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Electromembrane extraction using stabilized constant d.c. electric current—A simple tool for improvement of extraction performance

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ABSTRACT

This contribution presents an experimental approach for improvement of analytical performance of electromembrane extraction (EME), which is based on the use of stabilized constant d.c. electric current. Extractions were performed using a high voltage power supply, which provided stabilized constant d.c. current down to 1 μA and facilitated current-controlled transfer of ions of interest from a donor solution through a supported liquid membrane (SLM) into an acceptor solution. Repeatability of the extraction process has significantly improved for EME at constant electric current compared to EME at constant voltage. The improved repeatability of the extraction process was demonstrated on EME-capillary electrophoresis (EME-CE) analyses of selected basic drugs and amino acids in standard solutions and in human urine and serum samples. RSD values of peak areas of the analytes for EME-CE analyses were about two-fold better for EME at constant electric current (2.8–8.9%) compared to EME at constant voltage (3.6–17.8%). Other analytical parameters of the EME-CE methods, such as limits of detection, linear ranges and correlation coefficients were not statistically different for the two EME modes. Moreover, EME at constant electric current did not suffer from SLM instabilities frequently observed for EME at constant voltage.

1. Introduction

Liquid phase microextraction (LPME) has attracted significant attention in recent years due to its favorable features, e.g. reduced use of organic solvents, fast pretreatment process, low volumes of pretreated samples and reduced analysis costs. Various LPME pretreatment techniques were reported over the last two decades that made use of the outstanding characteristics of supported liquid membranes (SLM) [1–3], hanging drops of organic solvents [4,5], hollow fibres (HF) [6,7] and other microscale approaches [8].

One of the most perspective LPME techniques in pretreatment of biological samples is based on use of SLMs and was first described by Audunsson [1]. In subsequent years the technique has attracted other researchers [2,3] and has further developed into two sample pretreatment methods, namely HF-LPME [6,7,9] and electromembrane extraction (EME) [10–13]. These two methods are also based on extractions across thin SLMs. A porous inert supporting material (usually polypropylene (PP) or polytetrafluoroethylene (PTFE) sheet or HF) is impregnated with water immiscible organic solvent to form the SLM, which separates two compartments filled with aqueous donor and acceptor solutions. The transfer of analytes into the acceptor solution is driven either by diffusion in HF-LPME or by a combination of diffusion and electromigration in EME whereas interfering matrix components and solid particles are retained on the SLM. Solutions from the acceptor compartment can then be directly analyzed by HPLC, GC, CE and MS [7,9,11–13].

The extraction time in EME can be significantly reduced compared to HF-LPME due to an increased transfer rate of charged species on application of electric field [14]. The extraction times in EME are usually 5–15 min and an additional selectivity of the pretreatment process is achieved since only positively or negatively charged species could be transferred to the acceptor compartment. EME became very popular in recent years [10,15–19] and although the method is still in its infancy, several critical reviews [11–13] and one publication on theory of EME [20] can be found in the literature. This theoretical contribution is based on Nernst–Planck equation and also on earlier publications describing iontophoretic transport of drugs through cellular membranes [21,22] and considers electric field as one of the key factors for ion transfer in EME. EME is therefore normally performed at constant voltage in all applications these days. Another reason for the dominant use of constant voltage in EME may be the fact that voltages used are mostly units to hundreds volts and usual constant d.c. voltage power supplies or even common 9 V batteries [23] are used, which are readily

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available. Note, however, that extraction repeatability is often compromised for EME at constant voltage. Typical repeatability values (expressed as RSDs of peak areas) for a set of independent EME measurements are around 10% [16–18,24], however, significantly lower repeatability, with RSD values up to 30%, is often reported [10,25,26].

A thorough examination and discussion of the EME repeatability issue is given in this manuscript and it is suggested that possible source of the lower repeatability might be the operation mode of EME using constant voltage. At constant voltage, the electric current in the EME system varies for non-uniform extraction units based on the Ohm's law since the total resistance of the EME system is given by the low-conductive SLM. Any small difference in the SLM uniformity will therefore induce measurable alterations of the resulting electric current. The total electric charge (Q in Coulombs) passed through the system will be different for non-uniform SLMs and consequently the total amount of charged species transferred through the SLMs will be different in accordance with Faraday's law. A logical alternative offering higher extraction repeatability is therefore application of stabilized constant electric current, which eliminates variations of the driving d.c. electric current during EME. Use of constant electric current in EME was, however, not proposed until now and comparison of the two EME modes is also not available.

