Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 998 (2003) 61–72
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Liquid-phase microextraction of hydrophilic drugs by carrier-mediated transport

Tung Si Ho*, Trine Grønhaug Halvorsen, Stig Pedersen-Bjergaard, Knut Einar Rasmussen
School of Pharmacy, University of Oslo, P.O. Box 1068 Blindern, N-0316 Oslo, Norway

Received 4 April 2003; accepted 11 April 2003

Abstract

Basic studies on carrier-mediated transport as a mechanism to extract polar drugs by hollow fibre-based liquid-phase microextraction are presented for the first time. Hydrophilic alkaline drugs with log *P* (octanol/water partition coefficient) values less than 1 were selected as model substances. Sodium octanoate served as carrier and was added to the sample solution at pH 7 to form hydrophobic ion-pair complexes with the analytes. The ion-pair complexes were extracted into octanol as liquid membrane immobilised in the pores of the hollow fibre. Further extraction into an aqueous acceptor phase inside the lumen of the hollow fibre was facilitated by counter transport of protons from the acceptor solution to the sample solution. Protons from the acceptor solution released the analytes at the liquid membrane–acceptor interface and neutralized the carrier. The acceptor phase was analysed by capillary electrophoresis. The studies show that high extraction recoveries of ionic hydrophilic drugs can be obtained at a sample–acceptor volume ratio of 10. Linear calibration graphs and clean electropherograms indicate that carrier-mediated transport is a promising technique in microextraction of polar drugs from biological matrices.

Keywords: Liquid-phase microextraction; Extraction methods; Carrier-mediated transport; Sodium octanoate; Amphetamine; Morphine; Practolol

1. Introduction

Liquid–liquid extraction (LLE) has for many decades been a widely used and accepted sample preparation method for the analysis of drugs in biological fluids such as urine and plasma. Although LLE offers high reproducibility and high sample capacity it is considered to be a time- and labour-intensive procedure with a tendency for emulsion formation and with a poor potential for automation. In addition LLE requires large amounts of high purity solvents which are expensive and toxic and results in the production of hazardous laboratory waste.

Miniaturisation can prove a solution to the above-mentioned problems. In the last few years' efforts to miniaturise the LLE extraction has been directed towards reducing the solvent to aqueous phase ratio. This has led to the development of novel solvent microextraction techniques. In single-drop microextraction, which is discussed in a recent review [1],

*Corresponding author. Fax: +47-2285-4402.
E-mail address: tungsho@farmasi.uio.no (T.S. Ho).

0021-9673/03/$ – see front matter
doi:10.1016/S0021-9673(03)00634-4

the extractant phase is a drop of a water immiscible solvent suspended in the aqueous sample. Unfortunately for extraction of complex matrices the single-drop is not stable [2]. In order to develop devices suitable for routine analysis of biological samples Pedersen-Bjergaard and co-workers [3,4] developed a liquid-phase microextraction (LPME) device based on a liquid membrane housed in the pores of a hollow fibre. The device is suitable for both two-phase and three-phase extractions. Two-phase LPME is used to extract charged and neutral substances from aqueous sample solutions into an organic solvent present in the lumen of the hollow fibre. The technique is used to extract analytes from biological fluids [2,5,6] and environmental samples [7] prior to GC and GC–MS analysis. Three-phase LPME allows extraction of ionic analytes from aqueous sample solutions through a liquid membrane into an aqueous acceptor solution inside the hollow fibre. The acceptor solution is injected into HPLC, HPLC–MS or CE without further manipulation. The extraction involves pH adjustment of the sample solution to a pH where the analytes are uncharged. The analytes are extracted through the organic solvent immobilised in the pores of the hollow fibre and into the aqueous acceptor solution in the lumen of the fibre. The acceptor solution has a pH where the analytes are charged preventing them from back diffusion into the organic solvent. Applications involve extraction of drugs from biological fluids such as plasma and urine [3,8–11] and analytes from environmental samples [12–14]. The analytes are typically extracted from 0.5 to 5 ml of sample solution into 5–25 μl of acceptor solution. Substantial enrichment and sample clean up has been reported and the use of organic solvents has virtually been eliminated.

Common to the published methods are that the analytes are relatively hydrophobic. The extraction efficiency is governed by the partitioning of the analyte between the sample matrixes and the immobilised solvent and by the partitioning between the acceptor phase and the immobilised solvent. Hydrophobic analytes are easily extracted into organic solvents from aqueous sample solutions. In addition hydrophobic ionic analytes have large solubility differences in acidic and basic aqueous solutions. Consequently hydrophobic ionic analytes are extracted well into the aqueous acceptor phase in the three-phase system with recoveries of 60–80% [8–11]. On the other hand, polar analytes have low solubility in water immiscible organic solvents and small differences in their solubility in acidic and basic aqueous solutions. Polar analytes are therefore difficult to extract by three-phase LPME. The ability of LPME to exclude extraction of polar analytes contributes to the selectivity of the method.

