# Experiences with Carrier-Mediated Transport in Liquid-Phase Microextraction

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

## Abstract

**Different organic borates, phosphates, sulphates, and carboxylic acids are evaluated as extraction carriers in three-phase liquid-phase microextraction (LPME). Hydrophilic basic drugs form ion-pairs with the carriers and are extracted as ion-pair complexes into an organic liquid membrane of *n*-octanol or peppermint oil immobilized in the pores of a polypropylene hollow fiber. From this point, the basic drugs are released into a 20-µL solution of 50mM HCl placed inside the lumen of the hollow fiber (acceptor solution). Simultaneously, the carrier is neutralized by protons from the acceptor solution (protonated to maintain the charge balance). Both water-soluble and water-insoluble carriers are tested. One promising candidate among the water-soluble carriers is 1-heptanesulfonic acid. This is added to the sample solution to a final concentration of 25mM and served to ion-pair the analytes within the sample solution. Among the less water-soluble candidates, a mixture of di(2-ethylhexyl) phosphate (DEHP) and tris(2-ethylhexyl) phosphate (TEHP) serve as efficient carriers. Ten percent (w/w) of each of DEHP and TEHP are added to the organic liquid membrane, and these carriers principally worked through ion-pairing with the analytes at the interface between the sample solution and the organic liquid membrane. Several carriers are found to be compatible with human plasma samples, and bromthymol blue is particularly efficient in combination with these protein-containing matrices. Following optimization of the conditions for bromthymol blue, including saturation of the plasma samples with sodium sulphate, extraction recoveries between 45% and 75% are obtained for eight model drugs after 60 min of extraction. With bromthymol blue as the carrier, highly acceptable validation data are obtained for phenylpropanolamine and practolol extracted from human plasma.**

## Introduction

For several years, some interest has been focused on miniaturization of analytical liquid–liquid extractions to reduce the consumption of hazardous organic solvents and to improve automation. The first effort in this field of liquid-phase microextraction (LPME) was focused on single-drop microextraction, in which the extracting phase was a microdrop of a water-immiscible organic solvent suspended on the tip of a conventional microsyringe, immersed in an aqueous sample solution (1). Although single-drop microextraction proved to be a simple, inexpensive, fast, and virtually solvent-free sample pretreatment technique, problems with drop stability were often encountered, which has limited the applicability of this concept (2,3).

Recently, a novel microextraction technique was introduced (4,5) in which the microextracting phase (acceptor solution) was placed inside a porous hollow fiber for mechanical protection (hollow-fiber LPME). With this concept, the drop stability problem was eliminated. In hollow-fiber LPME, analytes are extracted from a small volume of a stagnant aqueous sample through an organic solvent impregnated in the pores of the hollow fiber (liquid membrane) and further into an acceptor solution inside the lumen of the hollow fiber. The chemical principle of hollow-fiber LPME is similar to the work on supported liquid membranes (6–10), but the two techniques differ significantly in terms of instrumentation and operation.

In hollow-fiber LPME, the acceptor solution is frequently aqueous, resulting in a three-phase extraction system (three-phase LPME). This system is suitable for ionic compounds with certain hydrophobic properties, as their neutral forms are highly soluble in the organic membrane and their ionic forms are highly soluble in the aqueous acceptor solution. This has been confirmed in several recent papers from different research groups (11–23). However as reported in the literature (19), polar compounds are poorly extracted in the three-phase LPME systems.

In order to enhance the transport of polar drugs through the organic liquid membrane in hollow-fiber LPME and to expand the application area of the technique, the first papers on carrier-mediated LPME of polar drugs were recently published (24,25). In carrier-mediated LPME, a hydrophobic ion-pair reagent (carrier) was dissolved into the sample solution to

* Author to whom correspondence should be addressed: email stig.pedersen-bjergaard@farmasi.uio.no.

form hydrophobic ion-pair complexes with the analytes. The ion-pair complexes were extracted into the organic liquid membrane and immobilized in the pores of the hollow fiber based on passive diffusion. In the contact region of the liquid membrane and the acceptor solution, the analytes were released from the ion-pair complex into the acceptor solution, whereas counter-ions (protons) present in a very high concentration in the acceptor solution ion-paired with the carrier in the contact area, and the new ion-pair complex was partially back-extracted into the sample. Protons were the driving force for the extraction, and a large excess of protons was necessary in order to maintain high extraction recoveries and to prevent the analytes from back-diffusion into the liquid membrane.

The first paper on carrier-mediated LPME, in which the technique was combined with capillary electrophoresis (CE), briefly demonstrated the principle and showed the performance for two polar model drugs with *n*-octanoic acid as carrier (24). In the second work, in which carrier-mediated LPME was combined with LC–MS, more polar drugs were included, and the concept was validated utilizing *n*-octanoic acid as carrier (25). From a method optimization point of view, knowledge on alternative extraction carriers is important, and because most work up to date has been reported with *n*-octanoic acid, the current work was addressed to initial studies of alternative extraction carriers. Special attention was devoted to their compatibility with human plasma samples. One of the most promising ones, namely bromthymol blue, was optimized and validated for quantitative applications.

