Journal of Chromatography A, 1217 (2010) 6661–6667

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Simultaneous extraction of acidic and basic drugs at neutral sample pH: A novel electro-mediated microextraction approach

Chanbasha Basheer[a], Jingyi Lee[b], Stig Pedersen-Bjergaard[c], Knut Einar Rasmussen[c], Hian Kee Lee[b,*]

[a] Department of Chemistry, King Fahd University of Petroleum and Minerals, KFUPM Box 1059, Dhahran 31261, Saudi Arabia
[b] Department of Chemistry, National University of Singapore, 3 Science Drive 3, Singapore 117543, Singapore
[c] School of Pharmacy, University of Oslo, P.O. Box 1068 Blindern, 0316 Oslo, Norway

ARTICLE INFO

Article history:
Available online 18 May 2010

Keywords:
Electromembrane extraction
Pharmaceuticals
Gas chromatography–mass spectrometry
Wastewater analysis

ABSTRACT

The simultaneous extraction of acidic and basic analytes from a particular sample is a challenging task. In this work, electromembrane extraction (EME) of acidic non-steroidal anti-inflammatory drugs and basic β-blockers in a single step was carried out for the first time. It was shown that by designing an appropriate compartmentalized membrane envelope, the two classes of drugs could be electrokinetically extracted by a 300 V direct current electrical potential. This method required only a very short 10-min extraction time from a pH-neutral sample, with a small amount (50 μL) of organic solvent (1-octanol) as the acceptor phase. Analysis was carried out using gas chromatography–mass spectrometry after derivatization of the analytes. Extraction parameters such as extraction time, applied voltage, pH range, and concentration of salt added were optimized. The proposed EME technique provided good linearity with correlation coefficients from 0.982 to 0.997 over a concentration range of 1–200 μg L^{-1}. Detection limits of the drugs ranged between 0.0081 and 0.26 μg L^{-1}, while reproducibility ranged from 6 to 13% (n = 6). Finally, the application of the new method to wastewater samples was demonstrated.

1. Introduction

With the growing concern over organic pollutants found in the aquatic environment, a great deal of research effort has been devoted to identify these pollutants, as well as to recognize and understand their fate and potential impacts on the environment [1,2]. Among these organic contaminants, pharmaceutical products have drawn special attention, especially in developed countries. Pharmaceuticals like analgesics, anti-inflammatories, anti-epileptics and β-blockers have been identified as emerging environmental pollutants of great concern [3–5]. This is because at the end of their application cycles, these products are discharged into sewage systems but are not totally removed even after treatment processes in sewage treatment plants. Consequently, traces of these drug residues can reach surface and groundwater [6,7]. The existence of pharmaceuticals in aquatic ecosystems presents a potential hazard to human health if they contaminate our drinking water. In view of this, our work aims to quantitatively and simultaneously evaluate both acidic and basic drugs in the aquatic environment for proper risk assessment. Our research is also driven by the demand for more environmentally benign procedures and the need for reduction in sample preparation time.

Miniaturization of liquid–liquid extraction to reduce solvent consumption and multistep extraction has lead to the development of liquid-phase microextraction (LPME) or single drop microextraction for non-polar compounds in clean water samples [8–12]. For complex samples, hollow fiber-protected LPME has been demonstrated to be effective [13]. For the determination of polar and ionizable compounds, three-phase liquid–liquid–liquid microextraction (LLLME) has been used, in which the final extract is an aqueous buffer. This technique is more suitable for analysis via reversed-phase high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) [14–16]. The inexpensive hollow fiber membranes are disposed after each extraction and this certainly precludes carryover effects. In all the above techniques, the extraction mechanism depends on the analytes partitioning between the sample (donor) solution and the extractant (acceptor) phase. Recently, an improvement of LLLME using electrokinetic membrane extraction was demonstrated: here, the electrokinetic migration is accomplished by the application of a direct current (d.c.) potential difference across the hollow fiber membrane [17,18]. Compared with conventional LLLME, electrokinetic transport was found to effectively enhance extraction speed, resulting in the equilibration time for electromembrane extraction (EME) to be much shorter [19–21]. Hitherto, only a single class of analytes (either acidic or basic drugs) has been extracted successfully using EME. In this paper, simultaneous extraction of acidic and basic analytes from wastewater samples is reported for the first time.

* Corresponding author. Tel.: +65 6516 2995; fax: +65 6779 1691.
E-mail address: chmleehk@nus.edu.sg (H.K. Lee).

