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Electrokinetic migration across artificial liquid membranes New concept for rapid sample preparation of biological fluids

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Abstract

Basic drug substances were transported across a thin artificial organic liquid membrane by the application of 300 V d.c. From a 300 μl aqueous donor compartment (containing 10 mM HCl), the drugs migrated through a 200 μm artificial liquid membrane of 2-nitrophenyl octyl ether immobilized in the pores of a polypropylene hollow fiber, and into a 30 μl aqueous acceptor solution of 10 mM HCl inside the lumen of the hollow fiber. The transport was forced by an electrical potential difference sustained over the liquid membrane, resulting in electrokinetic migration of drug substances from the donor compartment to the acceptor solution. Within 5 min of operation at 300 V, pethidine, nortriptyline, methadone, haloperidol, and loperamide were extracted with recoveries in the range 70–79%, which corresponded to enrichments in the range 7.0–7.9. The chemical composition of the organic liquid membrane strongly affected the permeability, and may serve as an efficient tool for controlling the transport selectivity. Water samples, human plasma, and human urine were successfully processed, and in light of the present report, electrokinetic migration across thin artificial liquid membranes may be an interesting tool for future isolation within chemical analysis.

Keywords: Electrokinetic migration; Artificial liquid membranes; Sample preparation; Biological fluids; Drugs

1. Introduction

It is well known that charged chemical and biochemical substances migrate in solution under the application of an electrical potential difference. This type of transport, which is called electrokinetic migration, is the basis for electrophoresis, and is also widely used for isolation purposes both in industrial applications (purification) and in the field of analytical chemistry (sample preparation). Frequently, isolation based on electrokinetic migration is carried out in an aqueous one-phase system. One important example of this is electrodialysis, where charged chemical substances are transferred from an aqueous donor compartment, through the pores of a thin polymeric membrane filled with the same aqueous medium, and into an aqueous acceptor compartment in direct contact with the rest of the system. Electrodialysis is an important industrial purification and desalting process, and has also been reported as a sample preparation technique in analytical chemistry [1–4]. Within the latter field, electrodialysis has been implemented in several different formats, including the use of porous hollow fibers as a semi-permeable membrane coupled on-line to capillary electrophoresis (CE) [5,6].

Alternatively, isolation based on electrokinetic migration may be accomplished in a two-phase system, involving transport across the interface between two different liquid phases. One electrode is located in each phase, and an electric field is imposed on the system. During their elektrokinetic migration towards the electrodes, charged chemical substances can cross the phase boundary effecting separation. Isolation into the separate phases is possible since the thermal currents that generate convective mixing are constrained to a single phase and do not cross the interface. The two phases may both be aqueous formed by spontaneous phase separation of different water-soluble polymers [7–13], but also two-phase systems consisting of an aqueous compartment and an organic solvent immiscible with water have been reported [14–21]. In the latter type of system, chemical substances are isolated from the aqueous phase into the organic solvent, or in the reverse direction. Electrokinetic migration in different aqueous/organic two-phase systems has mainly been

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studied with focus on fundamental understanding [14–21], but the principle has also been reported in the field of analytical chemistry where different drugs were "electroextracted" from solutions of ethyl acetate and into an aqueous buffer at the inlet of a capillary electrophoresis column by application of high voltages in a CE-instrument [22–24].

Electrokinetic migration may also be accomplished in a three-phase system, where chemical substances cross two phase boundaries during their migration from one aqueous solution, through a layer of an organic solvent, and into a second aqueous compartment. However, experimental results with this type of isolation system are scarce in the literature [25,26], and most work reported has been focused on extraction of metal ions in systems with relatively thick layers of organic solvent (≈0.2 cm). In this paper, we show for the first time electrokinetic migration in a three-phase system where chemical substances migrates across a thin artificial liquid membrane (≈200 μm) immobilized in the pores of a porous hollow fiber, and we also demonstrate for the first time that this type of separation system may be a very fast and selective concept for isolation and preconcentration of charged analytes from complicated biological samples. This new concept is called electro membrane isolation (EMI).