In this contribution, the two modes (constant d.c. voltage vs. constant d.c. electric current) are compared and the effect of the selected operation mode on the performance of the EME pretreatment process is evaluated. Two principally different EME systems are examined. In the first system, extractions of basic drugs are performed through a poorly conductive SLM and the total electric current in the EME system (ca. units of μA) is obtained predominantly by the transfer of the analytes through the SLM. In the second system, SLM composition and transfer of matrix inorganic ions through the SLM contribute significantly to the total electric current in the EME system (ca. 100 μA), whereas transfer of minor analytes (amino acids) has only a little effect on the total electric current.

2. Materials and methods

2.1. Instrumentation

2.1.1. Electromembrane extraction

The EME system is depicted in Fig. 1 and was described in detail earlier [19]. A 3 cm long piece of a polypropylene hollow fibre (Accurel PP 300/1200, Membrana, Wuppertal, Germany; wall thickness of 300 μm and internal diameter of 1200 μm) was used as a single use extraction unit. Before EME, each extraction unit was dipped for a given time into an organic solvent, the lumen was filled with 20 μL of an acceptor solution and excessive solvent was removed using lint-free medical wipe. After EME, the acceptor solution was collected from the lumen and was filled into a microvial for CE analysis.

In constant voltage mode, the EME system was operated by using ES 0300-0.45 power supply (Delta Elektronika BV, Zierikzee, The Netherlands, d.c. voltage 0–300 V at maximum current of 450 mA). The voltage applied to the EME system generated electric currents of approximately 1–190 μA. In constant electric current mode, the EME system was operated by using CZE1000R high voltage power supply (Spellman, Pulborough, UK, 0–300 μA stabilized d.c. current at 0–30,000 V). Stability of the electric current in both EME modes was continuously monitored using M-3800 (Metex, Seoul, Korea) digital multimeter. The CZE1000R high voltage power supply showed stable performance at constant currents down to 1 μA. All EME experiments were performed at ambient temperature of 25 ± 2 °C.

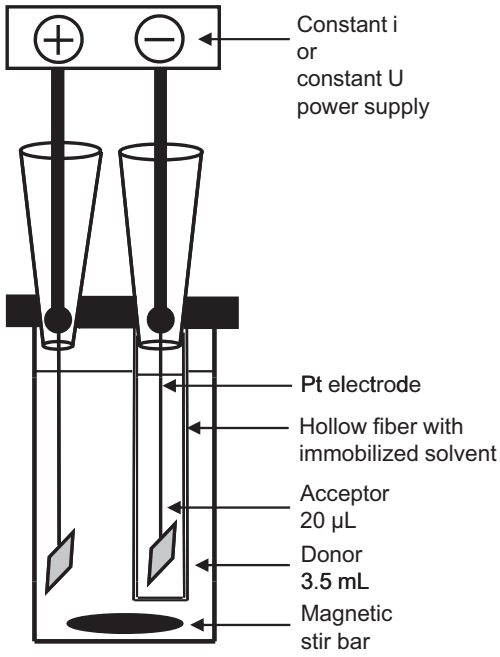

Fig. 1. Schematic drawing of the EME system.

2.1.2. Capillary electrophoresis

A P/ACE 5000 CE instrument (Beckman, Fullerton, CA, USA) equipped with UV–vis absorbance detector was operated at +12.5 kV (amino acids) and +15 kV (basic drugs) applied at the injection side of the separation capillary. Separation capillaries were fused-silica capillaries (75 μm ID, 375 μm OD, 27 cm total length and 20.3 cm effective length, Polymicro Technologies, Phoenix, AZ, USA). New separation capillaries were preconditioned with 1 M NaOH, DI water, and BGE solution by flushing for 10 min at 20 psi. Between two successive CE runs, the capillary was flushed with BGE solution for 1.5 min at 20 psi. Injections were carried out hydrodynamically by application of 0.5 psi for 2–5 s, which represents less than 4.6% of the total capillary volume (22–55 nL). All CE experiments were performed at 25 °C. Direct UV–vis detection of selected basic drugs and amino acids was performed at 200 nm. The CE system was controlled and data were acquired by P/ACE Station software.