## Experimental

### Chemicals

Amphetamine, morphine, phenylpropanolamine, and practolol were obtained from Norsk Medisinaldepot (Oslo, Norway). Metaraminol, cimetidine, sotalol, atenolol, sodium octanoate, dihexyl ether, 2-nitrophenyl octyl ether, dodecylacetate, methylorange, heptanoic acid sodium salt, salicylic acid sodium salt, cholic acid sodium salt, valproic acid sodium salt, di(2-ethylhexyl)phosphate, 1-heptanesulfonic acid sodium salt, 1-octanesulfonic acid sodium salt, lauryl sulfate sodium salt, bromthylmol blue sodium salt, methanol, and *n*-octanol were obtained from Sigma (St. Louis, MO). 2-Octanone, silicon oil AR 20, phenylboronic acid, tri-tert-butyl borate, tris(2-ethylhexyl)phosphate, triethyl phosphate, tridecyl phosphate, sodium 2-ethylhexyl sulphate, sodium sulphate, and 1-nonanesulfonic acid sodium salt were obtained from Fluka (Buchs, Switzerland). Hydrochloric acid, ortho-phosphoric acid, disodium hydrogen phosphate, and sodium chloride were obtained from Merck (Darmstadt, Germany). Tributyl phosphate, 1-naphthoic acid, phenylcinnamic acid, 1-naphthalenesulfonic acid, and triphenyl phosphate were obtained from Aldrich (Steinheim, Germany). Peppermint oil was obtained from Tamro (Oslo, Norway). Drug-free plasma was obtained from Ullevål University Hospital (Oslo, Norway). The purities of all the model drugs and carrier reagents exceeded 98%, and the other reagents used were of analytical grade.

### Standard solutions and biological samples

All solutions were prepared from 1-mg/mL stock solutions of amphetamine, morphine, phenylpropanolamine, metaraminol, cimetidine, sotalol, atenolol, and practolol in methanol. Test solutions of the drugs in pure water were obtained by direct dilution of the previously mentioned stock solutions to a drug concentration of 10 µg/mL. Plasma samples containing 10 µg/mL of each of amphetamine, morphine, phenylpropanolamine, metaraminol, cimetidine, sotalol, and atenolol were prepared by dilution of the stock solutions with drug-free plasma. All solutions were stored at 5°C and protected from light.

### CE

CE was performed with a MDQ instrument (Beckman, Fullerton, CA) equipped with a UV detector. Separations were accomplished in a 75-µm-i.d. fused-silica capillary (BGB Analytik, Anwil, Switzerland) with an effective length of 50 cm (total length of 60 cm), utilizing a 25mM 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 psi for 5 s. Detection was accomplished at 200 nm utilizing an 800- × 100-µm slit.

### LPME device

The LPME device has been described in detail in previous publications (4,5). LPME was carried out in 100-µL micro inserts (VWR International, West Chester, PA), which were placed into a conventional 2-mL sample vial (Supelco, Bellefonte, PA) equipped with a screw cap and a silicon septum. Two conventional medical syringe needles (0.8-mm o.d.) were inserted through the silicon septum in the screw cap, and the two ends were connected to each other by a 6.5-cm piece of Q3/2 Accurel KM polypropylene hollow fiber (Membrana, Wuppertal, Germany). One medical syringe needle served to introduce the acceptor phase to the lumen of the hollow fiber prior to extraction, whereas the other needle was utilized for collection of the acceptor phase after extraction. The inner diameter of the hollow fiber was 600 µm, the thickness of the wall was 200 µm, and the pore size was 0.2 µm. The acceptor phase volume was 20 µL, and the volume of organic phase immobilized in the pores was approximately 18 µL. The whole 6.5-cm piece of hollow fiber was curled into the sample solution for extraction. During extraction, the sample vials were vibrated at 1500 rpm using a Vibramax 100 (Heidolph, Kelheim, Germany).

### LPME procedures

LPME was carried out either by addition of carrier to the sample solution or to the organic liquid membrane. In the former case, a 50-µL volume of the sample solution was filled into the microinsert, followed by the addition of 50 µL of 50mM carrier dissolved in 25mM phosphate buffer that was adjusted to pH 7.0. The hollow fiber was dipped for 5 s in *n*-octanol, followed by ultrasonification for 15 s in a water bath to remove excess solvent. In cases in which the carrier was added to the organic liquid membrane, a 50-µL volume of the sample solution was filled into the microinsert followed by 50

µL of 25mM phosphate buffer adjusted to pH 7.0. The hollow fiber was dipped for 5 s in *n*-octanol, which contained the carrier. Subsequently, the fiber was subjected to ultrasonification for 15 s in a water bath to remove excess solvent and carrier.

Common for all experiments, 20 µL of 50mM HCl was injected into the lumen of the hollow fiber with a microsyringe. This solution served as the acceptor phase. The fiber was placed in the sample solution (micro insert), and the sample vials were vibrated at 1500 rpm for 60 min. After extraction, the total volume of acceptor phase (20 µL) was flushed into a 200-µL microinsert (Beckman) for the CE instrument by application of small head pressure on the medical steel needle used for sample introduction, and finally the extract was analyzed by CE.

### Calculations

The log *P* and the p$K_a$ values of the model drugs were calculated with ACD/Lab software manufactured by Advanced Chemistry Development Inc., (Toronto, Canada). The extraction recovery (*R*) was calculated according to the following equation:

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

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 collected in the acceptor solution, respectively. $V_a$ is the volume of acceptor phase, $V_s$ 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.