2. Experimental

2.1. Electro membrane isolation

The equipment used for electro membrane isolation is illustrated in Fig. 1. The d.c. power supply used was a model XFR 300-9 (Xantrex, Burnaby, Canada) with programmable voltage in the range 0–300 V, providing currents in the range 0–9 A. Simple steel wires with a diameter of 0.2 mm were used as electrodes in the sample and acceptor solutions, and were connected to the power supply. As sample compartment, 800 μl polypropylene vials with cap were used with a height of 35 mm and with an internal diameter of 5.2 mm (unknown supplier). The porous hollow fiber used for immobilization of the artificial liquid membrane and for housing the acceptor solution was a PP Q3/2 polypropylene hollow fiber (Membrana, Wuppertal, Germany) with an internal diameter of 1.2 mm, with a 200 μm wall thickness, and with 0.2 μm pores. The whole sample compartment was agitated at 1200 rpm during the experiments with a Vibramax 100 agitator (Heidolph, Kelheim, Germany).

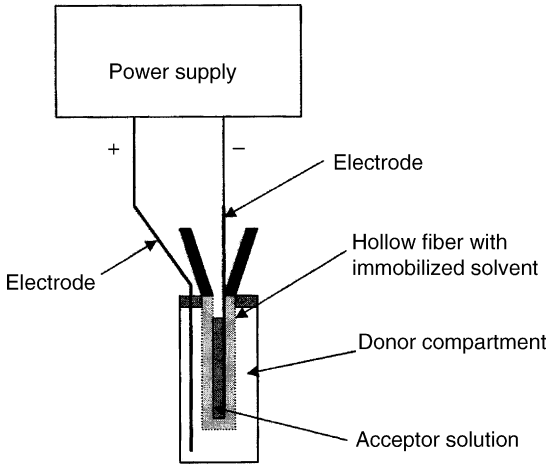

Fig. 1. Schematic illustration of the equipment for electro membrane isolation (EMI).

EMI was performed according to the following procedure; 300 μl acidified sample solution was filled into a polypropylene vial (sample compartment), and the positive electrode was placed in the sample. A 3.1 cm piece of polypropylene hollow fiber was closed in the lower end by mechanical pressure, whereas the upper end was connected to a 2.2 cm length pipette tip of polypropylene (Finntip 200 Ext from Thermo Electron, Vantaa, Finland) as a guiding tube. The hollow fiber and the guiding tube were then inserted through the cap of the polypropylene vial. The hollow fiber was dipped for 5 s in the organic solvent serving as the artificial liquid membrane (typically 2-nitrophenyl octyl ether), and excess of solvent was removed with a medical wipe. With a micro syringe, 30 μl of acceptor solution was filled into the hollow fiber, and the negative electrode was placed in the acceptor solution. Finally, the hollow fiber with acceptor solution was placed into the sample, and voltage (typically 300 V) was applied for 5 min. After EMI, the acceptor solution was collected with a micro syringe, and then transferred to a micro insert for the capillary electrophoresis instrument.

2.2. Capillary electrophoresis

Capillary electrophoresis was performed with a MDQ instrument (Beckman, Fullerton, CA, USA) equipped with a UV-detector. Separations were accomplished in a 75-μm-I.D. fused-silica capillary with an effective length of 20 cm (Beckman). The running buffer was 15 mM phosphate adjusted to pH 2.7 with ortho-phosphoric acid. The instrument was operated at 20 kV, which generated a current level of approximately 50 μA. Samples were introduced by hydrodynamic injection at 0.5 psi for 5 s. Detection was accomplished at 200 nm utilizing a 100 μm × 800 μm slit.

2.3. Chemicals

Pethidine hydrochloride, nortriptyline hydrochloride, methadone hydrochloride, haloperidol, and loperamide hydrochloride were all obtained from Sigma–Aldrich (St. Louis, MO, USA). 2-Nitrophenyl octyl ether, dihexyl ether, 2-octanone, silicone oil AS 4, and di(2-ethylhexyl)phosphate were from Fluka (Buchs, Switzerland). 1-Octanol and dodecyl acetate were purchased from Sigma, kerosene was from Norsk Medisinaldepot (Oslo, Norway), whereas soy-bean oil and peppermint oil were from a local pharmacy. Hydrochloric acid, disodium hydrogen phosphate dodecahydrate, and sodium dihydrogen phosphate monohydrate were from Merck (Darmstadt, Germany), and formic acid was from Fluka. Human plasma was obtained from Ullevål hospital (Oslo, Norway).

2.4. Standard solutions and biological samples

A stock solution containing 1 mg/ml of each of pethidine, nortriptylin, methadone, haloperidol, and loperamide was prepared in ethanol and stored at −20 °C protected from light. Sample solutions (in pure water) were prepared by dilution of this stock solution by 10 mM HCl. This stock solution was also utilized to spike human plasma and urine samples.