Carrier-mediated transport allows extraction of ionic analytes from an aqueous solution through a liquid membrane and into an aqueous acceptor solution. The carrier forms lipophilic complexes with the analytes that facilitates transport through the liquid membrane. Carrier-mediated transport has been frequently used in industrial applications for the removal of metal ions from wastewater. There is also an increasing use of carrier-mediated transport to recover pharmaceutical products from fermentation broths and reaction mixtures [15]. Applications include carrier-mediated liquid membrane extraction of polar acidic substances such as penicillin G [16,17], cephalosporin-C [18], cephalexin [19] and lactic acid [20]. Amines such as Aliquat-336, Amberlite LA-2 and tri-*n*-octylamine (TOA) were used as carriers to provide facilitated transport via ion-pairing mechanism. The carrier was dissolved in the membrane phase, which separated the aqueous feed and strip solutions. The feed and strip solutions were continuously pumped through the extraction unit.

Only a few applications on carrier-mediated transport in analytical-scale extractions have been reported. These are based on a supported liquid membrane device with a flowing donor solution and a stagnant acceptor solution. The carrier is dissolved in the membrane phase. Amino acids were extracted as cations with anionic carriers such as di-2-ethylhexyl phosphoric acid [21] and dodecylbenzenesulphonic acid [22]. Anionic analytes such as glyphosat metabolites were extracted with Aliquat 336 as cationic carrier [23,24]. Ionic carriers are associated with counter-ions to maintain electro neutrality in the apolar membrane phase. The transport mechanism is counter-coupled transport in which the driving force of mass transport over the membrane is created by the gradient of counter ion from the acceptor to the donor phase.

In this study, we outline for the first time the

possibility of using carrier-mediated transport to extract polar drugs from biological fluids in the three-phase LPME concept. Cationic hydrophilic drugs with log *P* (octanol/water partition coefficient) values below 1 were selected as model substances. Different carboxylic acids were evaluated as carriers via ion-pairing mechanism. The carriers were added to the sample solution to form hydrophobic ion-pair complexes that were extracted into the liquid membrane and the parent drugs were recovered in the aqueous acceptor phase. The effect of carrier concentration, pH of the donor phase, the nature of the immobilised organic phase and the acceptor phase and the phase ratio were studied using aqueous sample solutions. Finally, the model drugs were extracted from biological matrices such as plasma and urine.

2. Experimental

2.1. Chemicals

Amphetamine, morphine, and practolol were obtained from Norsk Medisinaldepot (Oslo, Norway). Sodium hexanonate, sodium octanoate, sodium decanoate, octanoic acid, 1-hexanol, 1-octanol, and 1-decanol were obtained from Sigma (St. Louis, MO, USA). Sodium hydroxide, sodium chloride, hydrochloric acid, ortho-phosphoric acid, disodium hydrogen phosphate, 1-nonanol, and methanol were obtained from Merck (Darmstadt, Germany). Formic acid was obtained from Riedel-de Haen (Hannover, Germany). 1-Pentanol and 1-dodecanol were obtained from Fluka (Buchs, Switzerland). Drug-free plasma was obtained from Ullevål University Hospital (Oslo, Norway).

2.2. Standard solutions and biological samples

All solutions were prepared from 1 mg/ml stock solutions of amphetamine, morphine, and practolol in methanol. Test solutions of the drugs in pure water were obtained by direct dilution of the above-mentioned stock solutions to a drug concentration of 1 μg/ml. Plasma and urine samples containing 16 μg/ml of each of amphetamine, morphine, and practolol were prepared by dilution of the stock solutions with drug-free plasma and drug-free urine, respectively. All solutions were stored at 5 °C protected from light.

2.3. Capillary electrophoresis

CE was performed with a MDQ instrument (Beckman, Fullerton, CA, USA) equipped with a UV detector. Separations were accomplished in a 60 cm (effective length 50 cm)×75-μm I.D. fused-silica capillary (BGB Analytik, Anwil, Switzerland) utilising a 25 m*M* phosphate buffer adjusted to pH 2.75 as the CE running buffer. The instrument was operated at 25 kV. All samples were introduced by hydrodynamic injection at 0.5 p.s.i. for 5 s (1 p.s.i.= 6894.76 Pa). Detection was accomplished at 200 nm utilizing an 800×100-μm slit.

2.4. LPME equipment

LPME was carried out either in conventional 2- or 4-ml sample vials (Supelco, Bellefonte, PA, USA) equipped with a screw cap and a silicon septum. Two conventional 0.8-mm O.D. medical syringe needles of steel were inserted through the silicon septum in the screw cap, and the two ends were connected to each other by either a 6.5- or 8.0-cm piece of Q3/2 Accurel KM polypropylene hollow fibre (Membrana, Wuppertal, Germany). One medical syringe needle served to introduce the acceptor phase to the lumen of the hollow fibre prior to extraction, whereas the other needle was utilised for collection of the acceptor phase after extraction. The 6.5-cm pieces of fibre were utilized in connection with 2-ml sample vials, whereas 8.0 cm was used with the 4-ml vials. The inner diameter of the hollow fibre was 600 μm, the thickness of the wall was 200 μm, and the pore size was 0.2 μm. The internal acceptor phase volume was either 20 or 25 μl with the 6.5- and 8.0-cm fibres, respectively, whereas the corresponding volumes of organic phase immobilised in the pores were approximately 18 and 23 μl. During extraction, the sample vials were vibrated at 1500 rpm using a Vibramax 100 (Heidolph, Kelheim, Germany).