## Results and Discussion

The different compounds tested as carriers in three-phase LPME are summarized in Tables I–IV. The majority of the carries have been reported previously for large-scale industrial membrane extraction applications in which the aqueous solutions on each side of the membrane are flowing. The carriers

**Table I. Extraction Recoveries with Borate Carriers from Pure Water Samples***

| | Recovery (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Carrier** | **Amphetamine** | **Phenylpropanolamine** | **Metaraminol** | **Cimetidine** | **Morphine** | **Sotalol** | **Atenolol** | **Practolol** |
| No carrier | 19 | 17 | 9 | 18 | 19 | 5 | 3 | 6 |
| *Carrier added to aqueous sample* | | | | | | | | |
| Phenylboronic acid | 40 | 27 | 12 | 51 | 30 | 6 | 3 | 10 |
| Tri-*tert*-butyl borate | 48 | 47 | 35 | 54 | 39 | 35 | 34 | 29 |

* *n* = 4 and relative standard deviation (RSD) < 20%.

**Table II. Extraction Recoveries with Carboxylic Acid Carriers from Pure Water Samples***

| | Recovery (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Carrier** | **Amphetamine** | **Phenylpropanolamine** | **Metaraminol** | **Cimetidine** | **Morphine** | **Sotalol** | **Atenolol** | **Practolol** |
| *Carrier added to aqueous sample* | | | | | | | | |
| Heptanoic acid | 55 | 52 | 30 | 17 | 12 | 12 | 6 | 15 |
| Salicylic acid | 81 | 72 | 32 | 67 | 44 | 10 | 4 | 17 |
| Octanoic acid | 84 | 83 | 67 | 64 | 70 | 31 | 19 | 46 |
| Valproic acid | 79 | 73 | 36 | 64 | 60 | 12 | 5 | 22 |
| 1-Naphthoic acid | 67 | 57 | 25 | 27 | 11 | 14 | 5 | 15 |
| Phenylcinnamic acid | 69 | 63 | 36 | 52 | 33 | 15 | 7 | 22 |
| Cholic acid | 67 | 64 | 43 | 59 | 50 | 20 | 10 | 28 |
| *Carrier added to organic liquid membrane* | | | | | | | | |
| Valproic acid | 72 | 73 | 36 | 64 | 60 | 13 | 5 | 16 |
| 1-Naphthoic acid | 64 | 42 | 18 | 22 | 10 | 11 | 3 | 16 |
| Phenylcinnamic acid | 69 | 71 | 50 | 48 | 33 | 29 | 12 | 31 |

* *n* = 4 and RSD < 20%.

were selected based on the presence of an anionic functional group capable of ion-pairing with the basic drugs and based on the presence of a significant hydrophobic moiety to ensure efficient extraction of the ion-pair complex into the organic liquid membrane. With these properties, pollution of the acceptor solution (50mM) was also efficiently suppressed. The anionic functional groups included borates, carboxylic acids, phosphates, and sulphates. Depending on the solubility characteristics of the carrier, it was either dissolved in the aqueous sample solution, in the organic liquid membrane (*n*-octanol), or in both in separate experiments. When dissolved in the aqueous sample solution, a 50-µL aliquot of 50mM carrier in phosphate buffer pH 7.0 was mixed with 50 µL of a pure water sample containing the model drugs, providing a final carrier concentration of 25mM within the donor compartment. This concentration level has previously been reported as optimal for octanoic acid (24,25) and was adapted without further optimization for the first part of this paper. When the carrier was dissolved in the organic liquid membrane, the concentration was either 50mM or 15% (w/w), depending on the actual solubility in *n*-octanol. For measurement of extraction performance, eight different basic drugs were selected as model compounds (as illustrated in Figure 1). For all of the compounds except amphetamine, log *P* values were between 0.0

**Table III. Extraction Recoveries with Phosphate Carriers from Pure Water Samples***

| | Recovery (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Carrier** | **Amphetamine** | **Phenylpropanolamine** | **Metaraminol** | **Cimetidine** | **Morphine** | **Sotalol** | **Atenolol** | **Practolol** |
| *Carrier added to organic liquid membrane* | | | | | | | | |
| Triethyl phosphate | 8 | 11 | 12 | 22 | 24 | 10 | 5 | 11 |
| Tris(2-ethylhexyl) phosphate | 10 | 15 | 18 | 27 | 26 | 9 | 8 | 13 |
| Tributyl phosphate | 59 | 47 | 35 | 10 | 19 | 15 | 4 | 16 |
| Triphenyl phosphate | 12 | 17 | 19 | 32 | 28 | 13 | 9 | 14 |
| Di(2-ethylhexyl) phosphate | 7 | 10 | 33 | 40 | 46 | 44 | 35 | 46 |
| Tridecyl phosphate | 5 | 14 | 42 | 35 | 37 | 40 | 33 | 34 |
| Di(2-ethylhexyl) phosphate + Tris(2-ethylhexyl) phosphate | 8 | 10 | 29 | 47 | 51 | 57 | 55 | 58 |
| Di(2-ethylhexyl) phosphate + Tributyl phosphate | 10 | 14 | 28 | 51 | 59 | 49 | 58 | 59 |

* $n = 4$ and RSD < 20%.