2.5. Calculation of recovery and enrichment

Recovery (R) during the electro membrane isolation was calculated according to the following equation for each analyte:

$$R = \frac{n_{a,final}}{n_{s,initial}} \times 100\% = \left(\frac{V_a}{V_s}\right)\left(\frac{C_{a,final}}{C_{s,initial}}\right) \times 100\% \quad (1)$$

where $n_{s,initial}$ and $n_{a,final}$ are the number of moles of analyte originally present in the sample and the number of moles of analyte finally colleted in the acceptor solution, respectively. V_a is the volume of acceptor phase, V_s is the volume of sample, $C_{a,final}$ is the final concentration of analyte in the acceptor phase, and $C_{s,initial}$ is the initial analyte concentration within the sample.

Enrichment (E) during the cross-membrane isolation was calculated according to the following equation for each analyte:

$$E = \frac{C_{a,final}}{C_{s,initial}} \quad (2)$$

3. Results and discussion

3.1. Initial experiments

The basic principle of electro membrane isolation is illustrated in Fig. 1. The sample solution (300 μl), which was acidified with HCl (pH ≈2) prior to EMI to ionize the basic analytes of interest, was filled into a small polypropylene tube, and a positive electrode was placed in this solution and connected to the power supply. A porous hollow fiber of polypropylene, which was sealed in the lower end by mechanical pressure, was dipped in 2-nitrophenyl octyl ether for 5 s to immobilize the organic solvent in the pores in the wall of the hollow fiber. This thin layer of organic solvent served as the artificial liquid membrane, the volume was approximately 15 μl, and the thickness was approximately 200 μm corresponding to the wall thickness of the hollow fiber. Inside the lumen of the hollow fiber, 30 μl of a 10 mM hydrochloric acid solution in water was injected which served as the acceptor solution, and a negative electrode connected to the power supply was placed in this acceptor solution. Finally, the hollow fiber was placed in the sample, and 300 V was applied over the electrodes for 5 min. The acceptor solution (30 μl) was collected after this by a micro syringe and transferred for analysis by capillary electrophoresis. Five different basic drugs were selected as model analytes, and their chemical structures are shown in Fig. 2. The model analytes were relatively hydrophobic substances as shown by their high log P values (1-octanol/water partition coefficients).

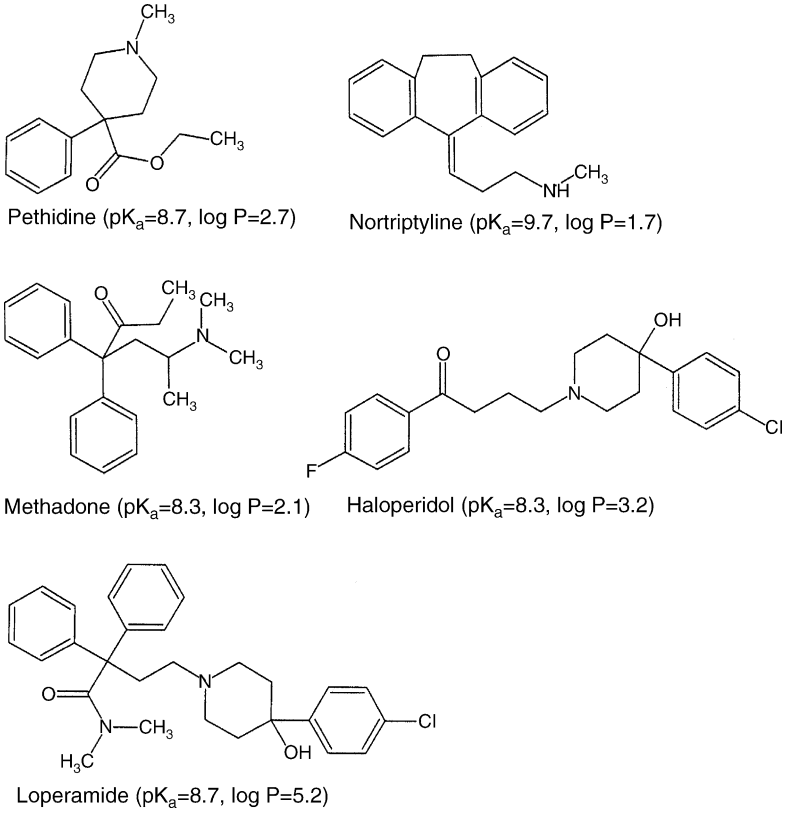

Fig. 2. Structures for the model compounds used.