0021-9673/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2010.04.066

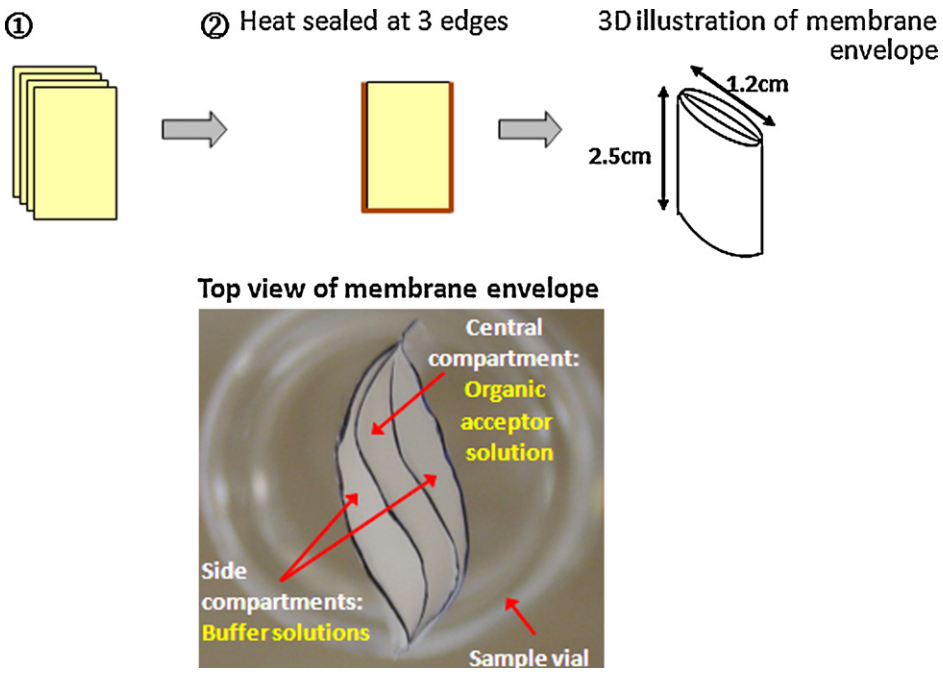

Fig. 1. Schematic representation of membrane envelope fabrication.

Although further work is required to fully understand the electrokinetic migration of the analytes in this novel system, we are able to successfully explore the fundamental extraction parameters in detail to give a better understanding of the theoretical concepts of the technique, in this paper. Conditions essential to extraction and derivatization were optimized and the optimized parameters were applied to the analysis of genuine environmental samples.

2. Experimental

2.1. Materials and chemicals

The Accurel 2E HF (R/P) polypropylene membrane sheet (157 μm thickness, 0.2 μm pore size) was supplied by Membrana (Wuppertal, Germany). The d.c. power supply used was a multichannel electrophoresis system MCE-PS468 from CE Resources (Singapore) with programmable voltage in the range 0–5 kV. The following chemicals were obtained from Sigma–Aldrich (Milwaukee, WI, USA): ibuprofen, naproxen, ketoprofen, propranolol hydrochloride (HCl) and norephedrine HCl. Alprenolol HCl was obtained from Sigma Chemicals (St Louis, MO, USA). Toluene was bought from Fisher Scientific (Loughborough, UK). HPLC-grade methanol, 1-hexane, dichloromethane (DCM) were from Tedia Company (Fairfield, OH, USA). 1-Octanol and ethyl acetate were obtained from Riedel-De Haen AG (Seelze-Hannover, Germany). Phosphoric acid was purchased from Carlo Erba (Milan, Italy). Sodium chloride (NaCl) was from Goodrich Chemical Enterprise (Singapore). The derivatization agent *bis*(trimethylsilyl)tri-fluoroacetamide (BSTFA), sodium dihydrogen-phosphate monohydrate and sodium hydroxide were purchased from Merck (Darmstadt, Germany). Ultrapure water was prepared on a Nanopure water purification system (Barnstead, Dubuque, IA, USA).

2.2. Standard solutions

Stock standard solutions of each analyte were prepared separately in methanol at 1000 mg L^{-1} concentration and stored at 4.0 °C. A working standard solution of a drug mixture at 10 mg L^{-1} concentration of each analyte was prepared by dilution with methanol. One molar phosphate buffer solutions were prepared at pH 2.0 and pH 12.0.

2.3. Electromembrane extraction

The extraction device was fabricated according to the schematic representation shown in Fig. 1. Firstly, four sheets of porous polypropylene membrane were combined and heat-sealed at three edges (two sides and the bottom) using an electrical heat sealer to give a three-compartment envelope. The dimension of the membrane envelope was 1.2 cm × 2.5 cm. The outer compartments were filled with acidic and alkaline buffer solutions (100 μL each), and the middle compartment was filled with acceptor phase (1-octanol, 50 μL). The outer skin of the membrane envelope was impregnated with toluene by dipping in the solvent for few seconds to form the supported liquid membrane (SLM) before placing it in an extraction vial containing sample solution for extraction. In order to perform EME, platinum electrodes of 1 mm diameter were used. The positive electrode was placed into the acidic buffer (pH 2) compartment and the negative electrode was placed into the alkaline buffer (pH 12) compartment. A d.c. potential difference (300 V) was applied for 10 min and the sample solution was agitated at 73 rad s^{-1} with a magnetic stirring bar (12 mm length, 4.5 mm i.d.). Fig. 2 shows the experimental setup for the simultaneous extraction of acidic and basic drugs. After extraction, the organic acceptor phase was collected and derivatized using BSTFA. Finally, 2 μL of the derivatized extract was injected into a gas chromatography–mass spectrometric (GC–MS) system for analysis.

2.4. GC–MS analysis

Since the extract, consisting of both acidic and basic drugs, was organic, LC was not amenable to its analysis. Using reversed-phase LC would involve solvent-exchange, and also possibly different columns for the analysis of the different classes of drugs. Therefore, GC–MS was selected for convenience and compatibility with direct injection of an organic acceptor phase.