**Table IV. Extraction Recoveries with Sulphate Carriers from Pure Water Samples***

| | Recovery (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Carrier** | **Amphetamine** | **Phenylpropanolamine** | **Metaraminol** | **Cimetidine** | **Morphine** | **Sotalol** | **Atenolol** | **Practolol** |
| *Carrier added to aqueous sample* | | | | | | | | |
| 1-Heptanesulfonic acid | 45 | 62 | 71 | 73 | 58 | 57 | 37 | 61 |
| Sodium 2-ethylhexyl phosphate | 13 | 27 | 45 | 48 | 43 | 52 | 45 | 45 |
| 1-Octanesulfonic acid | 32 | 49 | 34 | 38 | 36 | 33 | 21 | 33 |
| 1-Naphthalenesulfonic acid | 61 | 50 | 26 | 46 | 21 | 7 | 4 | 18 |
| 1-Nonanesulfonic acid | 27 | 29 | 19 | 49 | 42 | 13 | 8 | 18 |
| Sodium laurylsulphate | 14 | 28 | 43 | 40 | 34 | 54 | 46 | 46 |
| Bromthymol blue | 24 | 38 | 54 | 58 | 57 | 69 | 70 | 60 |
| Methyl orange | 54 | 59 | 49 | 64 | 54 | 39 | 24 | 51 |
| *Carrier added to organic liquid membrane* | | | | | | | | |
| Bromthymol blue | 41 | 56 | 64 | 47 | 50 | 66 | 51 | 62 |

* $n = 4$ and RSD < 20%.

and 1.1, and the drugs were poorly recovered in extractions without the addition of carriers (Table I). Thus, these drugs served as hydrophilic model drugs. On the other hand, amphetamine was included as a more hydrophobic substance (log $P$ = 1.8) and served as a marker for what happens to compounds efficiently extracted, even without the addition of carriers.

**Experiences from pure water samples**

In a first experiment, two different borates were tested as carriers (Table I). For both carriers, the solubility in aqueous solution was high, and, consequently, the carriers were added to the aqueous sample. Both compounds functioned as carriers, and provided, in most cases, elevated extraction recoveries when compared with non-carrier extractions. Tri-tert-butyl borate was the most efficient carrier and provided even significant recoveries for sotalol, atenolol, and practolol, which appeared to be the most difficult analytes. This difference, most likely, was because of the significantly higher hydrophobicity of the former, and tri-tert-butyl borate was superior because it provided highly hydrophobic ion-pair complexes with the analytes, which were readily extracted into the organic liquid membrane.

In a second experiment, the different carboxylic acids were evaluated in a similar way. Heptanoic acid, salicylic acid, and octanoic acid showed limited solubility in *n*-octanol (below 50mM) and were only added to the aqueous sample solution. On the other hand, valproic acid, 1-naphthoic acid, and phenylcinnamic acid, which all provided acceptable solubility both in water and in *n*-octanol, were added to the aqueous sample in one experiment and to the organic liquid membrane in a second experiment (50mM). The results are summarized in Table II. Also, the carboxylic acids worked as carriers and provided elevated extraction recoveries for most model drugs as compared with non-carrier extraction. Octanoic acid, in particular, provided excellent results for phenylpropanolamine, metaraminol, cimetidine, and morphine. However, for sotalol, atenolol, and practolol, recoveries were, in most cases, not a significant improvement when compared with non-carrier extractions, indicating that the carboxylic acids tested were not highly efficient as general carriers. Their relatively low hydrophobicity was probably the reason for this. Interestingly, recoveries with valproic acid, 1-naphthoic acid, and phenylcinnamic acid were comparable when the carriers were added to the aqueous sample or to the organic liquid membrane, although the amount of carrier in the first case was a factor of 3.3 higher than in the latter. This observation suggested that the carriers were distributed between the different phases according to their partition coefficients during the extraction, and that it was of minor practical importance if the carrier was added to the aqueous sample or to the organic liquid membrane.

In a third experiment, different phosphates were tested as carriers. Their solubility in water was extremely low, and the carriers were added only to the organic liquid membrane. Because of their excellent solubility in *n*-octanol, the carriers were dissolved in the organic liquid membrane at the 15% (w/w) level. As illustrated in Table III, some of the phosphates exhibited a substantial extraction enhancement because of carrier transport. Interestingly, the most efficient phosphates provided extraction selectivities different from the carboxylic acids and the borates. With some of the phosphates, the difficult compounds (sotalol, atenolol, and practolol) were efficiently extracted with recoveries exceeding 50%.

Finally, several sulfates were tested in carrier-mediated LPME as shown in Table IV. Generally, the compounds selected were poorly soluble in *n*-octanol, and the carriers were only added to the aqueous sample. Bromthymol blue was also tested because of very promising initial results, but this compound was insoluble at the 50mM level either in the aqueous sample or in the organic liquid membrane. Thus, for this particular compound, a concentration of 5mM was utilized within the sample in one experiment, whereas the concentration level in *n*-octanol was adjusted to 35mM in a second experiment. As seen in Table IV, several of the sulfates were strong carriers. For sotalol, atenolol, and practolol, bromthymol blue provided excellent results, and this carrier appeared to be an efficient general carrier together with 1-heptanesulfonic acid.