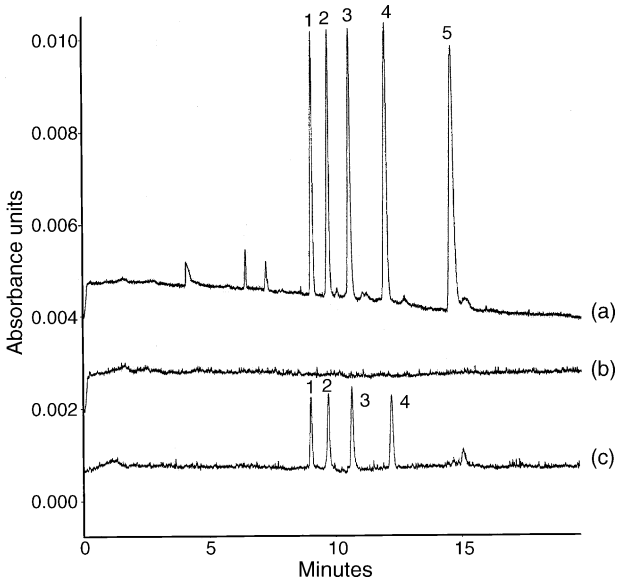

Fig. 3. Electropherograms demonstrating the strong effect of voltage on cross-membrane transport: (a) acceptor phase after 5 min of EMI at 300 V; (b) acceptor phase after 5 min of extraction with no voltage and with pH 2 in both the sample solution and in the acceptor (passive diffusion); and (c) acceptor phase after 5 min of extraction with no voltage and with pH 13 in the sample solution and pH 2 in the acceptor (passive diffusion).

In Fig. 3, an electropherogram of the acceptor solution after 5 min of EMI at 300 V is shown. This figure demonstrates that all the five model analytes were effectively transported across the artificial liquid membrane and trapped in the acceptor solution as an electrical potential difference was applied. Recovery values were in the range 70–79%. In a second experiment (Fig. 3b), the operation was repeated without application of the voltage. In this case, the only driving force for cross-membrane transport was passive diffusion, and this resulted in no detectable peaks. Clearly, the electrical potential difference was the driving force in EMI, whereas passive diffusion was undetectable. In another experiment without voltage, pH in the sample solution was adjusted to approximately 13 to deionize the model analytes, and the reason for this was to optimize their cross-membrane transport by passive diffusion [27]. An electropherogram from this experiment is shown in Fig. 3c. In this case, recovery values for four of the model analytes ranged between 18 and 26%, whereas loperamide was not detected. This experiment demonstrated that even if the conditions were optimized for cross-membrane transport based on passive diffusion, this process was significantly less effective than cross-membrane transport based on electrokinetic migration with an electrical potential difference. In other words, EMI appears to be a very rapid isolation technique capable of high analyte recoveries.

Agitation of the whole system was found to be important for the cross-membrane transport. With no agitation, recovery values for the five model drugs were in the range 8–10%, whereas they were improved to 70–79% as mentioned above when agitation at 1200 rpm was performed. This agitation speed was the maximum value obtainable with the current agitation system used.

3.2. Theoretical understanding

In order to enable EMI, the whole system comprising the sample solution, the artificial liquid membrane, and the acceptor solution should serve as an electrical circuit. The major electrical resistance of the system was associated with the artificial liquid membrane, and the solvent used here was critical in order to ensure penetration of the electrical energy. Thus, a solvent with a certain polarity or water content should be used to give sufficient electrical conductance, and to ensure penetration of the electrical field. Basically, the cross-membrane transport of model analytes increased with decreasing electrical resistance of the artificial liquid membrane. However, provided that the artificial liquid membrane and the model analytes were inert to electrode reactions, the following electrode processes occurred in the sample and acceptor solutions, respectively:

$$\text{Sample solution :} \qquad H_2O \rightarrow 2H^+ + \tfrac{1}{2}O_2 + 2e^-$$

$$\text{Acceptor solution :} \qquad 2H^+ + 2e^- \rightarrow H_2$$

thus, O_2 and H_2 were generated at the two electrodes, and this bubble formation increased with increasing current flow in the system (decreasing electrical resistance of the artificial liquid membrane). In other words, in order to suppress substantial bubble formation, the electrical conductance of the artificial liquid membrane should not be too high, but rather a compromise between the transport efficiency and the bubble formation tendency. 2-Nitrophenyl octyl ether as used above appeared to be a successful compromise in terms of electrical conductance, and with this solvent, no observable bubble formation was observed during visual inspection.