2.5. LPME procedure

Extractions were normally carried out in 4-ml vials according to following procedure; a 2-ml sample solution was filled into a vial followed by 2 ml of 50 m*M* ion-pair reagent dissolved in 25 m*M* phosphate buffer adjusted to pH 7.0. The hollow fibre was dipped for 5 s in 1-octanol followed by ultrasonification for 15 s in a water bath to remove excess of the solvent. Subsequently, 25 μl of 50 m*M* HCl (acceptor phase) was injected into the lumen of the hollow fibre with a micro syringe. The fibre was placed in the 4-ml vial, and during the 45-min of extraction, the sample vials were vibrated at 1500 rpm. After extraction, the total volume of acceptor phase (25 μl) was flushed into a 200-μl micro insert (Beckman) for the CE instrument by application of a small head pressure on the medical steel needle used for sample introduction, and finally the extract was analysed by CE.

2.6. Calculations

The $\log P$ and the pK_a values of the compounds were calculated with ACD/Lab software manufactured by Advanced Chemistry Development (Canada). The phase ratio (PR) between the sample solution and the acceptor solution was calculated according to the following formula:

$$\mathrm{PR} = V_d/V_a \quad (1)$$

where V_d is the volume of donor phase (volume of diluted sample) and V_a is the volume of acceptor phase. The extraction recovery (R) was calculated according to the following formula:

$$R = \frac{n_{a,final}}{n_{s,initial}} \times 100\% = (V_a/V_s) \times (C_{a,final}/C_{s,initial}) \times 100\% \quad (2)$$

where $n_{s,initial}$ and $n_{a,final}$ are the number of mole of analyte originally present in the sample and the number of mole of analyte finally collected in the acceptor solution, respectively. V_a is the volume of acceptor phase (same as used in Eq. (1)), V_s is the volume of undiluted 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. In cases where an internal standard was used, $C_{a,final}$ was determined by correcting the peak area of the drug of interest with the peak area of the internal standard.

The concentration enrichment (E) was calculated by the following formula:

$$E = C_{a,final}/C_{s,initial} = V_s R/100V_a$$

3. Results and discussion

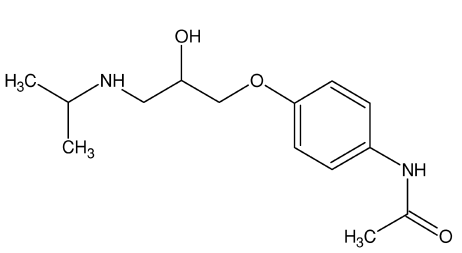
3.1. Theoretical considerations

The primary purpose of three-phase LPME is to provide enrichment and clean up of drugs from biological fluids, prior to HPLC, LC–MS and CE. The aqueous acceptor phase is injected directly into the analytical instrument without further manipulation. An acceptor solution free from substances that might interfere with the analysis or cause ion suppression in LC–MS is of particular importance. These considerations affect the selection of ion-pair carrier and demand a carrier that is insoluble in the acceptor solution. This carrier should bear a functional group with a charge opposite to the charge of the transported molecule. Such a carrier facilitates the analyte passing over the liquid membrane by formation of a neutral ion-pair complex soluble in organic solvents. Prior studies on ion-pair facilitated transport in large scale extractions are based on hydrophobic carriers, which are dissolved in the immobilised membrane phase. The sample solution is pumped through the narrow channels of the extraction units were it is brought in close contact with the membrane liquid to facilitate extraction.

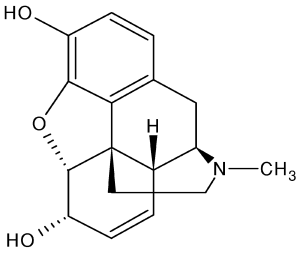
Close contact with the sample solution and the membrane cannot be achieved in the LPME device, which is based on stagnant donor and acceptor phases. The contact area is small because of the large sample to organic solvent volume ratio. These conditions are unfavourable for extraction. Carrier-mediated transport is carried out at a pH in the sample solution where both the analyte and the carrier is charged. Under these conditions ionic hydrophilic analytes are highly water-soluble and have a limited tendency to migrate towards a lipophilic membrane. Initial attempts in our laboratory to extract charged

analytes in the LPME device through a liquid membrane with a dissolved ionic carrier gave low recoveries. Therefore, the approach of adding the carrier directly to the sample solution was investigated. Practolol and morphine were selected as hydrophilic model substances. For comparison amphetamine was selected as a lipophilic model substance because it is easily extracted by passive diffusion. Several acidic substances were tested as carriers. The structure, pK_a values and log P values of the substances are shown in Fig. 1.

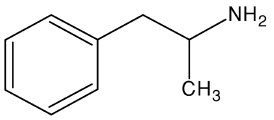
Carrier-mediated transport through a liquid membrane depends on a number of parameters such as the nature and concentration of the carrier, partition coefficient of the ion-pair complex, properties of the membrane liquid, nature and concentration of counter ions, diffusion coefficients, vibration speed, temperature, etc. A simplified working model for the extraction is shown in Fig. 2. The extraction is separated into three independent steps. The first step involves extraction of analyte from the sample solution to the membrane phase. The sample solution is added to buffer solution and the carrier. The buffer adjusts the pH of the sample solution to a pH where the analytes and the carrier are charged to promote formation of a hydrophobic ion-pair complex. In this experiment the sample solution has a pH of 7. The next step is extraction of the ion-pair complex into the membrane phase and diffusion of the complex through the membrane. The third step involves desorption of analyte to the acceptor phase. At the interface between the organic phase and the acceptor