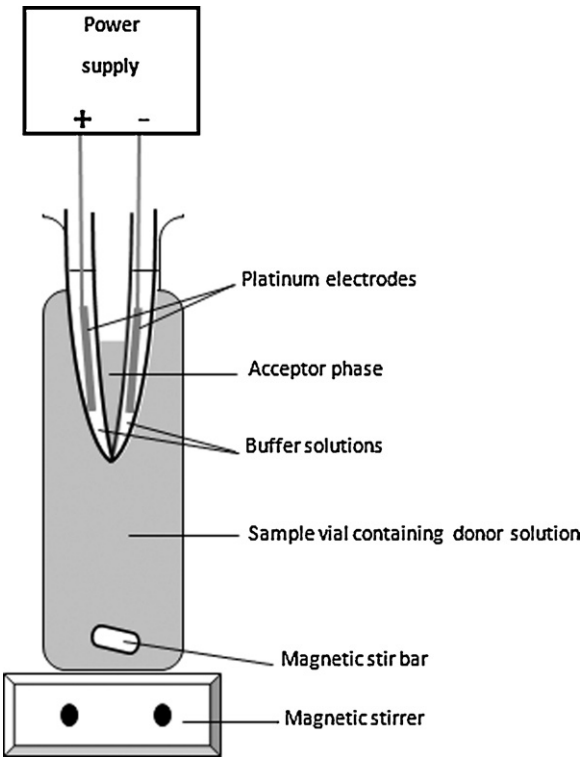

Fig. 2. Experimental setup of EME.

Analysis was carried out using a Shimadzu QP2010 GC–MS system equipped with a Shimadzu AOC-20i autosampler (Kyoto, Japan) and a DB-5MS fused silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) made by J&W Scientific (Folsom, CA, USA). High-purity (99.999%) helium was used as the carrier gas at a flow rate of 2.2 mL min^{-1}. The derivatized extract was injected into a split/splitless injector under splitless mode after a sampling time of 2 min (i.e. derivatized extract was retained in the injector port for 2 min). The injection temperature was set at 300 °C, with the MS interface temperature at 280 °C. The GC temperature program was as follows: initial temperature 70 °C, held for 2 min; then increased by 10 °C min^{-1} to 240 °C, held for 8 min; and a final increase at 10 °C min^{-1} to 300 °C, held for 5 min. Data acquisition was performed in full scan mode across a mass range of *m*/*z* 50–550 to confirm the retention times of the analytes, and selective ion monitoring mode was used for quantification. The most abundant ion present was selected as the quantitative ion, while a further two ions were used for the confirmation of individual compounds.

2.5. Solid-phase extraction (SPE)

Previously published conditions for SPE were used to carry out a comparative study between the extraction techniques [22]. Briefly, commercially available 200 mg capacity Oasis-HLB (*N*-vinylpyrrolidone/divinylbenzene) SPE cartridges from Waters (Milford, MA, USA) were used as received. SPE was performed using a 12-port vacuum manifold (Supelco, Bellefonte, PA, USA). Prior to extraction, the cartridges were conditioned with 5 mL ultrapure water, 5 mL 1-hexane, 5 mL ethyl acetate, 10 mL methanol and 10 mL ultrapure water. Extraction of 100 mL ultrapure water sample spiked with the acidic and basic drugs was carried out under vacuum. The approximate flow rate was 15 mL min^{-1}. After sample loading, the analytes were then eluted with 5 mL methanol and dried with anhydrous sodium sulfate. The extract was subsequently reduced to 1 mL by a stream of nitrogen. Derivatization was carried out with similar procedures as in the proposed EME approach and 2 μL of the extract was injected into the GC–MS system.

3. Results and discussion

Having designed the membrane envelope for simultaneous extraction, several initial experiments were performed to probe the feasibility of the proposed EME technique which involves the use of electrodes to attract analytes of the opposite charge (via electrokinetic force) into the buffer solutions. The analytes are subsequently extracted into the organic acceptor solvent via diffusion. To investigate the possibility of the pharmaceuticals undergoing such migration, they were spiked into sample solutions at pH 7.2 so that they would be sufficiently ionized (i.e. basic drugs were positively charged; acidic drugs were negatively charged). Under the application of voltage (300 V), the basic drugs migrated towards the negative electrode which was placed in the alkaline buffer (pH 12). The latter solution caused the basic analytes to lose their protons, and hence be deionized. Similarly and concurrently, negatively charged acidic drugs migrated towards the positive electrode that was placed in the acidic buffer (pH 2) and became deionized as well. In the middle compartment of the membrane envelope was an organic acceptor solvent, a better solvent for the deionized drugs than the buffer solutions. Hence, both basic and acidic drugs could be extracted simultaneously into the acceptor solvent.

In the first instance, the effectiveness of electric potential in extracting the drugs simultaneously was assessed. Experiments without the application of voltage showed that only small amount of norephedrine HCl and ibuprofen were extracted (Fig. 3a). In this case, the cross-membrane transport mechanisms for the analytes were mainly due to passive diffusion facilitated by sample agitation. Over a short period of time, this was not an efficient process. On the other hand, both acidic and basic drugs were effectively transported across the membranes and into the acceptor solution when an electrical potential difference was applied (Fig. 3b). EME has certainly demonstrated the simultaneous extraction of both classes of drugs. Apparently, diffusion due to the pH gradient and sample agitation alone was not sufficient to allow extraction. Potential difference has shown to be the main driving force in achieving such extractions in previous work [23,24].