In the sample solution, pH was adjusted into the acidic range to ensure that the basic model analytes (B) were totally protonated (BH^+). Upon application of the electrical potential difference, the protonated model analytes started their electrokinetic migration from the sample solution, and in the direction towards the negative electrode placed in the acceptor solution. In the aqueous sample solution, the electrical field strength (V/cm) was relatively low due to the low electrical resistance of this phase, but nevertheless, because the model analytes were totally protonated, they rapidly migrated towards the artificial liquid membrane. This rapid migration was also promoted by using a thin sample vial, which ensured a short migration distance to the artificial membrane. The different model analytes migrated with different velocity in the sample solution based on their charge-to-size ratio, but this was expected only to be a minor factor responsible for the differences observed in their individual transport efficiencies (recovery values).

Secondly, the model analytes crossed the interface to the artificial liquid membrane. An exact theoretical model for this electrokinetic phase-transfer is not yet available, but will be studied in detail in a future paper. Nevertheless, it is expected that the electrical field strength (V/cm) within the organic phase was high due to the electrical resistance of the organic solvent used. In spite of this, electrokinetic migration of the analytes through the interface and within the organic phase was somewhat sup-

pressed because partial deprotonation of the basic substances occurred in the non-polar medium. For compounds with a low degree of deprotonation, the electrokinetic migration through the artificial membrane was significant, whereas strongly deprotonating compounds showed very low electrokinetic migration and were effectively discriminated by the artificial liquid membrane. This phenomenon was expected to be a principal reason for the differences in extraction recoveries observed. In addition, differences in the charge-to-size ratios were also expected to affect the individual transport efficiencies inside the artificial liquid membrane.

Thirdly, the model analytes crossed the interface between the artificial liquid membrane and the acceptor solution. In the acceptor solution, the electrical field strength (V/cm) was relatively low, but strong protonation of the model analytes again resulted in strong electrokinetic migration. During initial EMI, electrokinetic migration through the artificial liquid membrane effectively increased the concentration of model analytes in the acceptor solution. However, after a certain period of time with maximum agitation of the equipment at 1200 rpm, the analyte recovery approached 70–79%, and no further gain in recovery was observed as function of time. The reason for this is currently not fully understood, but may be due to mass transfer resistance and built-up of a boundary layer of ions (from hydrochloric acid) at the interfaces at both sides of the artificial liquid membrane, or a result of back diffusion of analyte to the artificial liquid membrane. Analysis of the sample solution after EMI revealed that the majority of analyte not recovered in the acceptor solution was still present in the sample. This indicated that the former explanation was the most plausible, but future work will be directed to study this important aspect in more detail.

3.3. Effect of the organic phase

In order to further investigate the EMI system, experiments with different organic solvents as the artificial liquid membrane were conducted to study this part of the system. The results are summarized in Table 1. As pure solvents, 2-nitrophenyl octyl ether, dihexyl ether, 1-octanol, 2-octanone, and dodecyl acetate were tested as EMI candidates. 2-Nitrophenyl octyl ether provided high recoveries (70–79%) for all the model analytes, 1-octanol resulted in low recoveries (3–7%), whereas no analyte transport was observed through dihexyl ether and dodecyl acetate. With 2-octanone, electrokinetic migration was observed in some cases, but the results were unreliable with a large standard deviation. In order to increase the electrical conductance of the artificial liquid membrane, 5% di(2-ethylhexyl)phosphate was added to 2-nitrophenyl octyl ether, but this resulted in decreased recoveries as compared with pure 2-nitrophenyl octyl ether.

In addition to the pure solvents, we also tested some different commercial oils as the artificial liquid membrane (Table 1). Kerosene, silicone oil (phenyl-methyl polysiloxane), and soybean oil all failed and provided no electrokinetic migration of the model analytes, whereas high recoveries were observed for 4 of the model analytes based on the use of peppermint oil. Thus, peppermint oil appeared to be an interesting green-chemistry alternative to 2-nitrophenyl octyl ether although it is not a highly defined product. Because 2-nitrophenyl octyl ether provided high recovery for the entire model analytes studied, this solvent was used as the artificial liquid membrane through the rest of this work.