2.2. Reagents, BGE solutions, standards and body fluids

All chemicals were of reagent grade and DI water with resistivity higher than 18 MΩ cm was used throughout. Stock solution of 1.5 M Na^+ was prepared from NaCl (Pliva-Lachema, Brno, Czech Republic). Stock solutions of basic drugs (1000 mg/L, Sigma, Steinheim, Germany) were prepared from pure chemical (haloperidol) and from their hydrochloride salts (nortriptyline, loperamide) and were diluted with pure methanol (Sigma). Stock solutions of selected amino acids (10 mM, Sigma, and Fluka, Buchs, Switzerland): creatinine (Crea), arginine (Arg), histidine (His), tryptophan (Trp), and phenylalanine (Phe) were prepared from pure chemicals and were diluted with DI water. Concentration of stock solution of tyrosine (Tyr) was lowered to 1 mM due to its poorer solubility in DI water. Standard solutions for CE measurements were prepared from these stock solutions and were diluted with 10 mM HCl (Pliva-Lachema) for analyses of basic drugs and with 2.5 M acetic acid (Fluka) for analyses of amino acids. Standard donor solutions for EME experiments of basic drugs were prepared in 10 mM HCl and contained 30 mM Na^+ and 0.01–2 mg/L of the analytes. For EME of amino acids, standard donor solutions were prepared in 2.5 M

acetic acid and contained 6 mM Na^+ and 5–100 μM of the analytes. Organic solvents for liquid membranes, 1-ethyl-2-nitrobenzene (ENB) and bis(2-ethylhexyl)phosphate (DEHP), were obtained from Sigma and were of highest available purity. The solvents were used without any further purification. BGE solution for CE of basic drugs (15 mM phosphate buffer) was prepared weekly from 15 mM sodium dihydrogenphosphate (Fluka) and was adjusted to pH 2.9 with 1 M orthophosphoric acid (Pliva-Lachema). BGE solution for CE of amino acids (2.5 M acetic acid, pH 2.0) was prepared weekly from concentrated acetic acid (Fluka). All BGE solutions were kept refrigerated at 4 °C.

Human urine samples were obtained from volunteers at the Institute of Analytical Chemistry. Human serum samples were purchased as lyophilized powders from Sigma and were prepared according to supplier's instructions. Serum samples were stored at −20 °C, urine samples were used only at the day of collection and then were disposed off. Human urine was diluted 1:4 with 12.5 mM HCl and then was spiked with the three basic drugs to final concentration of 0.1 and 0.5 mg/L. Human serum was diluted 1:24 with 2.6 M acetic acid and endogenous concentrations of amino acids were determined.

3. Results and discussion

Schematic drawing of the EME device is illustrated in Fig. 1. In order to examine analytical parameters of EME at constant voltage and EME at constant electric current, two principally different model extraction systems were chosen, namely EME of basic drugs as proposed by [10,27] and EME of amino acids as proposed by [24].

3.1. EME of basic drugs

Extractions of basic drugs are the most frequent applications of EME in analysis of biological samples [11–13]. The EME systems use 2-nitrophenyl octyl ether (NPOE) and ENB as liquid membranes and acidic donor and acceptor solutions [10,27]. These conditions ensure positive charge of the basic drugs and their efficient transfer through the SLM. The electric current is usually very low and is predominantly generated by transport of the target analytes through a poorly conductive SLM. Various matrix components (such as inorganic salts, proteins and mineral acids used to adjust the pH of donor/acceptor solutions) have only negligible effect on the electric current since these species do not cross the interface [10]. In our experiments, we have adopted previously described EME conditions, which are described in captions to Fig. 2. Three basic drugs (nortriptyline, haloperidol and loperamide) were selected as suitable analytes for EME and after extraction, the cationic analytes were determined in acceptor solutions by CE using 15 mM phosphate buffer at pH 2.9 [10].

3.1.1. EME at constant voltage

In the first series of experiments, EME of basic drugs was performed at constant voltage of 4 V. The standard donor solutions of the three basic drugs in 10 mM HCl were prepared at four different concentrations (0.1, 0.5, 1 and 2 mg/L). 30 mM of Na^+ added to each standard donor solution corresponds to concentration of Na^+ in 1:4 diluted human body fluids. Electric current during the extractions was monitored and resulting current curves for five independent EMEs of basic drugs at 1 mg/L are shown in Fig. 2A. The electric currents show similar profiles but the absolute values differ significantly. The electric currents integrated over the 5-min extraction time for the extraction with highest (run 5) and lowest (run 1) electric currents differ by more than 10%. Similar results were also observed for electric currents generated during EMEs of the basic drugs at the other three concentrations. Corresponding electropherograms for the five EMEs of basic drugs at 1 mg/L are shown