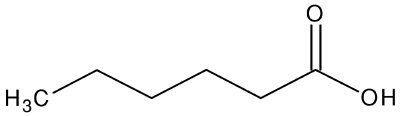
Practolol
Log P = 0.79
pK_a = 9.17



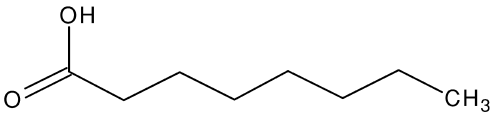
Morphine
Log P = 0.89
pK_a = 8.21



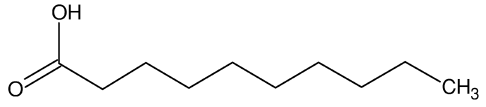
Amphethamine
Log P = 1.81
pK_a = 9.94



Hexanoic acid
Log P = 1.84
pK_a = 4.78



Octanoic acid
Log P = 2.90
pK_a = 4.78



Decanoic acid
Log P = 3.97
pK_a = 4.79

Fig. 1. Structure of the tested substances and their pK_a and log P values.

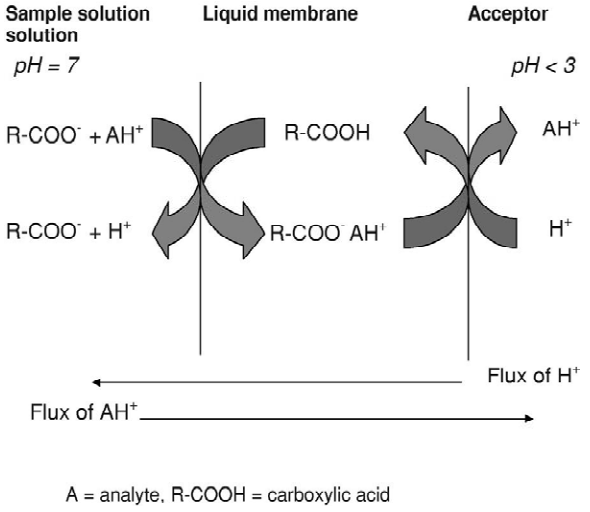


Fig. 2. Working model for carrier-mediated extraction.

phase the carrier releases the analyte by counter ion-exchange. The acceptor solution has an acidic pH and protons are used as counter ions. The protons replace the analytes and neutralize the carrier. The carrier diffuses to the sample side of the membrane, dissolves in the sample solution and repeats the cycle. This process occurs because the carrier is insoluble in the acidic acceptor solution. The analytes are trapped as salts in the acidic acceptor solution and a large excess of protons prevents them from being back extracted into the membrane phase.

In order to test the working model several experiments were carried out. The initial experiments were based on mixing 2 ml of the working solution containing the 1 μg/ml of analytes with 2 ml of phosphate buffer containing potential carriers. The volume of acceptor solution was 25 μl and the sample/acceptor phase ratio was 160. The LPME device was in all experiments vibrated for 45 min.

3.2. Basic experiments

Initially the test substances were extracted by passive diffusion of the analytes from the sample solution adjusted to pH 13 and 7 in respective experiments, through octanol immobilised in the pores of the hollow fibre and into hydrochloric acid as acceptor solution. The recoveries are summarized in Table 1. As expected amphetamine was extracted well at pH 13 while insignificant extraction occurred at pH 7 where the analyte was ionized. The recoveries of practolol and morphine were in both cases 1–3%. When sodium octanoate was added to the sample solution at pH 7 a significant increase in recovery occurred. The recoveries of practolol and morphine were 9 and 11%, respectively. Amphetamine was extracted with a recovery of 42%. This experiment was a first indication of successful carrier-mediated transport in LPME and prompted further optimization. Table 1 also show that the recoveries obtained after dissolution of octanoic acid as carrier in the membrane phase gave significantly lower recoveries than dissolution of sodium octanoate in the sample solution. This support the statement mentioned under theoretical considerations that the presence of the carrier only in the organic phase is insufficient to promote extraction because of

Table 1
Basic experiments

Additions to 2 ml of test solution	Organic phase	Recovery (%)[a]		
		Amphethamine	Morphine	Practolol
250 μl 2 *M* NaOH + 1.75 ml water	1-Octanol	77	2	3
2 ml 25 m*M* phosphate buffer, pH 7.0	1-Octanol	3	2	1
2 ml of 50 m*M* sodium octanoate in 25 m*M* phosphate buffer, pH 7.0	1-Octanol	42	11	9
2 ml 25 m*M* phosphate buffer, pH 7.0	1-Octanol containing 5% octanoic acid	22	3	3

Test solution: 1 μg/ml of amphethamine, morphine and practolol in water.
Acceptor phase: 25 μl of 50 m*M* HCl.
[a] RSD ($n = 3$) < 10%.

the low contact area between the sample and the organic phase.