3.4. Effect of pH in the acceptor solution

In a second experiment, different types of acceptor solutions were evaluated for EMI performance. The results are summarized in Table 2, and showed that 10 mM HCl provided the highest recoveries. Interestingly, also 10 mM formic acid served as an efficient acceptor solution providing results comparable with HCl. Although 10 mM HCl was used during the rest of this work, formic acid may be highly interesting in combination with LC–MS, and for applications where the analytes are unstable in strongly acidic solutions. Different phosphate buffers with pH in the range 6.0–8.0 were also tested, but these showed poor performance. With increasing pH, the electrokinetic migration into the acceptor solution was reduced due to partial deprotonation of the model analytes, and back-diffusion based on passive transport from the acceptor to the artificial liquid membrane was accelerated for the same reason.

Table 1
Recovery with different artificial liquid membranes

	Recovery (%)[a]				
	Pethidine	Nortriptyline	Methadone	Haloperidol	Loperamide
2-Nitrophenyl octyl ether	70	70	79	72	76
Dihexyl ether	nd	nd	nd	nd	nd
1-Octanol	3	4	7	3	7
2-Octanone	[b]	[b]	[b]	[b]	[b]
Dodecylacetate	nd	nd	nd	nd	nd
2-Nitrophenyl octyl ether + 5% di(2-ethylhexyl) phosphate	57	13	26	3	4
Kerosene	nd	nd	nd	nd	nd
Silicone oil AS 4	nd	nd	nd	nd	nd
Soy-bean oil	nd	nd	nd	nd	nd
Peppermint oil	13	73	73	78	79

[a] ($n = 3$), relative standard deviations were all below 15% RSD.
[b] Recovery was observed, but results were unreliable due to large standard deviations.

Table 2
Recovery with different acceptor solutions

	Recovery (%)[a]				
	Pethidine	Nortriptyline	Methadone	Haloperidol	Loperamide
100 mM HCl	60	40	83	72	80
10 mM HCl	70	70	79	72	76
1 mM HCl	44	27	31	23	22
10 mM HCOOH	56	70	63	52	61
10 mM phosphate pH 6.0	24	24	26	9	15
10 mM phosphate pH 7.0	19	8	9	nd	4
10 mM phosphate pH 8.0	nd	nd	nd	nd	nd

[a] ($n = 3$), relative standard deviations were all below 15% RSD.

3.5. Effect of pH in the sample

In a third experiment, different acid solutions and buffer solutions were tested as sample, and in all cases, the model analytes were spiked into the solution to a constant concentration level. The results are summarized in Table 3. As seen from the table, all the different sample solutions provided relatively high recoveries, which indicated that pH in the sample was not highly critical for the EMI process. Surprisingly, even the phosphate buffer at pH 8.0 gave high recoveries, even if the pH value in this case was close to the pK_a-values for several of the basic model analytes. Most probably, this supported the theoretical discussion above that electrokinetic migration in the sample, which was reduced at pH 8.0 due to deprotonation of the model analytes, was not the limiting step controlling the cross-membrane transport, and that transport limitations in the system was associated with the artificial liquid membrane. For the rest of this work, 10 mM HCl was utilized as the sample compartment.

3.6. Effect of time and voltage

In a fourth experiment, recovery of the different model analytes was studied as function of EMI time. The results are summarized in Fig. 4. In general, recoveries increased with increasing EMI time up to 5 min, where after recoveries leveled off or even decreased with increasing EMI time. The reason for the level off effect was discussed above (Section 3.2). The slight decrease in recoveries after more than 10 min of EMI probably was due to experimental inaccuracies or due to small losses of artificial liquid membrane. Recoveries for methadone and loperamide increased very rapidly with extraction time, whereas the other model analytes responded more slowly. Most probably, the observed behavior of the former compounds was a result of a relatively strong stability of their protonated species within the artificial liquid membrane, which in turn resulted in superior electrokinetic migration. A similar experiment on recovery versus time was conducted for transport based on passive diffusion only, without applying the electrical potential difference and adjusting pH in the sample to approximately 13 [27]. In this case 30–45 min of extraction was required before extraction recoveries leveled off. Thus, the speed of transport across the artificial liquid membrane was improved dramatically upon application of the electrical potential difference. During the rest of this work, 5 min was selected as EMI time.