Fig. 3c shows the chromatogram of the standards at the same concentration as that used for EME, with direct injection. Significant enrichment of the analytes by EME can be observed when comparing the chromatograms (b) and (c) in Fig. 3.

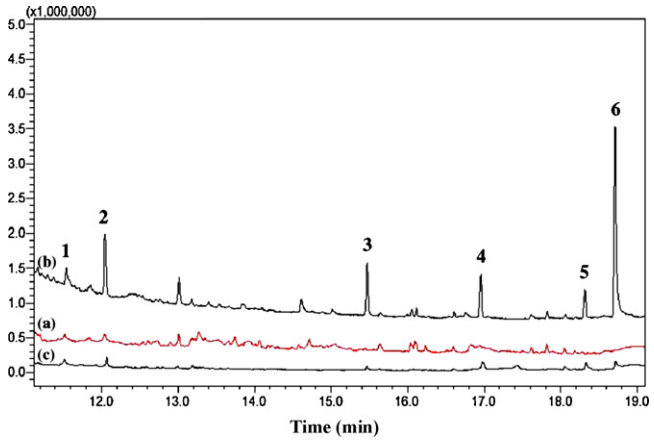

Fig. 3. GC–MS traces demonstrating the significant effect of voltage on cross-membrane transport: (a) extraction carried out at 0 V; (b) EME carried out at 300 V; (c) standard mixture of analytes at the same concentrations of analytes (direct injection). Peaks identification: (1) norephedrine HCl, (2) ibuprofen, (3) alprenolol HCl, (4) naproxen, (5) ketoprofen, (6) propanolol HCl.

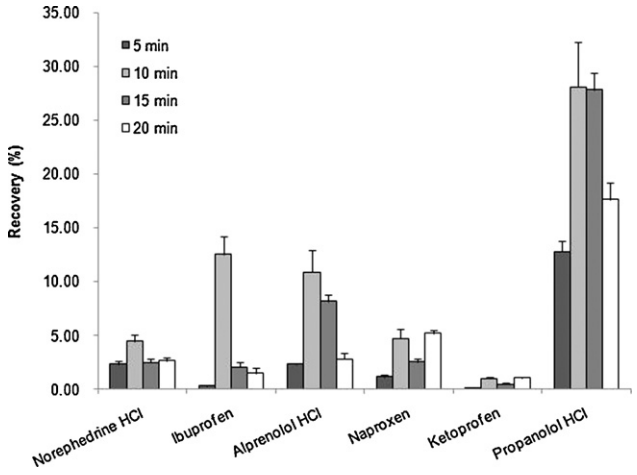

Fig. 4. Extraction time profile. Extraction conditions: 300 V voltage, toluene as SLM, 50 μL 1-octanol as acceptor phase, derivatized at room temperature for 1 h.

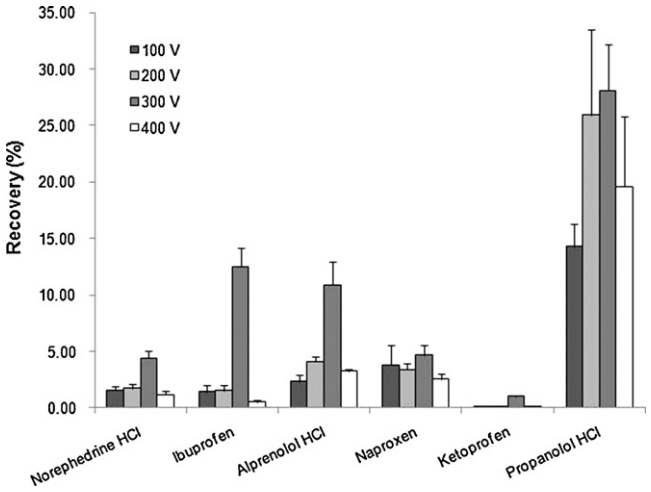

Fig. 5. Influence of applied voltage on EME. Extraction conditions: 10 min extraction time, toluene as SLM, 50 μL 1-octanol as acceptor phase, derivatized at room temperature for 1 h.

These experiments showed that cross-membrane transport based on electrokinetic migration with an applied voltage is undeniably more effective than cross-membrane transport based on passive diffusion. Moreover, this designed EME system is definitely capable of the concurrent extraction of both acidic and basic drugs, with high enrichment. As such, further optimization of the technique was performed and quantitative analysis was carried out. Conclusions were drawn by using the GC–MS chromatogram peak areas to represent extraction recoveries.

3.1. Extraction time

EME is a non-exhaustive extraction procedure where equilibrium-based distribution is expected. However, as this procedure is driven by electrokinetic migration, extraction time was predicted to be shorter than conventional LPME procedures [25]. As such, extraction time was varied from 5 to 30 min at constant applied voltage, to monitor EME. Fig. 4 shows that for most of the analytes, maximum recovery was attained after 10 min of extraction. Instead of observing a plateau representing the attainment of equilibrium, recovery of the analytes decreased after 10 min. This phenomenon can conceivably be explained by the saturation of analytes in the acceptor phase over time, which resulted in back-diffusion into the buffer solutions. Similar observations were reported in earlier papers as well [17,26]. Therefore, 10 min was selected as optimal extraction time.

