H^+ \quad (3)$$

$$B_w \rightleftharpoons B_o \quad (4)$$

BH^+, B_w, and B_o represent the protonated based (cationic acid), the free base in sample/acceptor, and the free base in SLM, respectively. Corresponding equilibrium equations are:

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

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

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

$$BH^+ \rightleftharpoons B_o + H^+ \quad (7)$$

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

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

$$BH^+_w \rightleftharpoons BH^+_o \quad (9)$$

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

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

$$BH^+_o \rightleftharpoons B_o + H^+_o \quad (10)$$

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

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

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

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

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

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

$$pH \ll pK_{aH} - \log P_{SLM} \quad (13)$$

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

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

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

3.2 | Experimental verification, recovery versus pK_{aH} – log P

In a set of practical experiments, we tested the validity of Equation 13 with the log P_{SLM} = log P approximation. For this purpose, we selected 12 nonpolar basic drug substances as model analytes. Their physicochemical properties are summarized in Table 1; the model analytes were within log P 2.19 to 4.89, and pK_{aH} – log P ranged between 3.02 and 8.18. The model analytes were extracted with the same pH in sample and acceptor, and pH 2.0, 3.0, 4.0, 5.0, 6.0, and 7.0 were tested. Extraction recoveries are summarized in Table 2 (model analytes ranged in order of decreasing pK_{aH} – log P). Recoveries ≥ 40% were considered as efficient extraction based on previous argumentation [27]. For seven of the model analytes (cocaine, nortriptyline, methadone, mianserin, promethazine, papaverine, and prochlorperazine), the experimental data were clearly in accordance with the theoretical discussion above. Thus, compounds with relatively large pK_{aH} – log P difference, were extracted efficiently at higher pH values than those with a smaller difference. In addition, for these compounds, pK_{aH} – log P served as a valid approximation for the highest pH where recoveries exceeded 40%. Methamphetamine, lidocaine, and pethidine deviated from this pattern, and these were extracted less efficiently than predicted by theory when pH was increased (discussed in more details in Section 3.4). Haloperidol and loperamide were extracted efficiently at slightly higher pH values than predicted by pK_{aH} – log P. Some of the data in Table 2 were influenced by the background buffer. Especially for cocaine, nortriptyline, and pethidine, mass transfer was less at pH 4.0 and 5.0 using acetate buffer than at pH 6.0 using phosphate buffer. This indicates the occurrence of additional ion-pairing equilibria.

TABLE 1 Model analytes and their physicochemical properties (data from www.scifinder.com)

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

[a]This particular value was obtained from www.drugbank.ca

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

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

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

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

[a]Phosphate buffer.
[b]Acetate buffer.

TABLE 3 Experimental NPOE/water (log P_{NPOE}) and n-octanol/water (log $P_{OCTANOL}$) partition coefficients, and theoretical log P

Analytes	log P_{NPOE}	log $P_{OCTANOL}$	vlog P[b]
Methamphetamine	2.05	2.23	2.20
Cocaine	–[a]	–[a]	2.28
Nortriptyline	4.38	>4.5	3.97
Lidocaine	2.21	3.00	2.20
Pethidine	2.67	2.63	2.19
Methadone	>4.5	–[a]	3.93
Mianserin	>4.5	–[a]	3.83
Haloperidol	3.95	3.93	3.76
Promethazine	>4.5	–[a]	4.89
Loperamide	>4.5	–[a]	4.15
Papaverine	3.56	3.10	2.93
Prochlorperazine	4.36	>4.5	4.64

[a]Not measured for this substance.
[b]From www.scifinder.com.

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

3.4 | The link between experiments and predictions

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

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

$$BH_o^+ + H_2PO_4^- \rightleftharpoons BH^+H_2PO_{4,o}^- \quad (14)$$

$$BH_o^+ + CH_3COO^- \rightleftharpoons BH^+CH_3COO_o^- \quad (15)$$

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

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

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

[a]Sample pH: 2.0

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

3.5 | Selectivity with different pH in sample and acceptor

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

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

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

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

4 | CONCLUDING REMARKS

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

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

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

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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