ELSEVIER

Journal of Chromatography A, 665 (1994) 259–268

JOURNAL OF CHROMATOGRAPHY A

Automated system for the trace analysis of organic compounds with supported liquid membranes for sample enrichment

J.Å. Jönsson*[,a], L. Mathiasson[a], B. Lindegård[a], J. Trocewicz[b], A.-M. Olsson[c]

[a]*Analytical Chemistry, University of Lund, POB 124, S-221 00 Lund, Sweden*
[b]*Chemical Physics, UMCS, Plac MCS 3, PL-20-031 Lublin, Poland*
[c]*Bioanalytical Department, Kabi-Pharmacia, S-223 63 Lund, Sweden*

Abstract

Supported liquid membranes, mounted in flow systems, can be used for selective and efficient extraction and enrichment of various types of analytes. The basic principle is a continuous extraction from an aqueous phase into an organic solvent, immobilized in a porous hydrophobic membrane, followed by a continuous back-extraction to a stagnant aqueous acceptor phase on the other side of the membrane. The entire acceptor phase is then used for further analysis.

The ASTED (Gilson) automated sample preparation system was modified by exchanging the dialysis unit for a supported liquid membrane unit. This permits a direct connection to a liquid chromatographic column with minimal sample losses. The instrument can be loaded with up to 60 samples, which are automatically processed before the final chromatographic analysis. With this equipment, the same sample can pass the membrane extractor several times (while the acceptor phase remains stagnant) to give a higher recovery. When one sample is chromatographed the next one is enriched simultaneously.

The technique was evaluated for the extraction of a basic drug and its metabolite from water solutions with the purpose to be applied to blood plasma samples. The recovery depends on the time used for the enrichment. With enrichment times similar to the chromatographic run, or *ca.* 15 min, the recovery was *ca.* 35%.

1. Introduction

Automation of a complete analysis is desirable when a large number of samples regularly must be handled, as often is the case in *e.g.* bioanalysis. Automation of a trace analysis procedure often leads to reduced costs, better reproducibility and a reduced risk of contamination. When biological samples are handled, the risk of being infected is also smaller with a closed, automated system.

Classical workup techniques, such as liquid–liquid extraction and column fractionation [1,2] are difficult to automate. The most successful approach has been to use flow systems as Autoanalyzers (Technicon) and systems based on flow injection analysis (FIA) techniques for relatively simple applications [3,4].

Solid phase extraction (SPE) [5] is a relatively new technique, which in many cases is very efficient for sample cleanup, especially for lipophilic compounds, but also in this case, automation is not straightforward. A few commercial robotic systems, notably the ASPEC (Gilson, Villiers-le-Bel, France), Millilab (Millipore, Bedford, MA, USA) and Benchmate (Zymark,

* Corresponding author.

0021-9673/94/$07.00
SSDI 0021-9673(93)E0071-2

Hopkinton, MA, USA), are available for SPE and related sample workup operations in combination with HPLC [6]. The combination of automated on-line sample workup with GC is not common.

A novel approach for selective extraction and enrichment is the supported liquid membrane (SLM) technique [7–9]. This technique has been applied to various classes of compounds, including amines [10,11], acids [12–14] and metals [8]. It can handle complex biological matrices as urine [10], blood plasma [11] and faeces [13] and be connected on-line to gas [10,11] or liquid [12,14] chromatographic equipment. A notable feature is that chromatograms obtained from blank samples containing these matrices are very similar to chromatograms from distilled water blanks, showing a very efficient cleanup. The SLM principle is superficially similar to dialysis and these techniques share the efficient rejection of macromolecular matrix components. However, in dialysis [3,9], low molecular components nonselectively pass the membrane and are diluted in the acceptor liquid (and, with the ASTED, later concentrated on a SPE cartridge). The SLM technique gives a selective enrichment in a small volume.