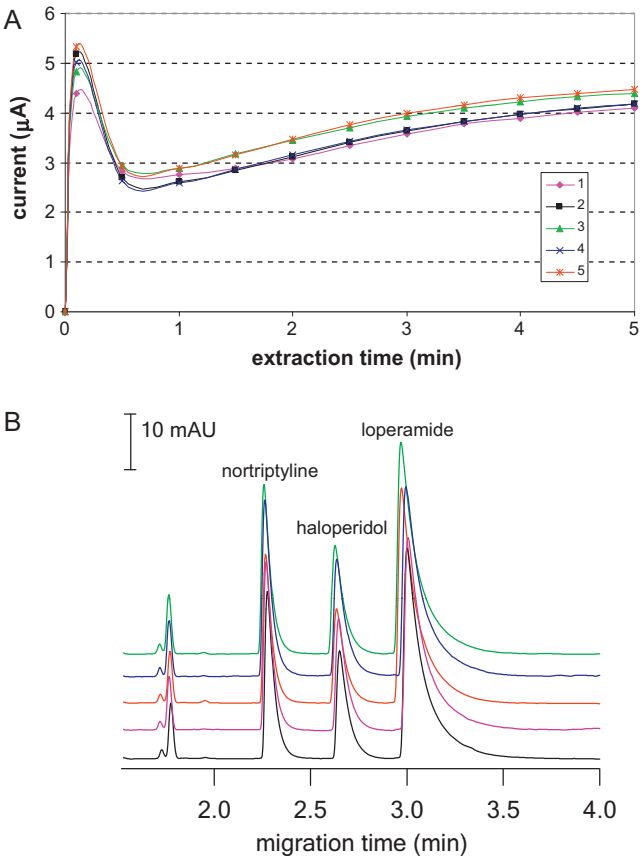

Fig. 2. (A) Electric current measured during 5 consecutive EMEs of basic drugs at constant voltage. (B) Corresponding electropherograms for the five extractions. EME conditions: liquid membrane: ENB, impregnation time: 10 s, agitation: 750 rpm, extraction voltage: 4 V, extraction time: 5 min, acceptor solution: 10 mM HCl. Donor solutions for EME were prepared in 10 mM HCl with 30 mM Na^+. Concentration of basic drugs is 1 mg/L. CE conditions: BGE solution: 15 mM phosphate buffer (pH 2.9), voltage: +15 kV, injection: 0.5 psi for 5 s, UV–vis detection at 200 nm.

in Fig. 2B and indeed, they show significant differences in quantitative measures of the CE method–in peak areas and peak heights. Repeatability of the EME-CE method, expressed as RSD values of peak areas, is summarized in Table 1 and is considerably higher than the repeatability (also included as RSD values in Table 1) of the CE system only, i.e., injecting standard solutions without the EME pretreatment step. Drug-free urine samples (diluted 1:4 with 12.5 mM HCl) were spiked with the three basic drugs to final concentrations of 0.1 and 0.5 mg/L and EME-CE analyses were performed at the same conditions as above. Repeatability of the method for analyses of spiked urine is also summarized in Table 1 along with other analytical parameters of the method, such as absolute recovery [10], limits of detection (LOD) and correlation coefficients of calibration curves.

3.1.2. EME at constant electric current

The same experimental conditions as in the Sections 3.1 and 3.1.1 were used except for application of constant electric current at 4.5 μA. This value corresponds to the average electric current measured at constant voltage in Section 3.1.1. Electric current was integrated over the 5-min extraction time and differences were less than 0.5%. Fig. 3A depicts electropherograms of five consecutive EMEs of the standard donor solution. The CE separation looks almost identical to that in Fig. 2B, note however, that a significant improvement of the analytical signal repeatability is observed. Electropherograms of urine samples, diluted 1:4 with 12.5 mM HCl, and spiked with 0.5 mg/L of the three basic drugs exhibit also an

Table 1
Analytical parameters of EME of basic drugs at constant voltage and constant electric current followed by CE analysis. EME and CE conditions as for Figs. 2 and 3. $n = 5$, calibration range = 0.01–0.5 mg/L.