**Theoretical considerations of carrier selection**

Based on the experience reported previously, some of the most promising carriers for hydrophilic drugs appeared to be tri-tert-butyl borate, octanoic acid, di(2-ethylhexyl) phosphate–tris(2-ethylhexyl) phosphate, di(2-ethylhexyl) phos-

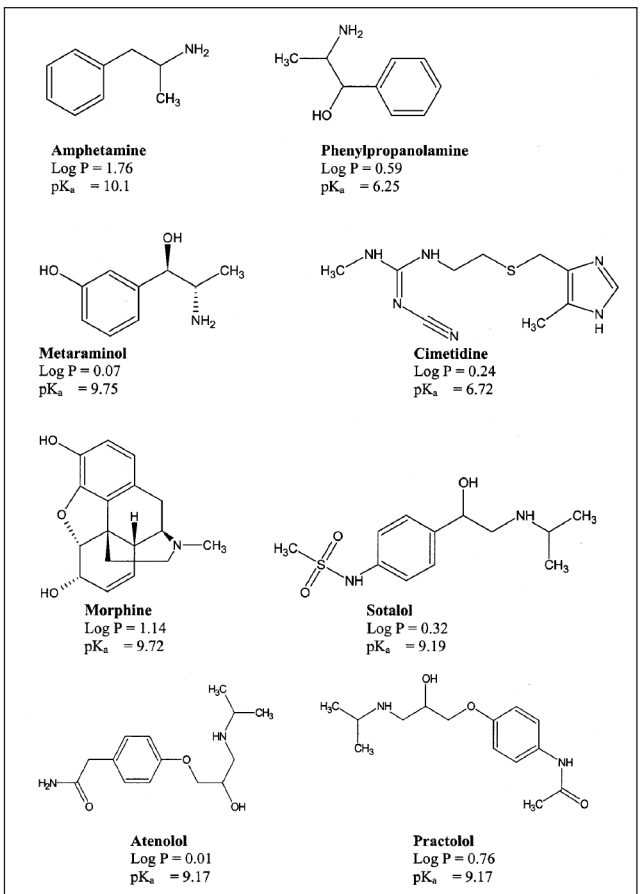


**Figure 1.** Structure, log $P$, and p$K_a$ values for the model drugs.

phate–tributyl phosphate, heptanesulfonic acid, and bromthymol blue. These carriers generally provided high recoveries but differed somewhat in terms of selectivity. Thus, selectivity tuning may be accomplished in the future by careful selection of the carrier.

Because the partitioning of the different carriers between neutral aqueous solution and *n*-octanol differed substantially, the extraction mechanism was expected to be somewhat different with the different carriers. For heptanesulfonic acid, the solubility in aqueous solution at pH 7.0 was about a factor of 300 higher than in *n*-octanol, as found by a simple solubility test. Because of this, heptanesulfonic acid in the unpaired state principally remained in the aqueous sample compartment during extraction, and it is, therefore, anticipated that this carrier principally ion-paired with the analytes in the sample and, subsequently, was extracted into the organic phase as ion-pairs with the basic drugs. A similar mechanism was proposed for octanoic acid, for which the solubility in aqueous solution at pH 7.0 was a factor of 15 higher than in *n*-octanol. For the phosphates, on the other hand, the water solubility was extremely low, and they were expected to remain in the organic liquid membrane during extraction. Thus, with these carriers, the ion-pair formation most probably occurred at the interface between the sample and the organic liquid membrane, or inside the latter. With bromthymol blue, the solubility in aqueous solution at pH 7.0 was determined to 5mM, whereas the corresponding value in *n*-octanol was 35mM. For extractions with bromthymol blue, the carrier was distributed both in the sample and in the organic liquid membrane, with prevalence for the latter. Thus, with bromthymol blue, the ion-pairing was expected to occur both in the sample solution, at the sample–membrane interface, and inside the organic liquid membrane. A similar mechanism was expected with tri-tert-butyl borate, which showed unlimited miscibility with *n*-octanol and a 55mM solubility in the phosphate buffer pH 7.0.

### Optimization of extractions with bromthymol blue as carrier

Among the most promising carriers reported previously, it

**Table V. Extraction Recoveries with Different Organic Liquid Membranes with Bromthymol Blue Used as Carrier***

| | Recovery (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Carrier | Amphetamine | Phenylpropanolamine | Metaraminol | Cimetidine | Morphine | Sotalol | Atenolol | Practolol |
| *n*-Octanol | 24 | 38 | 54 | 58 | 57 | 69 | 70 | 60 |
| *n*-Dihexyl ether | 47 | 21 | 12 | 9 | 26 | 4 | 3 | 6 |
| 2-nitrophenyl octyl ether | 65 | 33 | 12 | 17 | 23 | 25 | 7 | 13 |
| 2-octanone | 7 | 15 | 16 | 29 | 25 | 22 | 5 | 11 |
| Dodecylacetate | 82 | 64 | 21 | 10 | 14 | 30 | 7 | 14 |
| Silicon oil AR20 | – | – | – | – | – | – | – | – |
| Peppermint oil | 49 | 76 | 72 | 75 | 62 | 76 | 78 | 81 |

* $n = 4$ and RSD < 20%.