3.3. Modification of the sample solution

The first step in method optimization was modification of the sample solution. Modification of the sample solution includes selection of a suitable anionic carrier to be added to the sample solution, optimization of carrier concentration and optimisation of the pH. The selection of carrier is of vital importance for the success of the method. First of all the carrier must be easily soluble in the sample solution. It should form neutral ion-pair complexes with the analytes and be sufficiently hydrophobic to promote extraction of the ion-pair complex into the membrane phase. The ion-pair complex should be stable upon migration through the membrane and the analytes should be easily released from the ion-pair complex at membrane–acceptor interface. Different carboxylic acids were tested as they are insoluble in acidic acceptor phases and do not pollute the final extract. All acids have pK_a values below 5 and they are fully ionized at neutral pH in the sample solution. The acids were dissolved as sodium salts in 50 m*M* phosphate buffer, pH 7. The final concentration of carrier in the sample solution was 25 m*M*. Sodium hexanonate is well dissolved in the sample solution and it has the lowest log *P* value. The recoveries of morphine and practolol were only 1–2%. The hydrophobicity of hexanoic acid is probably too low to promote formation of hydrophobic ion-pair complexes. The highest recovery was obtained with sodium octanoate. Sodium octanoate is sufficiently soluble at neutral pH and it has a suitable hydrophobicity. The major problem using sodium decanoate is its low solubility at neutral pH. Precipitation was observed during extraction and the recoveries were low. Further optimisation was therefore focused on sodium octanoate as anionic carrier.

Ion-pair formation is dependent on the concentration of counter ion in the sample solution and extractions were performed in the range of 1–75 m*M* of sodium octanoate in the final sample solution. Fig. 3A shows that a concentration of sodium octanoate of 25 m*M* in the sample solution gave the highest recovery. At higher concentrations of sodium octanoate solubility problems occurred. The acceptor

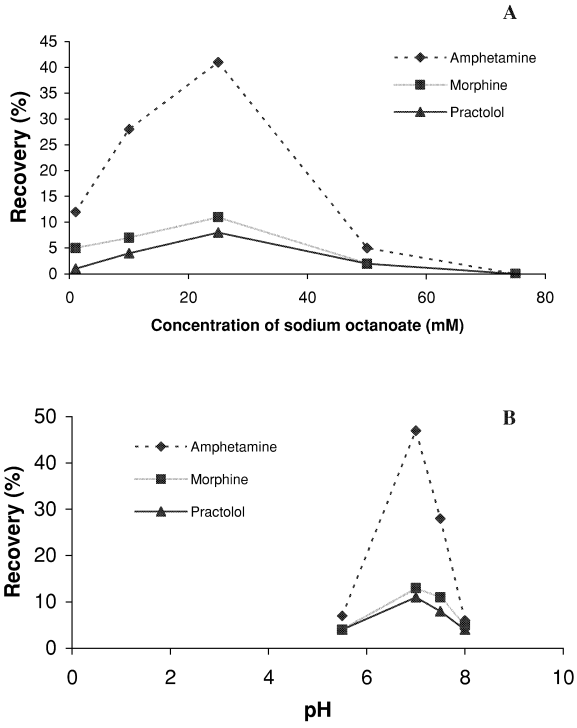


Fig. 3. Influence of the concentration of sodium octanoate (A) and pH (B) on the extraction recovery. Relative standard deviations were within 10% for the data points.

solution became whitish probably because of precipitation of octanoic acid, and a rapid loss of recovery was observed. Fig. 3B shows recoveries with 25 m*M* sodium octanate at different pH values in the range of 5.5–8.0 in the sample solution. The decreased recoveries at pH above 7.0 are due to deprotonation of the analytes while decreased recoveries at a pH below 7.0 are due to protonation of the carrier. These conditions reduce ion-pair formation. Further experiments were conducted at pH 7.0 in the sample solution added sodium octanoate to a final concentration of 25 m*M*.

3.4. Liquid membranes

Several factors have to be considered when choosing organic solvents to be immobilised as liquid membranes. The ion-pair complexes formed in the sample solution should have high partition coefficients into the membrane. Other substances present in the sample solution which may interfere with the

analysis, should have low partition coefficients. Solvents of low viscosity are preferred as low viscosity provides large diffusion coefficients and high flux through the membrane. In addition the water solubility should be as low as possible and the solvent should have a high boiling point to avoid evaporation during handling. In previous work on LPME solvents such as dihexyl ether or 1-octanol have been preferred. In general, dihexyl ether was shown to provide higher recoveries than octanol in the extraction of hydrophobic drugs by passive diffusion. In this study octanol showed higher recoveries than dihexyl ether, and different alcohols were screened. The recoveries are presented in Table 2. The highest extraction recoveries were obtained from octanol and nonanol as organic phases. The higher water solubility and polarity of pentanol and hexanol is unfavourable. The partitioning of ion-pair complexes into the higher alcohols is probably low because of too high hydrophobicity of these substances. Further studies were therefore focused on octanol as solvent.

3.5. Composition of the acceptor phase

The transport mechanism in the extraction is counter-coupled transport in which the driving force of mass transport over the membrane is created by the gradient of counter ion from the acceptor to the donor phase. Protons act as counter ions in this experiment. Strong acids added to the acceptor solution, produces a large excess of protons which replace the analyte from the ion-pair complex at the membrane–acceptor interface and neutralize the carrier. The carrier diffuses back across the liquid membrane, dissolves in the sample solution and repeats the process. The analytes are trapped as salts in the acceptor phase. A large excess of protons is necessary in order to prevent the analytes from ion-pair formation with the carrier and back extraction into the membrane phase.