	Nortriptyline	Haloperidol	Loperamide
RSD (%), PA (10 mg/L)[a]	3.3	2.8	4.3
Constant voltage (4 V)			
RSD (%), PA (0.1 mg/L)[b]	8.1	8.3	9.7
RSD (%), PA (0.5 mg/L) [b]	10.9	11.2	7.8
RSD (%), PA (1 mg/L) [b]	9.4	9.5	7.8
RSD (%), PA (2 mg/L) [b]	11.4	10.2	8.3
RSD (%), PA (0.1 mg/L) [c]	11.9	11.8	8.9
RSD (%), PA (0.5 mg/L) [c]	8.7	7.5	6.4
Absolute recovery (%) at 0.5 mg/L[b]	12.6	9.8	15.8
Absolute recovery (%) at 0.5 mg/L[c]	10.7	9.7	17.6
r^2 [b]	0.9979	0.9988	0.9991
LOD (mg/L)[b]	0.004	0.004	0.002
Constant electric current (4.5 μA)			
RSD (%), PA (0.1 mg/L)[b]	6.7	6.1	6.6
RSD (%), PA (0.5 mg/L) [b]	5.6	4.3	5.3
RSD (%), PA (1 mg/L) [b]	6.1	7.1	5.7
RSD (%), PA (2 mg/L) [b]	3.8	3.2	7.4
RSD (%), PA (0.1 mg/L) [c]	3.8	7.4	5.4
RSD (%), PA (0.5 mg/L) [c]	3.7	4.3	4.2
Absolute recovery (%) at 0.5 mg/L[b]	24.0	22.5	20.6
Absolute recovery (%) at 0.5 mg/L[c]	17.1	15.8	21.7
r^2 [b]	0.9987	0.9991	0.9998
LOD (mg/L)[b]	0.002	0.002	0.001

PA – peak area.
[a] CE-UV injection at 0.5 psi for 5 s.
[b] Standard solution.
[c] Spiked urine sample.

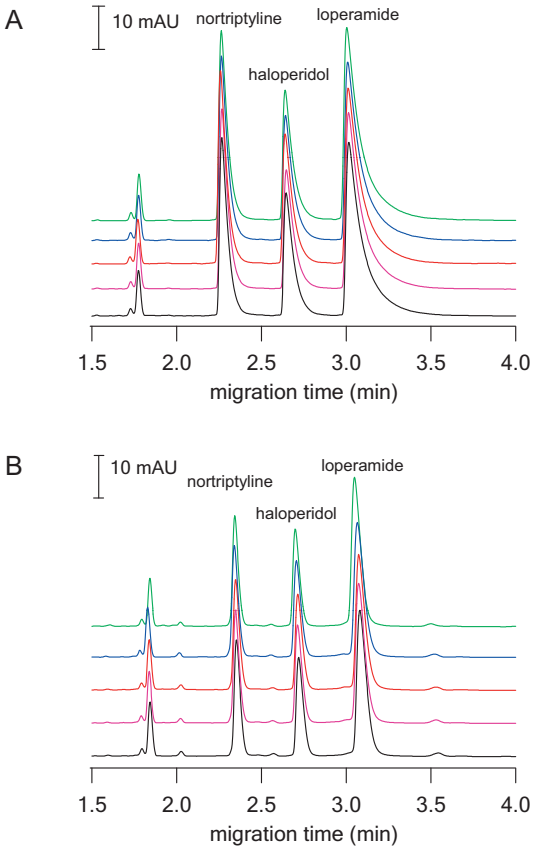

Fig. 3. Five consecutive EME-CE analyses of basic drugs extracted at constant electric current in (A) standard donor solutions and (B) human urine. Concentration of basic drugs is 1 mg/L in (A) and 0.5 mg/L in (B). EME and CE conditions as for Fig. 2 except for application of constant electric current (4.5 μA) during EME pretreatments.

excellent repeatability of the analytical signals as can be seen in Fig. 3B. All analytical parameters are summarized in Table 1.

Repeatability (as RSD values) of the analytical performance of the EME-CE at constant electric current (3.2–7.4%) is two-fold better compared to EME at constant voltage (6.4–11.9%) and approaches RSD values achieved with CE system only. Absolute recoveries and LODs were slightly better for EME at constant electric current and excellent linearity was achieved in the calibration range 0.01–0.5 mg/L for both EME modes.

3.2. EME of amino acids

EME was shown to be an efficient method for determination of endogenous concentrations of amino acids in body fluids [24]. The total electric current is one to two orders of magnitude higher than during EME of basic drugs due to higher conductivity of the SLM and due to transfer of analytes as well as matrix inorganic ions (e.g. Na^+) from donor to acceptor solution. Macromolecular bio-species are retained on the SLM and do not contribute to the total electric current [24]. Experimental conditions for EME of amino acids from body fluids were comprehensively optimized previously [24] and were only slightly modified in our experiments and are reported in captions to Fig. 4.