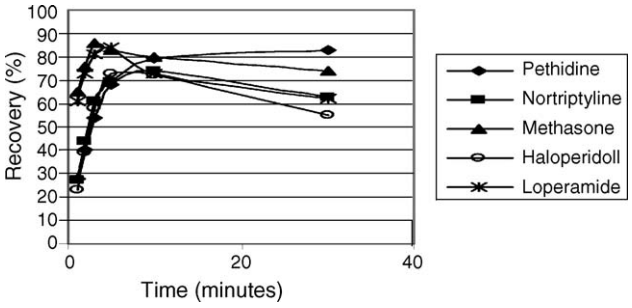

Fig. 4. Effect of time on cross-membrane transport (recovery versus EMI time).

In another experiment, recoveries were investigated as function of the applied electrical potential difference. These results are demonstrated in Fig. 5. For pethidine, haloperidol, and nortriptyline, relatively high recoveries were obtained with only

Table 3
Recovery with different compositions of the sample solution

	Recovery (%)[a]				
	Pethidine	Nortriptyline	Methadone	Haloperidol	Loperamide
100 mM HCl	72	65	78	65	68
10 mM HCl	70	70	79	72	76
1 mM HCl	64	59	71	63	65
10 mM HCOOH	68	69	76	69	73
10 mM phosphate pH 6.0	61	57	73	58	57
10 mM phosphate pH 7.0	63	62	71	58	49
10 mM phosphate pH 8.0	63	60	72	60	50

[a] ($n = 3$), relative standard deviations were all below 15% RSD.

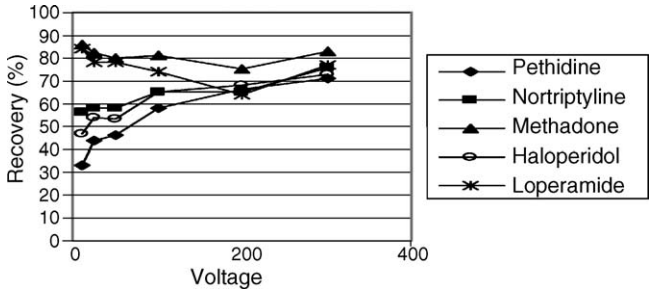

Fig. 5. Effect of voltage on cross-membrane transport recovery versus EMI voltage).

10 V as the potential difference, but recoveries increased further as the voltage was increased up to the upper limit at 300 V for the power supply. In their protonated form, their tendency to deprotonate in the artificial liquid membrane was relatively high, and high electrical potential differences were required to promote efficient migration through the artificial membrane. For methadone and loperamide in contrast, the highest recovery was obtained at 10 V, whereas recoveries decreased slightly as the applied voltage was increased up to 300 V. The latter compounds most probably showed a higher degree of protonation in the artificial liquid membrane, and consequently, lower electrical potential differences were required for effective electrokinetic migration. A few experiments were also carried out with another power supply operated at 500–1000 V, but in this case the system suffered from bubble formation at the electrodes and corresponding instability problems. In one occasion, sparking was observed. Thus, electrical potential differences above 300 V were found to be inappropriate, and during the rest of this work, 300 V was used.

3.7. Performance characteristics and system robustness

On the basis of the experiments discussed above, the highest performance of EMI for the model analytes was obtained utilizing 2-nitrophenyl octyl ether as the artificial liquid membrane, an acceptor solution of 10 mM HCl, a sample solution containing 10 mM HCl, 300 V potential difference, and 5 min of EMI time. In this case, the model analytes were transferred to the acceptor solution with 70–79% recoveries (Table 4). Since the model analytes were transferred from a 300 μl sample volume to a 30 μl acceptor phase solution, the corresponding enrichment values (E) ranged between 7.0 and 7.9 (Table 4).

To evaluate the practical applicability of the proposed EMI technique, repeatability and linearity were investigated utilizing standard solutions of the model analytes in 10 mM HCl. In a first experiment, the repeatability was studied ($n = 6$) at two different concentration levels (100 and 1000 ng/ml). As illustrated in Table 4, relative standard deviations were in the range 4.6–10.5% at the 100 ng/ml level, and in the range 5.4–16.0% at the 1000 ng/ml level. The repeatability was acceptable and comparable with values reported for miniaturized analytical extraction procedures [27]. In a second experiment, linearity was investigated in the concentration range 100 ng/ml–2.5 μg/ml. Excellent linearity was obtained with r^2-values ranging from 0.9999 to 0.9988.