**Table VI. Extraction Recoveries from Human Plasma Samples***

| | Recovery (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Carrier | Amphetamine | Phenylpropanolamine | Metaraminol | Cimetidine | Morphine | Sotalol | Atenolol | Practolol |
| *Carrier added to aqueous sample* | | | | | | | | |
| Bromthymol blue | 27 | 46 | 21 | 45 | 28 | 17 | 19 | 32 |
| Tri-tert-butyl borate | 46 | 33 | 29 | 39 | 26 | 17 | 8 | 25 |
| Octanoic acid | 49 | 45 | 42 | 43 | 37 | 25 | 11 | 21 |
| *Carrier added to organic liquid membrane* | | | | | | | | |
| Bromthymol blue | 27 | 32 | 43 | 46 | 28 | 29 | 21 | 41 |
| Valproic acid | 64 | 55 | 26 | 70 | 36 | 11 | 4 | 17 |
| Di(2-ethylhexyl) phosphate + Tributyl phosphate | – | – | – | – | – | – | – | – |

* $n = 4$ and RSD < 25%.

was decided to optimize extraction with bromthymol blue added to the sample solution. In a first experiment, pH in the sample was optimized utilizing different phosphate buffers in the range of pH 6.0 to 8.0. In this range, a pH value of 7.6 provided the highest recovery. In a second experiment, the concentration of bromthymol blue was optimized in the range 1–7mM. The solubility of bromthymol blue was limited to approximately 7mM in aqueous solution at pH 7.6, and recovery values were found to increase with increasing concentration of bromthymol blue up to concentrations between 5 and 7mM. In a third experiment, different organic solvents were tested as the organic liquid membrane as illustrated in Table V. As shown in this table, *n*-octanol and peppermint oil were the most successful organic phases, providing high recovery values for all the model drugs. The results from this experiment supported earlier observations that LPME based on carrier-mediated transport is highly affected by the chemical nature of the organic liquid membrane. The reason for this is probably that several different criteria should be fulfilled for the organic liquid membrane, including high complex-formation constants, intermediate solubility, and reasonable stability of the analyte-carrier complexes.

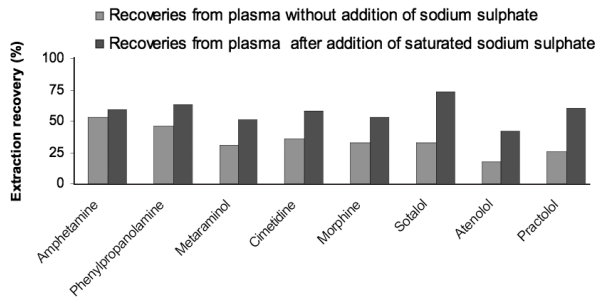


**Figure 2.** Extraction recoveries obtained from plasma samples with and without $Na_2SO_4$ addition.

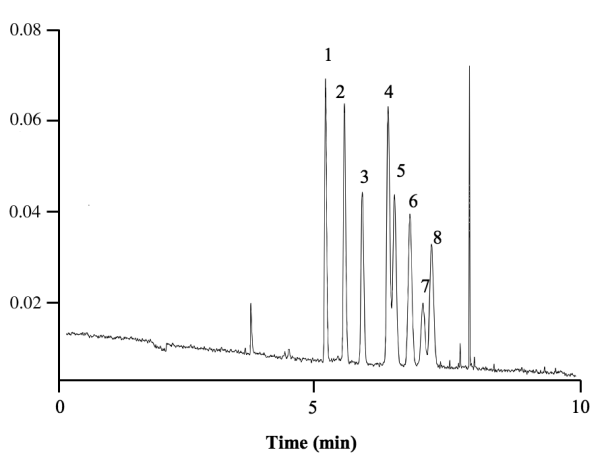


**Figure 3.** Electropherogram obtained from plasma sample saturated with $Na_2SO_4$ of 8 model drugs. Peak numbers: amphetamine, 1; phenylpropanolamine, 2; metaraminol, 3; cimetidine, 4; morphine, 5; sotalol, 6; atenolol, 7; and practolol, 8.

### Compatibility with human plasma samples

In a subsequent series of experiments, some of the most promising carriers were tested for extraction of the eight model drugs from human plasma samples. The results are summarized in Table VI. In most cases, the carriers were compatible with the plasma samples and provided extraction of all the eight model drugs. However, analyte recoveries were in most cases reduced when compared with extraction from pure water. There may be several different reasons for this. First, the carrier may have interactions with proteins and other components of the sample matrix, reducing the level of carrier–analyte interactions responsible for the extraction process. Secondly, the drugs may have interactions with the proteins, which were only partially suppressed in the extraction system. Third, the viscosity of plasma is higher than for water, and this will contribute to reduced analyte diffusion within the sample compartment. With the combination of di(2-ethylhexyl) phosphate and tributyl phosphate as carrier, the analytes were not extracted at all, and precipitations were observed in the sample after extraction. In addition, close examination of the fiber after extraction revealed precipitation also on the surface of the fiber. Clearly, this carrier combination was not compatible with the plasma samples and caused precipitation of the plasma proteins. This was also the case with several of the other phosphates (results not shown), which indicates that this class of carriers is not applicable for human plasma samples in general.