3.2.1. EME at constant voltage

Donor solutions were prepared as standard solutions of six amino acids (5–100 μM) in 2.5 M acetic acid with addition of 6 mM Na^+. Concentration of Na^+ in standard donor solutions corresponds to 1:24 diluted human serum. Electric current during EME was measured as a function of extraction time and resulting curves are depicted in Fig. 4A. The electric current values were almost identical within the first 30 s and thereafter significant differences were observed. Integration of the electric current over the 5-min extraction time showed that the difference between the highest (run 1) and lowest (run 2) total electric current was more than 10%. Serious differences in electric currents were also observed for consecutive EMEs of amino acids at other concentrations from the selected range (5, 50 and 100 μM). Electropherograms corresponding to the five consecutive extractions of the standard donor solution containing 10 μM of amino acids are depicted in Fig. 4B. Several additional peaks can be observed in the electropherograms between 2.75 and 3.25 min. These peaks originate from the EME system as was proven by EME of a blank donor solution and do not interfere with quantitative analyses.

Repeatability of the EME-CE method is summarized in Table 2 and varies between 3.6 and 17.8%. The repeatability values are considerably higher than those (also included in Table 2) for the CE system only, i.e., injecting standard solutions without the EME pretreatment step. Calibration measurements, LODs and absolute recoveries [10] of amino acids are also summarized in Table 2. Human serum was diluted 1:24 with 2.6 M acetic acid (see Section 2.2) and endogenous concentrations of amino acids were determined using the EME-CE method. During EMEs of real samples through SLMs made of ENB and DEHP, emulsification of the SLM and unstable electric currents due to excessive electrolysis were observed previously [24,27]. Addition of the ENB/DEHP solution forming the liquid membrane into the HF lumen prior to filling it with acceptor solution was necessary in order to avoid collapse of the EME system [24,27]. The same behavior was also observed when the diluted serum was extracted at 40 V in our experiments. EME of the serum samples was therefore performed after the initial addition of 3 μL of the ENB/DEHP solution into the HF.

Table 2
Analytical parameters of EME of amino acids at constant voltage and constant electric current followed by CE analysis. EME and CE conditions as for Figs. 4 and 5. $n = 5$, calibration range = 5–100 μM.

	Crea	Arg	His	Trp	Phe	Tyr
RSD (%), PA (100 μM)[a]	5.7	5.8	4.9	6.6	4.8	7.0
Constant voltage (40 V)						
RSD (%), PA (5 μM)[b]	13.0	8.2	17.8	9.9	6.1	8.8
RSD (%), PA (10 μM) [b]	7.5	6.9	13.2	6.6	6.8	8.0
RSD (%), PA (50 μM) [b]	6.6	6.3	4.8	7.1	3.6	8.6
RSD (%), PA (100 μM) [b]	8.6	5.9	5.8	7.3	6.6	11.3
RSD (%), PA[c]	6.4	4.5	8.1	5.2	3.6	10.5
Absolute recovery (%) at 50 μM[b]	3.7	1.1	0.9	4.4	2.6	0.2
r^2 [b]	1.0000	1.0000	0.9999	0.9999	1.0000	0.9999
LOD (μM) [b]	1	3	3	0.6	0.8	2
Constant electric current (90 μA)						
RSD (%), PA (5 μM)[b]	7.8	7.7	8.9	5.8	3.8	3.4
RSD (%), PA (10 μM) [b]	4.8	4.2	4.1	3.7	5.5	6.3
RSD (%), PA (50 μM) [b]	3.2	5.6	4.6	5.1	3.5	6.7
RSD (%), PA (100 μM) [b]	5.3	3.1	5.2	2.8	3.3	5.6
RSD (%), PA[c]	4.2	4.9	4.9	4.6	4.0	4.8
Absolute recovery (%) at 50 μM[b]	3.5	1.4	1.1	4.5	3.0	0.2
r^2 [b]	0.9997	0.9998	0.9999	0.9999	0.9999	0.9999
LOD (μM) [b]	0.9	2.5	2.2	0.5	0.7	1.5

PA – peak area.
[a] CE-UV injection at 0.5 psi for 2 s.
[b] Standard solution.
[c] Unspiked human serum sample.