3.2. Applied voltage

Preliminary experimentation had shown that applied voltage was an important criterion for efficient extraction of both the acidic and basic drugs simultaneously. Experiments with different applied potentials were performed over the range from 100 to 400 V (with current less than 50 μA). The results are summarized in Fig. 5. Generally, electrokinetic migration of the analytes into the acceptor solvent improved as voltage was increased from 100 to 300 V. This observation followed the modified Nernst–Planck equation, which predicts the improvement in the flux of analytes with increasing potential difference [27]. However, a further increment to 400 V showed a decrease in the performance of EME. Similar deviation has been reported in a recent paper [28]. This can be explained by the bubble formation at the electrodes due to electrolysis, causing the flux of the analytes to be unstable and hence hindering further improvement in extraction recoveries. 300 V was used for the rest of the study to permit maximum concurrent recoveries of all the drugs.

3.3. Derivatization conditions

Derivatization of the pharmaceuticals with BSTFA is necessary to enhance the volatility of these high molecular weight drugs, as well as to prevent peak tailing in the GC–MS analysis. In this procedure, improper derivatization had been reported due to excessive reagent and presence of moisture [29,30]. Hence, it is of importance that this derivatization step is done with care.

The amount of time required for derivatization was first optimized. Experiments were first carried out at room temperature, and substantial performance was observed after derivatization for 1 h. To reduce derivatization time, the mixture was heated at 70 °C in a water bath and derivatization time was significantly reduced to 30 min as a result (Fig. 6). Moreover, derivatization was most efficient at 70 °C for 30 min. In addition, the influence of different volumes of BSTFA added was investigated. Different extract:BSTFA volume ratios of 1:1, 1:2 and 3:1 were tested. Best results were achieved with a 1:1 ratio. When a higher ratio of BSTFA was used, column bleeding occurred due to the derivatization of the siloxane groups on the GC column. This led to undesirable damages to the column. When a low amount of BSTFA was used, incomplete derivatization occurred. Therefore, derivatization was carried out

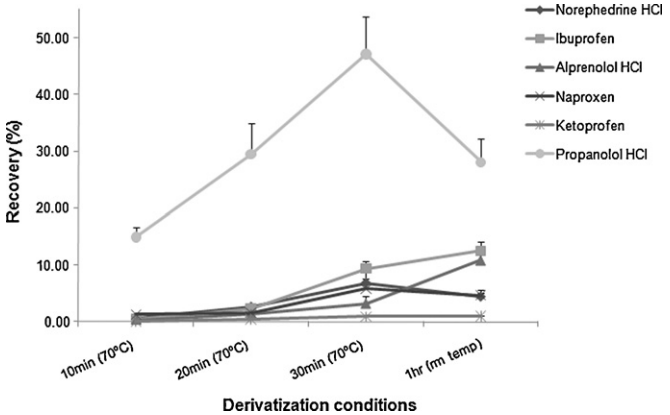

Fig. 6. Derivatization conditions for EME. Extraction conditions: 300 V voltage, 10 min extraction time, toluene as SLM, 50 μL 1-octanol as acceptor phase.

according to the following optimized condition: BSTFA was added in a 1:1 ratio to the extracted acceptor phase in a micro-vial that was subsequently capped and sealed tightly with Parafilm (Menasha, WI, USA). Utilizing a Vibramax100 vibrator (Heidolph, Kelheim, Germany), the mixture was well stirred and heated in 70 °C water bath for 30 min. In this procedure, complete derivatization was observed.

3.4. Supported liquid membrane

In conventional LLLME techniques, the SLM acts as a medium between the donor and acceptor phase to help speed up the mass transfer of the analytes [14–16]. This same approach was also adapted in our EME system and several considerations have to be made when choosing the appropriate organic solvent. Particularly in EME, it is crucial for the organic solvents used to have sufficient electrical conductance so as to allow a continuous electric field in the entire system. In our study, organic solvents were used for both the SLM and acceptor phase. Secondly, the organic solvent should have suitable chemical properties with the analytes to enable their proper phase transfer and electrokinetic migration across the membranes. Moreover, any leakage of the SLM during sample agitation is undesirable. Hence it is important to choose a solvent that is immiscible with water and compatible with the envelope material, so that the solvent can be well confined within the porous wall of the polypropylene membrane.

From the literature, four commonly used solvents were selected and utilized as the SLM. Results obtained with 1-octanol were irreproducible and this was probably due to the compatibility of alcohols with acidic analytes only as reported by Balchen et al. [31]. For DCM and 1-hexane, lower recoveries were observed. This could be attributed to the loss of these relatively more volatile organic solvents when Joule heating, as a result of applied voltage over time, occurred during extraction [32]. This phenomenon was more prominent with these two solvents as their boiling points are much lower than the rest. Satisfactory simultaneous extraction of all the acidic and basic analytes was achieved when toluene was tested. Hence, it was chosen as the SLM for subsequent analysis.