Interactions between the carrier and proteins were suspected to be the major reason for the lower recoveries from plasma, and, in a subsequent experiment, sodium sulphate ($Na_2SO_4$) was added to the sample in an attempt to reduce the level of interactions. The sample was saturated with $Na_2SO_4$, and bromthymol blue was used as carrier dissolved in the organic liquid membrane (*n*-octanol). Interestingly, $Na_2SO_4$ served as a very efficient extraction enhancer (as illustrated in Figure 2), providing excellent electropherograms from plasma (as shown in Figure 3). Especially for sotalol, atenolol, and practolol, recoveries were improved by more than a factor of 2, and the recoveries now ranged between 45% and 75%. Most likely, $Na_2SO_4$ served to suppress interactions between bromthymol blue and proteins, which enhanced the availability of the carrier towards the analytes. The addition of sodium chloride (NaCl) was also tested, but this resulted in suppression of the extraction process. In conclusion, future extraction from plasma should be performed after saturation with $Na_2SO_4$.

### Validation

As a final experiment, validation was accomplished with bromthymol blue as the carrier for extraction of phenylpropanolamine and practolol from human plasma, with sotalol as the internal standard. As shown from the results in Table VII, the plasma standard curve for phenylpropanolamine was found to be linear in the range of 0.5 to 10 µg/mL, with a correlation coefficient of 0.994, and the plasma standard curve for practolol was found to be linear in the range of 0.5 to 10 µg/mL, with a correlation coefficient 0.997. Interday precision was studied at three concentration levels and with six replications for each, and intraday precision was carried out on

**Table VII. Validation Data for Phenylpropanolamine and Practolol from Human Plasma Samples**

| Drug | Concentration | Intraday precision RSD (%) ($n$ = 6) | Interday precision RSD (%) ($n$ = 18) | Linear range (µg/mL) | $r$ |
|---|---|---|---|---|---|
| Phenypropanolamine | 0.5 µg/mL | 4.6 | 11.5 | 0.5–10 | 0.994 |
| | 2.5 µg/mL | 6.9 | 5.5 | | |
| | 10 µg/mL | 6.2 | 5.4 | | |
| Practolol | 0.5 µg/mL | 5.7 | 5.9 | 0.5–10 | 0.997 |
| | 2.5 µg/mL | 3.3 | 4.0 | | |
| | 10 µg/mL | 3.0 | 2.9 | | |

three consecutive days at three concentration levels, with 18 replications each. As seen in Table VII, the intra- and interday precision for phenylpropanolamine in plasma were in the range of 4.6–6.9% relative standard deviation (RSD) and 5.4–11.5% RSD, respectively. The intra- and interday precision for practolol in plasma were in the range of 3.0–5.7% RSD and 2.9–5.9% RSD, respectively.

## Conclusion

The present work has evaluated a broad range of potential carriers for the recovery of hydrophilic substances in LPME. Different organic borates, phosphates, sulphates, and carboxylic acids were demonstrated to work as extraction carriers, enhancing the extraction of hydrophilic basic drugs. Both water-soluble carriers added to the sample solution and water-insoluble carriers added to the organic liquid membrane were used. The former type of carrier is expected to ion-pair within the sample solution, whereas the latter most probably ion-paired with the analytes at the interface between the sample solution and the organic liquid membrane. The compatibility of the carriers with human plasma samples was studied, and some of the carriers were not bio-compatible, resulting in precipitation of proteins on the surface of the fibers and within the bulk sample solutions. Fortunately, others were compatible with human plasma samples and extracted the hydrophilic model compounds selected. Compared with extraction from pure water samples, the performance was initially reduced with plasma samples, most likely because of interactions between the carrier and plasma proteins, but this was efficiently suppressed by saturation of the plasma samples with sodium sulphate. Thus, with sodium sulphate, all basic drugs were extracted from plasma samples with recoveries in the range of 45% to 75%. The fundamental studies performed in this paper support that the carrier-mediated LPME may be an interesting alternative for future extractions of hydrophilic basic drugs from biological samples. Additionally, the concept may also be used in future laboratory studies of carrier-mediated transport, which play an important role both in biological systems and in industrial applications.