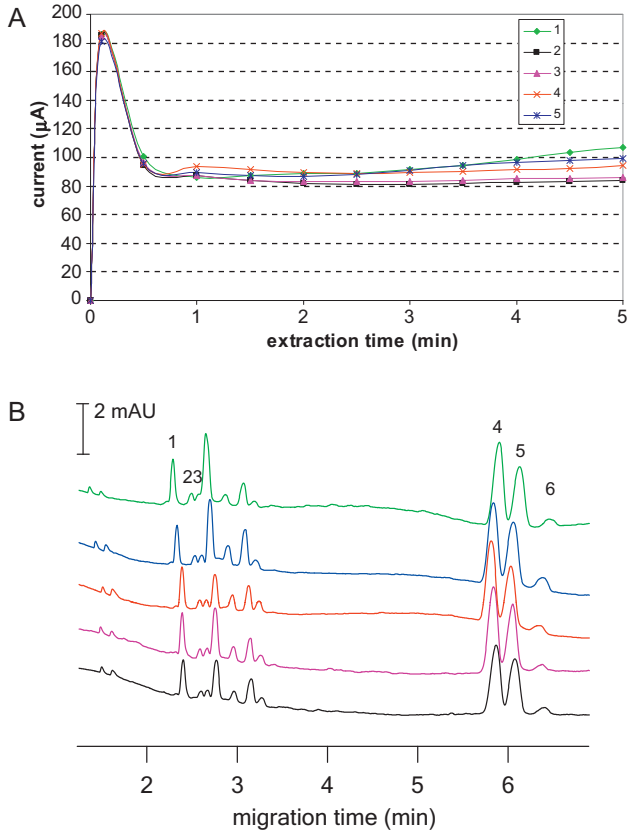

Fig. 4. (A) Electric current measured during 5 consecutive EMEs of amino acids at constant voltage. (B) Corresponding electropherograms for the five extractions. EME conditions: liquid membrane: ENB/DEHP (85:15, v/v), impregnation time: 10 s, agitation: 750 rpm, extraction voltage: 40 V, extraction time: 5 min, acceptor solution: 2.5 M acetic acid. Donor solutions for EME were prepared in 2.5 M acetic acid with 6 mM Na^+. Concentration of amino acids is 10 μM. CE conditions: BGE solution: 2.5 M acetic acid (pH 2.0), voltage: +12.5 kV, injection: 0.5 psi for 2 s, UV–vis detection at 200 nm. Peak description: 1 – Crea, 2 – Arg, 3 – His, 4 – Trp, 5 – Phe, 6 – Tyr.

3.2.2. EME at constant electric current

The selected value of constant electric current for EME of amino acids (90 μA) was the average plateau value as depicted in Fig. 4A and was used in all subsequent extractions. Other experimental conditions for EME and CE were same as in Sections 3.2 and 3.2.1. Fig. 5 depicts electropherograms for five consecutive extractions of the standard donor solution containing 10 μM of the six amino acids. Electric current was monitored and differences integrated over the 5-min extraction time were lower than 0.1%.

Analytical parameters of the EME-CE method are summarized in Table 2. RSD values of peak areas of the six amino acids are about two-fold better for EME at constant electric current (2.8–8.9%) compared to EME at constant voltage (3.6–17.8%). Absolute recovery, LODs and linear ranges are comparable for the two EME modes.

EME of human serum was performed at 1:24 dilution with 2.6 M acetic acid. Excellent EME performance was achieved without the initial addition of the ENB/DEHP solution into the lumen of the HF and corresponding repeatability values are also summarized in

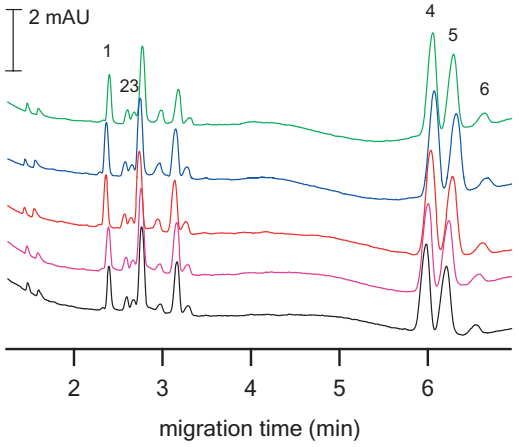

Fig. 5. Five consecutive EME-CE analyses of 10 μM amino acids extracted at constant electric current in standard donor solutions. EME and CE conditions as for Fig. 4 except for application of constant electric current (90 μA) during EME pretreatments.

Table 2. SLM depletion, excessive gas formation at electrodes due to electrolysis and extraction system collapse (previously described for EME at constant voltage [24,27], see also Section 3.2.1) was not observed for EME at constant electric current. We assume that the SLM stability is enhanced for EME at constant electric current, since no sudden current changes (such as the initial increase and drop (see Figs. 2A and 4A), which might break the SLM integrity) take place. The process of SLM depletion is, however, not yet fully understood and should be examined more in future.