Here we present a novel technical solution for automated analysis of ionizable substances. It is based on the SLM technology for sample workup with the membrane unit incorporated into a modified ASTED (Gilson) equipment, directly connected to a HPLC system. The object is to process small sample volumes (<1 ml) and to obtain a final extract with a volume compatible with a direct injection into a HPLC. Due to low concentrations, a high transfer rate through the membrane is needed, and the time for the enrichment should be in the same range of time as the chromatographic run. Three basic compounds, the drug Amperozide and two related compounds (Kabi-Pharmacia AB, Lund, Sweden), were used as model substances. Amperozide is an amine (see under Chemicals) with a pK_a of 6.8.

In a forthcoming publication, the application of this methodology to blood plasma samples will be presented.

2. Experimental

2.1. Apparatus

An ASTED (Automated Sequential Trace Enrichment of Dialysates) instrument (Gilson Medical Electronics, Villiers-le-Bel, France) was modified in the following way: the original dialysis membrane holder was exchanged for a custom made holder for the supported liquid membrane, shown in Fig. 1. This consisted of two 10 × 25 × 70 mm PVDF (polyvinyliden fluoride) blocks with identical machined grooves having the dimensions 0.1 × 2.5 × 40 mm, forming channels with a nominal volume of 10 μl each. The membrane (6 × 46 mm) was placed between the blocks, which were clamped together with six bolts. To prevent stoppage in the acceptor channel, especially when high donor flow rates were used, a thin polyethene spacer was placed between the acceptor block and the membrane. Low-volume connections for standard Altex type fittings were provided in the blocks.

The original dialysis membrane holder in the ASTED instrument was replaced with the liquid membrane unit. The original SPE cartridge, connected to the injection valve, was replaced with a 30 μl loop of stainless steel tubing. The parts of the ASTED equipment needed for this application can also be purchased separately

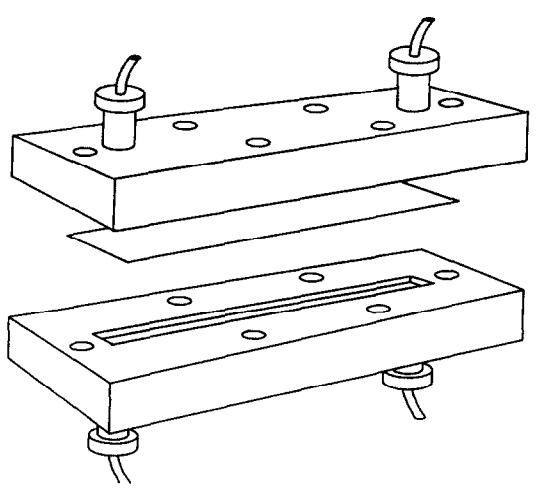


Fig. 1. Membrane unit.

(Model 231 sample handling unit and an extra syringe pump). The hardware related to the dialysis membrane as well as the special ASTED software is not needed.

The porous PTFE membrane used (Model TE35, 0.2 μm, Schleicher and Schuell, Dassel, Germany), was cut to fit in the holder and soaked for 15 min in the selected solvent. After mounting, a washing sequence was run to ensure that excess solvent was washed away from the membrane surfaces.

The equipment for the analysis consisted of a HPLC pump (Model 2150, LKB, Bromma, Sweden), a column (150 mm × 4.6 mm I.D., packed with 5 μm Nucleosil C_{18}, Hichrom), a variable-wavelength UV detector (Spectroflow, Kratos, Ramsey, NJ, USA) and a strip-chart recorder (Model 2210, LKB, Bromma, Sweden). The mobile phase flow-rate was usually 0.65 ml/min and the UV detector was set at 265 nm. Baseline separation of the compounds studied was obtained in about 15 min.