3.5. Acceptor phase solvent

Similar criteria apply to the selection of the organic acceptor phase. Moreover, the organic solvent should be non-volatile and amenable to GC–MS analysis. Experiments with four organic solvents, 1-octanol, toluene, 1-hexane and ethyl acetate were tested. Due to the relative higher volatilities of the solvents, except toluene and 1-octanol, none of the solvents were available for collection after 10 min of extraction. Comparing toluene with 1-octanol, 1-octanol has a higher boiling point (i.e. 195 °C) and a better electrical permittivity ($\varepsilon = 3.4$), which explains its better performance in allowing extraction of the two classes of analytes from the respective buffer solutions.

3.6. pH in buffer compartments

The acidic drugs used in our experiments have pK_a values ranging from 4.2 to 4.6, while the basic drugs have pK_a values at approximately 9.5 [33–35]. Acidic drugs are in their neutral form at pH values lower than their pK_a values. As for basic drugs, they are in their neutral forms at pH values higher than their pK_a values. Without adjusting the sample pH, different acidic and alkaline phosphate buffers ranging from 2 to 12 were tested. It was found that in the acidic buffer compartment and the alkaline buffer compartment, extraction efficiency was most effective with the use of phosphate buffers at pH 2 and pH 12, respectively. When buffers at pH 4 and 10 were used, small quantities of the drugs were extracted. Most probably, drugs that have migrated into the buffer solutions were not efficiently extracted into the organic acceptor phase due to poorer deionization. This happens when the buffer pHs are too close to the pK_a values of the analytes. This indicates that the drugs have to be in neutral form in order to migrate effectively into the organic acceptor solvent. Another experiment was done with pH values at 6 and 8. Not surprisingly, almost none of the drugs were extracted. This again further supports our understanding of simultaneous extraction using the designed EME system.

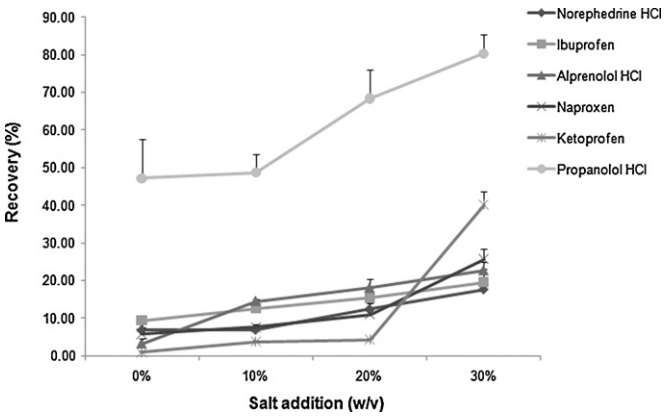

Fig. 7. Effect of NaCl addition on EME. Extraction conditions: 300 V voltage, 10 min extraction time, toluene as SLM, 50 μL 1-octanol as acceptor phase, derivatized at 70 °C for 30 min.

3.7. Addition of NaCl

In conventional liquid–liquid extraction, the "salting-out" effect [36] can often improve extraction performance by reducing the solubility of polar analytes. With the addition of salt, water molecules form hydration spheres around the ionic salt molecules. As such, the concentration of water available to dissolve analyte molecules is reduced, and the overall increase in ionic strength will also drive the analytes to the organic extractant. Various concentrations of NaCl ranging from 0 to 30% (w/v) were evaluated. Fig. 7 shows the effect of salt addition on EME. Here, increasing extraction efficiency was observed with addition of salt.

With the use of higher concentration of NaCl, the following electrolytic reactions were expected to occur at both electrodes:

$$\text{Cathode reaction}: \ 2H^+(aq) + 2e^- \rightarrow H_2(g)$$

$$\text{Anode reaction}: \ 2Cl^-(aq) \rightarrow Cl_2(g) + 2e^-$$

However, no bubble formation was observed around the electrodes; similar observations were reported in earlier works [18,31]. With the selected SLM, it is possible that chloride ions were not transported across it. Moreover, the current that was flowing through the system was kept at an appropriate level (i.e. low μA). As such, chlorine gas evolution at the anode was not of concern in this case. On the basis of the results, 30% (w/v) NaCl was added to the aqueous sample to achieve optimal extraction of both the acidic and basic pharmaceuticals.

4. Method validation

In order to assess the practical applicability of the proposed EME method, the optimized extraction conditions were adopted to evaluate its quantitative performance. The linearity of the method was tested at five different concentration levels, ranging from 1 to 200 $\mu g\,L^{-1}$. External calibration plots were constructed and good

Table 1
Quantitative performance of EME and in comparison with SPE.

Analyte	Correlation coefficient	RSD (%, $n=6$)	Enrichment factor	Recovery (%)		
				EME	SPE (170 μg L^{-1})	SPE (5 μg L^{-1})
Norephedrine HCl	0.997	12	81	18	6	6
Ibuprofen	0.982	13	90	20	10	11
Alprenolol HCl	0.997	11	104	23	18	12
Naproxen	0.989	10	118	26	22	11
Ketoprofen	0.997	9	185	40	73	22
Propanolol HCl	0.993	6	370	80	53	54

linearity with correlation coefficients between 0.982 and 0.997 were obtained. The precision of the method was evaluated by performing six consecutive analyses at various analyte concentrations and the relative standard deviations ranged from 6 to 13%.