Acceptor solutions made up of different concentrations of hydrochloric acid and formic acid were studied. Formic acid has the advantage of being LC–MS friendly and acceptor phases made of formic acid can be injected directly into LC–MS. Extraction recoveries are shown in Fig. 4. Extractions into pure water as acceptor phase failed because proton concentration is too low to provide counter ion-exchange. The recovery is dependent on the proton concentration and Fig. 4A shows that an optimal recovery was obtained with 50 m*M* HCl as acceptor phase. Formic acid which has a pK_a value of 3.8, is

Table 2
Influence of organic solvent on the extraction recovery

Organic phase	Recovery (%)[a]		
	Amphethamine	Morphine	Practolol
1-Pentanol	0	0	0
1-Hexanol	1	1	1
1-Octanol	42	11	9
1-Nonanol	43	8	8
1-Decanol	32	5	4
1-Dodecanol	13	1	1

Sample solution: 2 ml of test solution added 2 ml of 50 m*M* sodium octanoate in 25 m*M* phosphate buffer, pH 7.0.
Acceptor phase: 25 μl 50 m*M* HCl.
[a] RSD ($n=3$)<10%.

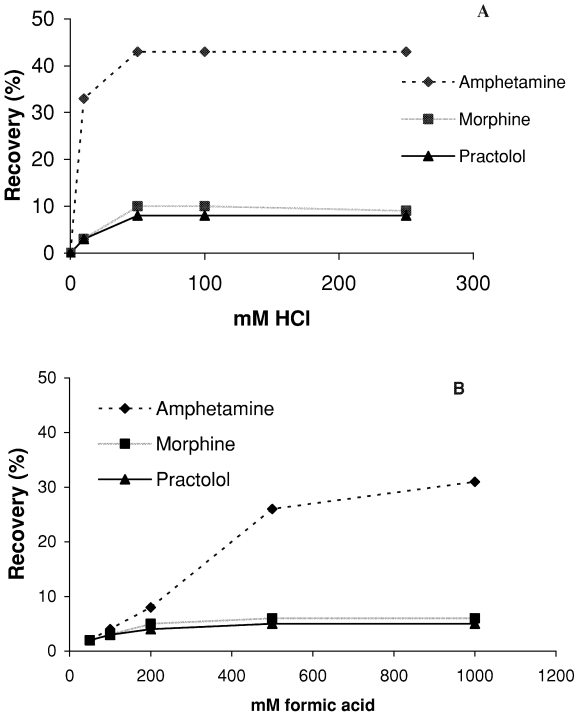


Fig. 4. Influence of the concentration of hydrochloric and (A) and formic acid (B) on the extraction recovery. Relative standard deviations were within 10% for the data points.

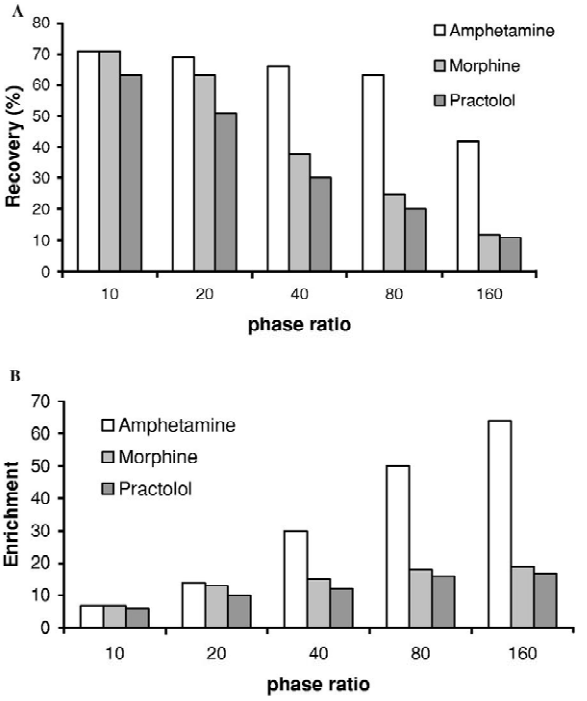


Fig. 5. Influence of the sample–acceptor phase ratio on the extraction recovery (A) and the concentration enrichment (B). Relative standard deviations were within 10% for the data points.

much weaker acid than hydrochloric acid and the recoveries were lower as shown in Fig. 4B.

3.6. Extractions at different phase ratios

All preliminary studies have been conducted at a sample–acceptor phase ratio of 160. The average recoveries using 50 m*M* HCl as acceptor phase were 42% for amphetamine, 11% for morphine and 9% for practolol. These recoveries follows the log *P* values of the analytes, amphetamine has the largest log *P* value while practolol have the lowest log *P* value. The recoveries correspond to concentration enrichments from the sample solution to the acceptor solution of 67 for amphetamine, 18 for morphine and 14 for practolol. Although the recoveries for morphine and practolol were low, high concentration enrichments were achieved.