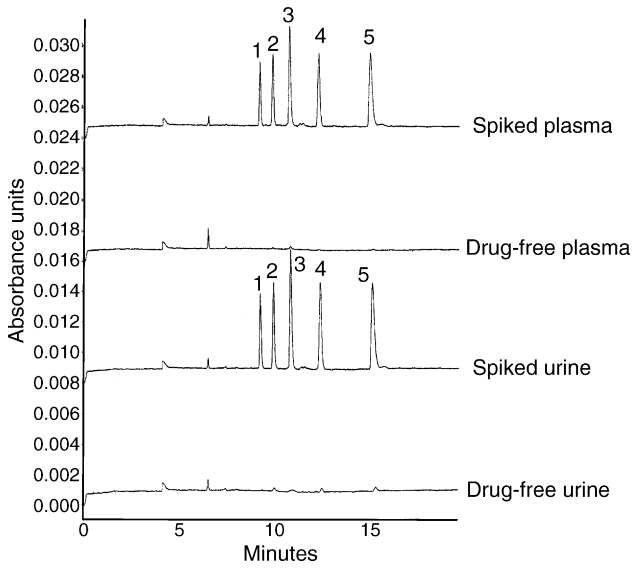

Fig. 6. EMI from human plasma and urine. All drugs were present at 1 μg/ml: (1) pethidine; (2) nortriptyline; (3) methadone; (4) haloperidol; and (5) loperamide.

As a final system check, EMI of 1 μg/ml of methadone was accomplished from 1) pure 10 mM HCl, 2) 10 mM HCL containing 10 μg/ml pethidine, and 3) 10 mM HCl containing 2% (w/w) NaCl. The corresponding recovery values for methadone were 77, 80, and 83%. Within the experimental inaccuracies of the experiment, EMI of methadone was not found to be affected by the presence of the aforementioned matrix components.

3.8. Experiences with biological samples

To finish the current evaluation of EMI, the model analytes were spiked into samples of human blood plasma and urine. In both cases, 100 μl of biological fluid was mixed with 200 μl of 15 mM HCl to give a final concentration of 10 mM HCl in the sample compartment. Subsequently, the samples were subjected to EMI with the same conditions as reported above. Fig. 6

Table 4
EMI performance and validation data

	Pethidine	Nortriptyline	Methadone	Haloperidol	Loperamide
Recovery (%)	70	70	79	72	76
Enrichment	7.0	7.0	7.9	7.2	7.6
Repeatability ($n = 6$) 100 ng/ml	9.5	6.0	4.6	5.5	10.5
Repeatability ($n = 6$) 1000 ng/ml	9.3	16.0	13.1	10.1	5.4
Linearity (r^2) 100–2500 ng/ml	0.9999	0.9992	0.9992	0.9988	0.9996

demonstrates the electropherograms obtained after analysis of the resulting acceptor solutions. Clearly, the model analytes were effectively isolated even from the biological samples, and the system was found to be compatible with the complicated samples. The results from urine were comparable with similar results from pure sample solutions of 10 mM HCl. Thus, in the case of urine, the sample matrix was found not to affect the recoveries obtained. Also for plasma, recoveries were comparable with similar results from pure sample solutions of 10 mM HCl, except for nortriptyline where plasma experiments resulted in slightly lower recoveries. Most probably, this arose from the protein binding of the drug.

Interestingly, very few matrix components were observed in the corresponding electropherograms of drug-free plasma and urine. This supported that the majority of endogenous substances were effectively discriminated or blocked by the artificial liquid membrane, and in turn suggests that EMI may be a highly selective sample preparation method producing very clean extracts.

4. Conclusion

The present work has for the first time demonstrated that electrokinetic migration across thin artificial liquid membranes may be a very powerful concept for isolation, enrichment, and clean-up of drug substances from complicated biological samples. This technique has been named electro membrane isolation (EMI). Compared with passive diffusion, electrokinetic migration appeared to be a much more efficient transport mechanism, providing high analyte recoveries in very short time. EMI was found to be compatible with complicated biological samples like human plasma and urine, and preliminary validation data supported that this concept may be utilized as a sample preparation technique for analytical measurements. Work is in progress to evaluate EMI for other types of analytes including biochemical substances, to develop new types of artificial liquid membranes, to extensively validate the concept, and to develop a clear theoretical understanding of the mechanisms involved. Also, work is in progress to develop other technical formats of EMI as the current version is very preliminary. With these efforts, EMI may become a future sample preparation method providing very rapid, simple, and selective isolation of chemical and biochemical substances from complicated samples with almost no consumption of organic solvents.

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