The enrichment factor (EF) and recovery (R) were calculated based on the following equations:

$$\mathrm{EF} = \frac{C_{\mathrm{a,final}}}{C_{\mathrm{d,initial}}}$$

$$R = \frac{n_{\mathrm{a,final}}}{n_{\mathrm{d,initial}}} \times 100\% = \frac{V_{\mathrm{a}}}{V_{\mathrm{d}}} \frac{C_{\mathrm{a,final}}}{C_{\mathrm{d,initial}}} \times 100\%$$

where $C_{\mathrm{a,final}}$ is the final analyte concentration in the acceptor solvent and $C_{\mathrm{d,initial}}$ is the initial analyte concentration within the sample solution. $n_{\mathrm{a,final}}$ is the amount of analyte enriched in the acceptor phase while $n_{\mathrm{d,initial}}$ is the total amount originally present in the sample. V_{a} is the volume of acceptor phase and V_{d} is the sample volume.

EFs of 81–370 that corresponded to recoveries ranging from 18 to 80% were achieved. A comparative study was done with SPE at two concentration levels according to the procedure as mentioned. SPE gave recoveries that were either comparable or lower. EME is shown to have higher capacity for both classes of analytes even though it is an equilibrium-based extraction approach. The lower recoveries of SPE are mainly due to its multistep procedure and its non-selectivity in extracting both acidic and basic analytes concurrently [37]. All these results are summarized in Table 1.

Limits of detection (LODs), calculated based on signal-to-noise ratio of 3, were found to be in the range of 0.0081–0.26 μg L^{-1}. The comparison between LODs of EME with other reported techniques is also summarized in Table 2. Results show that by using EME, LODs obtained were either equivalent or much lower when compared with previously reported LPME and solid-phase microextraction (SPME) procedures.

Table 2
Comparison of LODs with other techniques.

Analyte	LODs		
	Our work (μg L^{-1})	LPME (μg L^{-1})	SPME (μg L^{-1})
Norephedrine HCl	0.13	300[a]	–
Ibuprofen	0.13	0.1[b]	0.2[d]
Alprenolol HCl	0.18	–	0.82[e]
Naproxen	0.26	70[c]	5.0[d]
Ketoprofen	0.027	55[c]	–
Propanolol HCl	0.0081	–	0.82[e]

Since LODs are obtained from different references, there is some inconsistency in the significant figures reported.
[a] LPME–CE–ultraviolet detection (UV) [38].
[b] LPME–HPLC–UV [39].
[c] Dynamic LPME–HPLC–UV [40].
[d] SPME–GC–MS [41].
[e] SPME–LC–MS [42].

5. Wastewater sample analysis

Wastewater samples were collected from drains and extracted using the proposed method. The concentrations of the pharmaceuticals detected are summarized in Table 3. Wastewater samples spiked at a concentration level of 5 μg L^{-1} were also analyzed under the optimum EME conditions. The enrichment factor (EF) and recovery (R) from the spiked wastewater samples were calculated based on the following equations:

$$\mathrm{EF} = \frac{C_{\mathrm{a,final}} - C_{\mathrm{a,found}}}{C_{\mathrm{d,initial}}}$$

$$R = \frac{V_{\mathrm{a}}}{V_{\mathrm{d}}} \frac{C_{\mathrm{a,final}} - C_{\mathrm{a,found}}}{C_{\mathrm{d,initial}}} \times 100\%$$

where $C_{\mathrm{a,final}}$ is the final analyte concentration in the acceptor solvent, $C_{\mathrm{a,found}}$ is the analyte concentration found from unspiked real sample and $C_{\mathrm{d,initial}}$ is the initial analyte concentration within the sample solution. V_{a} is the volume of acceptor phase and V_{d} is the sample volume.

The results are tabulated in Table 3. The EFs and recoveries of the analytes from the spiked real samples were comparable to that from spiked ultrapure water. This clearly shows that interferences from real samples do not affect the selectivity of our EME system. It is obvious that the present method is rugged and applicable to environmental samples.

Table 3
Wastewater analysis.

Analyte	Unspiked real sample concentrations (μg L^{-1})	Spiked real sample at 5 μg L^{-1}	
		Enrichment	Recovery (%)
Norephedrine HCl	Not detected	89	19
Ibuprofen	Not detected	56	12
Alprenolol HCl	5.4	122	26
Naproxen	7.1	121	26
Ketoprofen	Not detected	148	32
Propanolol HCl	Not detected	350	76

6. Conclusion

In the present study, for the first time, a method based on electrokinetic migration has been developed for the simultaneous determination of trace levels of acidic and basic drugs in water samples at neutral pH. Compared with passive diffusion, electrokinetic migration is a much more effective transport mechanism, providing high extraction efficiencies in very short time. Moreover, with the specially designed and fabricated membrane envelope, we have performed effective extraction of the two different classes of analytes in a single step. The polypropylene membrane also acts as a filtering device, and with the selected SLM, only the analytes were allowed to pass through the walls of the membrane. As such, the system provides proper sample clean-up and also allows relatively dirty samples to be handled. Although future work is required to fully understand the kinetics of the analytes via EME, this analytical technique developed herein is certainly a rapid and powerful tool for both qualitative and quantitative determination of trace level pharmaceutical residues in wastewater samples.

Acknowledgements

J.L. is grateful to National University of Singapore (NUS) for the award of a research scholarship. The authors acknowledge the financial support of NUS for this research.