2.2. Operation

The operation is described with reference to Fig. 2. Sample from one of the vials (1), usually 800 μl, is sucked into the needle (2) by the syringe pump (3) after mixing with basic donor buffer (4). The needle is then moved to the injection port (5) and the sample is slowly pressed into the donor channel (6) of the membrane unit, where the unprotonated amines (drug compounds) are extracted into the membrane and subsequently re-extracted into the acceptor (7), where they are protonated and irreversibly trapped in a stagnant acidic buffer. The sample passes into a coil (8), large enough to accommodate the entire sample. The sample may then be sucked back through the donor channel, passing the membrane again and into a second coil (9). These steps can easily be repeated, permitting extractions of the same sample. After the extraction is completed, the donor channel is washed with sample-free donor solution. This step can remove uncharged compounds which may have been transferred into the acceptor phase together with the amines [8]. Finally, the acceptor volume, containing the extracted amines, is transferred by means of the second syringe (10) into the loop injector valve (11) and injected into the HPLC column (12), which is connected to the pump (13). Before a new sample cycle is started, both the donor and the acceptor channels are rinsed with donor (4) and acceptor (14) buffers, respectively.

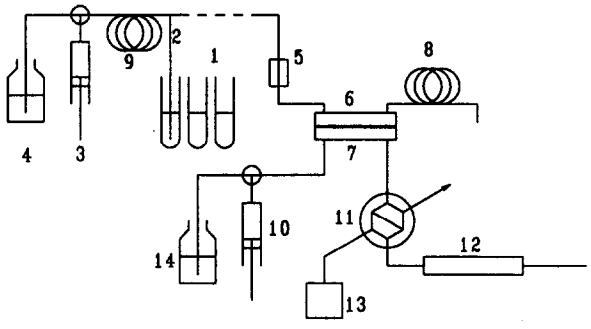


Fig. 2. Experimental setup. For details see the text.

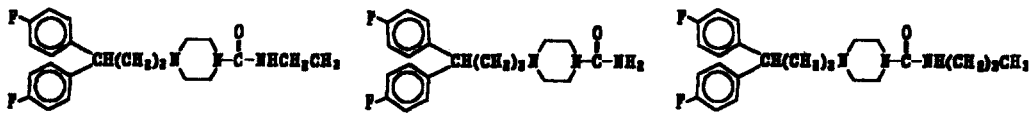
The sequence of operation of the equipment was programmed into the ASTED microprocessor. The complete program can be obtained from the corresponding author of this paper.

With the ASTED, up to 60 samples can be processed, and five different reagents can be automatically added to the samples.

2.3. Chemicals

Amperozide, 4-[4,4-bis(4-fluorophenyl)butyl]-N-ethyl-1-piperazinecarboxamide (**I**), a main metabolite, 4 - [4,4 - bis(4 - fluorophenyl)butyl] - 1-piperazinecarboxamide (**II**), and an analogue compound intended for use as an internal standard, 4-[4,4-bis(4-fluorophenyl)butyl]-N-butyl-1-piperazinecarboxamide (**III**), were obtained as hydrochlorides from Kabi-Pharmacia. Their purity was >99%. Stock solutions were prepared in water (200 μg/ml) and were stable for at least several months when kept in a refrigerator.

The donor solution contained 12.5 m*M* EDTA and NaOH to the desired pH, usually 9.0. To suppress adsorption, 3.8 m*M* $(NH_4)_2SO_4$ was added in most cases. The acceptor solution



I II III

contained 0.5 m*M* $(NH_4)_2SO_4$ and H_2SO_4 to give a pH of usually 2.3.

The membrane was impregnated by soaking for 15 min in either *di*-hexyl ether (Sigma, St. Louis, MO, USA) or in a solution of 5% (w/w) of TOPO, trioctyl phosphine oxide (Fluka, Buchs, Switzerland), in *di*-hexyl ether.

The mobile phase for the liquid chromatography was ammonium phosphate buffer (pH 8.2) in methanol (E. Merck, Darmstadt, Germany, HPLC grade) (55:155, v/v). To prevent bubble formation, the mobile phase was degassed with helium. All water was purified using a Milli-Q unit (Millipore, Bedford, MA, USA) and all chemicals were of analysis grade or better.