4. Conclusion

This work has for the first time presented electromembrane extractions performed at stabilized constant d.c. electric current. The current-controlled transfer of analytes from donor to acceptor solutions across supported liquid membranes has significantly enhanced repeatability of the extraction process. Repeatability of the EME-CE system (using EME at constant electric current) was just slightly worse than that of the CE system only, demonstrating that reproducibility of EME at constant electric current was very good and its contribution to the total analytical imprecision was not significant. Other analytical parameters of EME-CE methods were comparable for both EME modes. The improved analytical performance of EME at constant electric current was demonstrated on extraction and CE analyses of selected basic drugs and amino acids in standard solutions and human urine and serum samples. Moreover, EME at constant electric current does not suffer from SLM instabilities frequently reported in experiments with constant voltage [24,27]. All these results suggest that, if possible, EME should be preferably performed at constant electric current.

Acknowledgments

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References

[1] G. Audunsson, Anal. Chem. 58 (1986) 2714.
[2] J.Å. Jönsson, L. Mathiasson, TrAC 18 (1999) 318.
[3] J.Å. Jönsson, L. Mathiasson, TrAC 18 (1999) 325.
[4] H.H. Liu, P.K. Dasgupta, Anal. Chem. 68 (1996) 1817.
[5] M.A. Jeannot, F.F. Cantwell, Anal. Chem. 68 (1996) 2236.
[6] S. Pedersen-Bjergaard, K.E. Rasmussen, Anal. Chem. 71 (1999) 2650.
[7] J.Y. Lee, H.K. Lee, K.E. Rasmussen, S. Pedersen-Bjergaard, Anal. Chim. Acta 624 (2008) 253.
[8] S. Dadfarnia, A.M.H. Shabani, Anal. Chim. Acta 658 (2010) 107.
[9] S. Pedersen-Bjergaard, K.E. Rasmussen, J. Chromatogr. A 1184 (2008) 132.
[10] S. Pedersen-Bjergaard, K.E. Rasmussen, J. Chromatogr. A 1109 (2006) 183.
[11] A. Gjelstad, LCGC N. Am. 28 (2010) 92.
[12] A. Gjelstad, S. Pedersen-Bjergaard, Bioanalysis 3 (2011) 787.
[13] P. Kubáň, A. Šlampová, P. Boček, Electrophoresis 31 (2010) 768.
[14] A. Gjelstad, T.M. Andersen, K.E. Rasmussen, S. Pedersen-Bjergaard, J. Chromatogr. A 1157 (2007) 38.
[15] L. Xu, P.C. Hauser, H.K. Lee, J. Chromatogr. A 1214 (2008) 17.
[16] S. Nojavan, A.R. Fakhari, J. Sep. Sci. 33 (2011) 3231.
[17] S. Seidi, Y. Yamini, T. Baheri, R. Feizbakhsh, J. Chromatogr. A 1218 (2011) 3958.
[18] P. Kubáň, L. Strieglerová, P. Gebauer, P. Boček, Electrophoresis 32 (2011) 1025.
[19] I.K. Kiplagat, T.K.O. Doan, P. Kubáň, P. Boček, Electrophoresis 32 (2011) 3008.
[20] A. Gjelstad, K.E. Rasmussen, S. Pedersen-Bjergaard, J. Chromatogr. A 1174 (2007) 104.
[21] J.C. Keister, G.B. Kasting, J. Membr. Sci. 29 (1986) 155.
[22] G.B. Kasting, J.C. Keister, J. Control. Release 8 (1989) 195.
[23] I.J.O. Kjelsen, A. Gjelstad, K.E. Rasmussen, S. Pedersen-Bjergaard, J. Chromatogr. A 1180 (2008) 1.
[24] L. Strieglerová, P. Kubáň, P. Boček, J. Chromatogr. A 1218 (2011) 6248.
[25] A. Gjelstad, K.E. Rasmussen, S. Pedersen-Bjergaard, J. Chromatogr. A 1124 (2006) 29.
[26] M. Balchen, A. Gjelstad, K.E. Rasmussen, S. Pedersen-Bjergaard, J. Chromatogr. A 1152 (2007) 220.
[27] A. Gjelstad, K.E. Rasmussen, S. Pedersen-Bjergaard, Anal. Bioanal. Chem. 393 (2009) 921.