In extractions based on passive diffusion the recovery is based on the sample–membrane and the membrane–acceptor partition coefficients and on the phase ratios. The recovery increases with decreasing sample–acceptor phase ratios. The concentration enrichment is dependent on the recovery and on the sample–acceptor phase ratio. To study the recoveries and enrichments in extractions based on carrier-

Table 3
Recoveries after extractions of plasma and urine

Sample solution	Recovery (%)[a]		
	Amphethamine	Morphine	Practolol
100 μl of plasma added 100 μl of 50 m*M* sodium octanoate in 25 m*M* phosphate buffer, pH 7.0	71	57	45
100 μl of urine added 100 μl of 50 m*M* sodium octanoate in 25 m*M* phosphate buffer, pH 7.0	32	34	30
100 μl of urine added 100 μl of 50 m*M* sodium octanoate in 100 m*M* phosphate buffer, pH 7.0	27	52	46

Organic phase: 1-octanol.
Acceptor phase: 20 μl of 50 m*M* HCl.
[a] RSD ($n=3$) $<10\%$.

mediated transport, extractions at sample–acceptor phase ratios in the range of 10–160 were investigated. The results are shown in Fig. 5. The recoveries increase strongly by reducing the phase ratio. This could be explained by a decreased diffusion distance for the analytes as the volume of sample decrease. At a phase ratio of 10, the recoveries of morphine and practolol increased to 73 and 63%, respectively. The recovery in extracting amphetamine by carrier-mediated transport was similar to that obtained by extracting amphetamine by passive diffusion. The concentration enrichments decrease with decreasing phase ratios and were about 7 at a phase ratio of 10 for all test substances. These results show that carrier-mediated transport can be used to extract polar drugs with high recoveries and acceptable enrichments at low phase ratios. Extractions at a phase ratio of 10 with 500 m*M* formic acid as acceptor phase showed a recovery of 58% for amphetamine, 27% for morphine and 25% for practolol. These results show that more studies on LC–MS friendly acceptor phases are necessary to increase their extraction recovery.

3.7. Extractions of biological matrices

The previous experiments were based on extractions of the test substances dissolved in pure water and in a final experiment the test substances were extracted from human urine and plasma. The extractions were carried out at a phase ratio of 10 by adding 100 μl of 25–100 m*M* phosphate buffer, pH 7.0, containing 50 m*M* sodium octanoate to 100 μl of urine and plasma. Table 3 shows recoveries from plasma of 71, 57 and 45% for amphethamine, morphine and practolol, respectively. The recoveries are slightly lower than those obtained from extractions of the water solution. The recoveries from urine were dependent on the buffer capacity. Table 3 shows that the recoveries for morphine and practolol increased as a function of buffer strength and optimal recoveries were obtained after addition of 100 m*M* phosphate buffer containing 50 m*M* sodium octanoate. At these conditions no significant difference in the recoveries of morphine and practolol from plasma and urine was observed. The recovery of amphetamine from urine was lower than the recovery from plasma and was virtually unaffected by the buffer concentration.

Figs. 6 and 7 show electropherograms of extracts of 100 μl of drug-free plasma and urine and electropherograms of 100 μl of plasma and urine containing 16 μg/ml of the test substances. Both matrices contain a large variety of substances that might interfere in the analysis. However, clean electropherograms with no interfering substances were seen. The quantitative aspect of carrier-mediated transport was investigated by studying the linearity of calibrations graphs from plasma and urine. The concentration range studied was 1–25 μg/ml. A volume of 100 μl of plasma and urine was extracted. The sample–acceptor volume ratio was 10. Calibration graphs were set up by plotting the peak areas against the concentration of test substance in

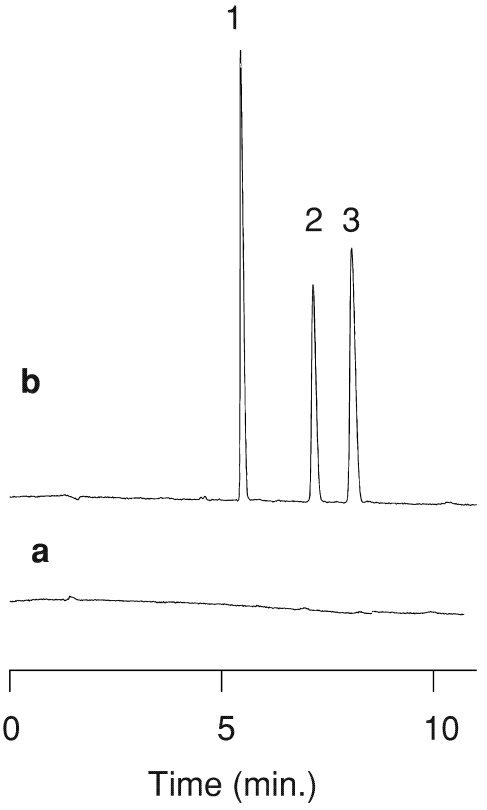


Fig. 6. Electropherograms of 100 μl of drug-free human plasma (a) and 100 μl of human plasma (b) spiked with 16 μg/ml of amphetamine (1), morphine (2) and practolol (3). For experimental conditions see text.

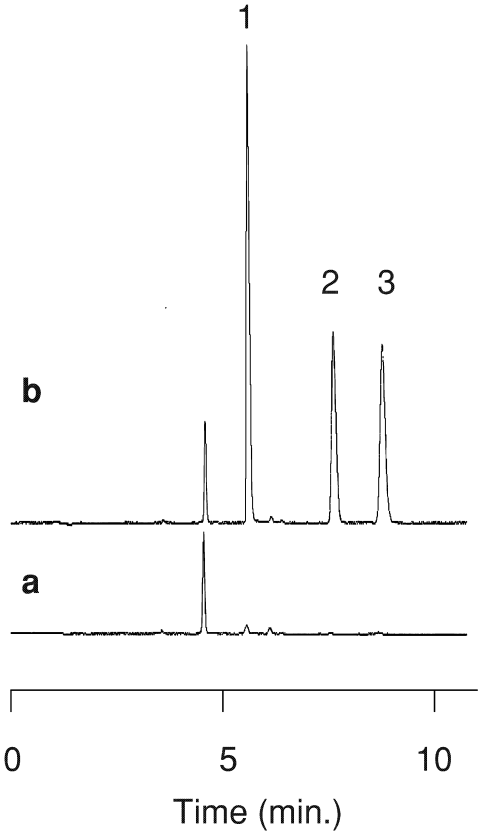


Fig. 7. Electropherograms of 100 μl of drug-free human urine (a) and 100 μl of human urine (b) spiked with 16 μg/ml of amphetamine (1), morphine (2) and practolol (3). For experimental conditions see text.