## References


1. M.A. Jeannot and F.F. Cantwell. Mass transfer characteristics of solvent extraction into a single drop at the tip of a syringe needle. *Anal. Chem.* **69:** 235–39 (1997).
2. E. Psillakis and N. Kalogerakis. Developments in single-drop microextraction. *Trends Anal. Chem.* **21:** 53–63 (2002).
3. E. Psillakis and N. Kalogerakis. Solid-phase microextraction versus single-drop microextraction for the analysis of nitroaromatic explosives in water samples. *J. Chromatogr. A* **938:** 113–20 (2001).
4. S. Pedersen-Bjergaard and K.E. Rasmussen. Liquid–liquid–liquid microextraction for sample preparation of biological fluids prior to capillary electrophoresis. *Anal. Chem.* **71:** 2650–56 (1999).
5. K.E. Rasmussen, S. Pedersen-Bjergaard, M. Krogh, H. Grefslie Ugland, and T. Grønhaug. Development of a simple in-vial liquid-phase microextraction device for drug analysis compatible with capillary gas chromatography, capillary electrophoresis and high-performance liquid chromatography. *J. Chromatogr. A* **873:** 3–11 (2000).
6. G. Audunsson. Aqueous/aqueous extraction by means of a liquid membrane for sample cleanup and preconcentration of amines in a flow system. *Anal. Chem.* **58:** 2714–23 (1986).
7. G. Nilvé, G. Audunsson, and J.Å. Jönsson. Sample preparation by means of a supported liquid membrane for the determination of chlorophenoxyalkanoic acids. *J. Chromatogr.* **471:** 151–60 (1989).
8. J.Å. Jönsson and L. Mathiasson. Supported liquid membrane techniques for sample preparation and enrichment in environmental and biological analysis. *Trends Anal. Chem.* **11:** 106–14 (1992).
9. J.Å. Jönsson and L. Mathiasson. Membrane-based techniques for sample enrichment. *J. Chromatogr. A* **902:** 205–25 (2000).
10. J.Å. Jönsson and L. Mathiasson. Membrane extraction in analytical chemistry. *J. Sep. Sci.* **24:** 495–507 (2001).
11. T.G. Halvorsen, S. Pedersen-Bjergaard, and K.E. Rasmussen. Liquid-phase microextraction and capillary electrophoresis of citalopram, an antidepressant drug. *J. Chromatogr. A* **909:** 87–93 (2001).
12. T.G. Halvorsen, S. Pedersen-Bjergaard, J.L.E. Reubsaet, and K.E. Rasmussen. Liquid-phase microextraction combined with flow-injection tandem mass spectrometry Rapid screening of amphetamines from biological matrices. *J. Sep. Sci.* **24:** 615–22 (2001).
13. L. Zhu, L. Zhu, and H.K. Lee. Liquid–liquid–liquid microextraction of nitrophenols with a hollow fiber membrane prior to capillary liquid chromatography. *J. Chromatogr. A* **924:** 407–14 (2001).
14. L. Zhao, L. Zhu, and H.K. Lee. Analysis of aromatic amines in water samples by liquid–liquid–liquid microextraction with hollow fibers and high-performance liquid chromatography. *J. Chromatogr. A* **963:** 239–48 (2002).
15. L. Zhu, K.H. Ee, L. Zhao, and H.K. Lee. Analysis of phenoxy herbicides in bovine milk by means of liquid–liquid–liquid microextraction with a hollow-fiber membrane. *J. Chromatogr. A* **963:** 335–43 (2002).
16. L. Hou, X. Wen, C. Tu, and H.K. Lee. Combination of liquid-phase microextraction and on-column stacking for trace analysis of amino alcohols by capillary electrophoresis. *J. Chromatogr. A* **979:** 163–69 (2002).
17. T.S. Ho, S. Pedersen-Bjergaard, and K.E. Rasmussen. Liquid-phase microextraction of protein-bound drugs under non-equilibrium conditions. *Analyst* **127:** 608–13 (2002).
18. S. Andersen, T.G. Halvorsen, S. Pedersen-Bjergaard, and

K.E. Rasmussen. Liquid-phase microextraction combined with capillary electrophoresis, a promising tool for the determination of chiral drugs in biological matrices. *J. Chromatogr. A* **963:** 303–12 (2002).
19. T. Kuuranne, T. Kotiaho, S. Pedersen-Bjergaard, K.E. Rasmussen, A. Leinonen, S. Westwood, and R. Kostiainen. Feasibility of a liquid-phase microextraction sample clean-up and liquid chromatographic/mass spectrometric screening method for selected anabolic steroid glucuronides in biological samples. *J. Mass Spectrom.* **38:** 16–26 (2003).
20. A. Bjørhovde, T.G. Halvorsen, S. Pedersen-Bjergaard, and K.E. Rasmussen. Liquid-phase microextraction of drugs from human breast milk. *Anal. Chim. Acta* **491:** 155–61 (2003).
21. T.G. Halvorsen, S. Pedersen-Bjergaard, J.L.E. Reubsaet, and K.E. Rasmussen. Liquid-phase microextraction combined with liquid chromatography–mass spectrometry. Extraction from small volumes of biological samples. *J. Sep. Sci.* **26:** 1520–26 (2003).
22. L. Hou and H.K. Lee. Dynamic three-phase microextraction as a sample preparation technique prior to capillary electrophoresis. *Anal. Chem.* **75:** 2784–89 (2003).
23. X. Wen, C. Tu, and H.K. Lee. Two-step liquid–liquid–liquid microextraction of nonsteroidal antiinflammatory drugs in wastewater. *Anal. Chem.* **76:** 228–32 (2004).
24. T.S. Ho, T.G. Halvorsen, S. Pedersen-Bjergaard, and K.E. Rasmussen. Liquid-phase microextraction of hydrophilic drugs by carrier-mediated transport. *J. Chromatogr. A* **998:** 61–72 (2003).
25. T.S. Ho, J.L.E. Reubsaet, H.S. Anthonsen, S. Pedersen-Bjergaard, and K.E. Rasmussen. Liquid-phase microextraction based on carrier mediated transport combined with liquid chromatography–mass spectrometry: new concept for the determination of polar drugs in a single drop of human plasma. *J. Chromatogr. A* **1072:** 29–36 (2005).

Manuscript received August 30, 2005;
revision received December 14, 2005.