References

[1] N. Paxéus, Water Res. 30 (1996) 1115.
[2] H.R. Rogers, Sci. Total Environ. 185 (1996) 3.
[3] C.G. Daughton, T.A. Ternes, Environ. Health Perspect. 107 (1999) 907.
[4] O.A.H. Jones, N. Voulvoulis, J.N. Lester, Environ. Toxicol. 22 (2001) 1383.
[5] T. Heberer, Toxicol. Lett. 131 (2002) 5.
[6] H.J. Stan, T. Heberer, Analusis 25 (1997) M20.
[7] T.A. Ternes, Water Res. 32 (1998) 3245.
[8] M.A. Jeannot, F.F. Cantwell, Anal. Chem. 68 (1996) 2236.
[9] Y. He, H.K. Lee, Anal. Chem. 69 (1997) 4634.
[10] M. Ma, F.F. Cantwell, Anal. Chem. 71 (1999) 388.
[11] H.L. Lord, J. Pawliszyn, J. Chromatogr. A 902 (2000) 17.
[12] L. Zhao, H.K. Lee, J. Chromatogr. A 919 (2001) 381.
[13] J. Lee, H.K. Lee, K.E. Rasmussen, S. Pedersen-Bjergaard, Anal. Chim. Acta 624 (2008) 253.
[14] T. Grønhaug-Halvorsen, S. Pedersen-Bjergaard, K.E. Rasmussen, J. Chromatogr. A 909 (2001) 87.
[15] S. Andersen, T. Grønhaug-Halvorsen, S. Pedersen-Bjergaard, K.E. Rasmussen, J. Chromatogr. A 963 (2002) 303.
[16] A. Bjørhovde, T. Grønhaug-Halvorsen, S. Pedersen-Bjergaard, K.E. Rasmussen, Anal. Chim. Acta 491 (2003) 155.
[17] S. Pedersen-Bjergaard, K.E. Rasmussen, J. Chromatogr. A 1109 (2006) 183.
[18] A. Gjelstad, K.E. Rasmussen, S. Pedersen-Bjergaard, J. Chromatogr. A 1124 (2006) 29.
[19] S. Pedersen-Bjergaard, K.E. Rasmussen, Anal. Chem. 71 (1999) 2650.
[20] S. Pedersen-Bjergaard, K.E. Rasmussen, M. Krogh, H. Grefslie Ugland, T. Grønhaug-Halvorsen, J. Chromatogr. A 873 (2000) 3.
[21] L. Zhao, L. Zhu, H.K. Lee, J. Chromatogr. A 963 (2002) 239.
[22] S. Weigel, R. Kallenborn, H. Hühnerfuss, J. Chromatogr. A 1023 (2004) 183.
[23] J. Lee, F. Khalilian, H. Bagheri, H.K. Lee, J. Chromatogr. A 1216 (2009) 7687.
[24] C. Basheer, S.H. Tan, H.K. Lee, J. Chromatogr. A 1213 (2008) 14.
[25] A. Gjelstad, T.M. Andersena, K.E. Rasmussen, S. Pedersen-Bjergaard, J. Chromatogr. A 1157 (2007) 38.
[26] I.J.Ø. Kjelsen, A. Gjelstad, K.E. Rasmussen, S. Pedersen-Bjergaard, J. Chromatogr. A 1180 (2008) 1.
[27] A. Gjelstad, K.E. Rasmussen, S. Pedersen-Bjergaard, J. Chromatogr. A 1174 (2007) 104.
[28] M. Balchen, L. Reubsaet, S. Pedersen-Bjergaard, J. Chromatogr. A 1194 (2008) 143.
[29] D. Li, J. Park, J.R. Oh, Anal. Chem. 73 (2001) 3089.
[30] Y. Shao, P. Marriott, H. Hugel, Chromatogr. Suppl. 57 (2003) 349.
[31] M. Balchen, A. Gjelstad, K.E. Rasmussen, S. Pedersen-Bjergaard, J. Chromatogr. A 1152 (2007) 220.
[32] L. Xu, P.C. Hauser, H.K. Lee, J. Chromatogr. A 1214 (2008) 17.
[33] J.E. Sinsheimer, L.G. Dring, R.T. Williams, Biochem. J. 136 (1973) 763.
[34] M. Yazdanian, K. Briggs, C. Jankovsky, A. Hawi, Pharm. Res. 21 (2004) 293.
[35] A. Trinh, L. Marlatt, D.S. Bell, The Reporter 24.1 (2006) 8.
[36] H.L. Lord, J. Pawliszyn, Anal. Chem. 69 (1997) 3899.
[37] D.M. Pavlović, S. Babić, A.J.M. Horvat, M. Kaštelan-Macan, Trends Anal. Chem. 26 (2007) 1062.
[38] L. Hou, X. Wen, C. Tu, H.K. Lee, J. Chromatogr. A 979 (2002) 163.
[39] X. Wen, C. Tu, H.K. Lee, Anal. Chem. 76 (2004) 228.
[40] M. Cruz-Vera, R. Lucena, S. Cárdenas, M. Valcárcel, J. Chromatogr. A 1202 (2008) 1.
[41] M. Moeder, S. Schrader, M. Winkler, P. Popp, J. Chromatogr. A 873 (2000) 95.
[42] H. Kataoka, S. Narimatsu, H.L. Lord, J. Pawliszyn, Anal. Chem. 71 (1999) 4237.