plasma and urine. The calibration graphs were linear with regression coefficients in the range of 0.9985–0.9987 for urine and 0.9957–0.9975 for plasma. The linear calibration graphs indicate that LPME based on carrier-mediated transport can be used in analytical extractions of polar analytes from biological matrices.

4. Concluding remarks

This is the first report on carrier-mediated transport as a mechanism to extract polar drugs in the stagnant LPME mode. The analytes were extracted as ion-pair complexes into octanol as membrane phase from neutral sample solutions added sodium octanoate as carrier. At the liquid membrane–acceptor interface the analytes are released from the ion-pair complex by counter ion-exchange. Protons in the acceptor solution served as counter ions. After counter ion-exchange the neutral octanoic acid diffuses back to membrane–sample interface, dissolves in the sample solution and repeats the extraction cycle. The analytes are trapped in the acceptor solution where a large excess of protons prevents them from back extraction into the membrane phase. Successful extraction is dependent on the properties and the concentration of carrier added to the sample solution, the pH of the sample solution, the nature of the liquid membrane and on the concentrations of protons in the acceptor phase. The recoveries are dependent on the sample–acceptor phase ratio and phase ratios of 10 provides recoveries similar to those obtained in extractions of hydrophobic drugs by passive diffusion. The concentration enrichments decreases with decreasing phase ratio and were about 7 at a phase ratio of 10. The results show that the model substances were successfully extracted from biological matrices such as urine and plasma. Electropherograms of extracts from human plasma and urine were free from interfering substances and calibrations graphs were linear. The results show that LPME based on carrier-mediated transport has potential to be a valuable method in the analysis of polar drugs from biological matrices.

References

[1] E. Psillakis, N. Kalogerakis, Trends Anal. Chem. 21 (2002) 53.
[2] K.E. Kramer, A.R.J. Andrews, J. Chromatogr. B 760 (2001) 27.
[3] S. Pedersen-Bjergaard, K.E. Rasmussen, Anal. Chem. 71 (1999) 265.
[4] K.E. Rasmussen, S. Pedersen-Bjergaard, M. Krogh, H.G. Ugland, T. Grønhaug, J. Chromatogr. A 873 (2000) 3.
[5] H.G. Ugland, M. Krogh, K.E. Rasmussen, J. Chromatogr. B 749 (2000) 85.
[6] L. de Jager, A.R.J. Andrews, Anal. Chim. Acta 458 (2002) 311.
[7] G. Shen, H.K. Lee, Anal. Chem. Anal. Chem. 74 (2002) 648.
[8] S. Pedersen-Bjergaard, K.E. Rasmussen, Electrophoresis 21 (2000) 579.
[9] T.G. Halvorsen, S. Pedersen-Bjergaard, K.E. Rasmussen, J. Chromatogr. B 760 (2001) 219.

[10] T.G. Halvorsen, S. Pedersen-Bjergaard, K.E. Rasmussen, J. Chromatogr. A 909 (2001) 87.
[11] S. Andersen, T.G. Halvorsen, S. Pedersen-Bjergaard, K.E. Rasmussen, J. Chromatogr. A 963 (2002) 303.
[12] L. Zhu, L. Zhu, H.K. Lee, J. Chromatogr. A 924 (2001) 407.
[13] L. Zhao, L. Zhu, K.K. Lee, J. Chromatogr. A 963 (2002) 239.
[14] L. Zhu, K.H. Ee, L. Zhao, H.K. Lee, J. Chromatogr. A 963 (2002) 335.
[15] R. Basu, K.K. Sirkar, J. Membr. Sci. 75 (1992) 131.
[16] Z. Lazarova, B. Syska, K. Schügerl, J. Membr. Sci. 5202 (2002) 151.
[17] R.-S. Juang, S.-H. Lee, R.-C. Shiau, J. Membr. Sci. 146 (1998) 95.
[18] G.C. Sahoo, N.N. Dutta, N.N. Dass, J. Membr. Sci. 157 (1999) 251.
[19] G.C. Sahoo, A.C. Gosh, N.N. Dutta, Proc. Biochem. 32 (1997) 265.
[20] R.-S. Juang, R.-H. Huang, R.- Rzong Wu, J. Membr. Sci. 136 (1997) 89.
[21] P. Wierczorek, K.Å. Jönsson, L. Mathiasson, Anal. Chim. Acta 346 (2001) 191.
[22] P. Wieczorek, J. Membr. Sci. 127 (1997) 87.
[23] P. Dzygiel, P. Wierczorek, J. Chromatogr. A 889 (2000) 93.
[24] P. Dzygiel, P. Wierczorek, J. Sep. Sci. 24 (2001) 561.