3. Results and discussion

3.1. Chromatograms

Fig. 3a shows a chromatogram of compounds **I–III**, enriched with the liquid membrane technique, after optimization of various parameters

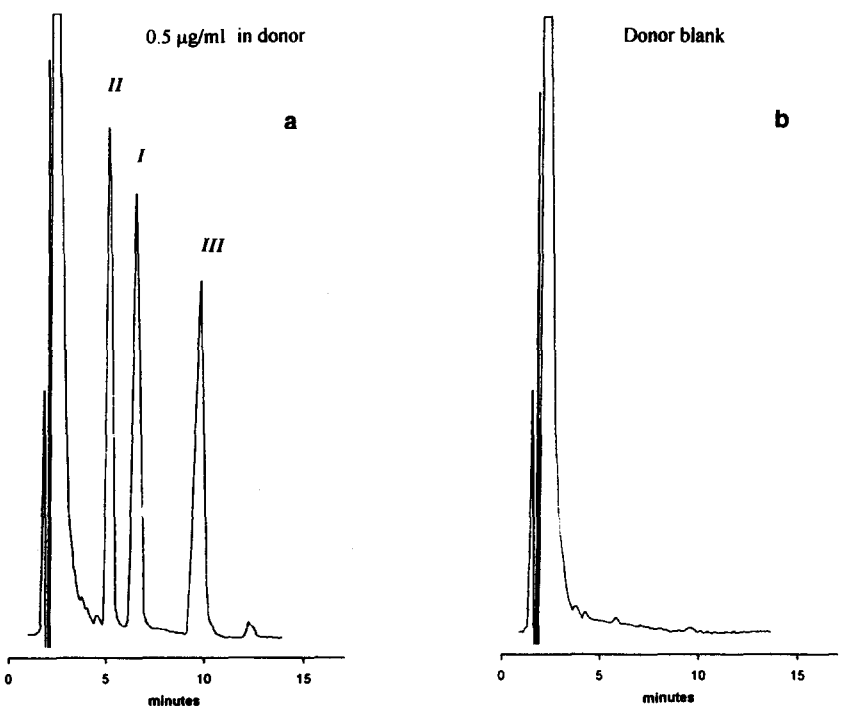


Fig. 3. (a) Chromatogram of compounds **I** (Amperozide), **II** (its metabolite) and **III**; 0.5 µg/ml each in donor buffer after enrichment as described in the text. (b) Chromatogram after enrichment of pure donor buffer.

as discussed below. Fig. 3b shows the corresponding blank.

The peaks appearing in the beginning of the chromatogram are solvent peaks, caused by the HPLC system and not by the liquid membrane procedure, as they turn up also in a direct injection on the column.

3.2. Optimization of dimensions and flow-rates

Strategies for the optimization of the recovery (expressed as extraction efficiency) are reviewed in ref. 15. In the present case, the basic requirements were:

(1) it must be possible to analyze small sample volumes (<1 ml), as required by future bioanalytical applications; (2) the extract volume should be limited to *ca.* 10 μl, since an on-line connection with complete sample transfer to HPLC is desired; (3) the sample workup should be finished within the time of the chromatographic run, which means that the analysis time is determined by the chromatographic separation; and (4) the extraction should be as selective as possible, leading to cleaner blanks and lower detection limits.

The first two conditions necessitate the use of a small extraction unit, with a channel volume of *ca.* 10 μl. As the membrane area should be as large as possible [15], this calls for shallow channels. The minimum depth that can be machined and is practical to use is *ca.* 0.1 mm, which leads to the dimensions stated above. The limited sample volume demands a high extraction efficiency in order to meet the requirement of a low detection limit. This is contrary to applications of the supported liquid membrane technique to environmental samples such as river water, where a low detection limit may be achieved by incomplete extraction of large sample volumes [7,8,15–17].

The efficiency of liquid membrane extraction depends on a number of experimental parameters [7,8,15], such as flow-rate, dimensions of the membrane unit, chemical composition of the phases, kinetic and thermodynamic properties, etc.:

$$E = 1 - \exp\left[\frac{3D_D}{\pi h_D K k_M} \ln\left(1 + K k_M \sqrt{\frac{2\pi h_D}{3 D_D \phi}}\right) - \sqrt{\frac{6 D_D}{\pi h_D \phi}}\right] \quad (1)$$

Here, E is the extraction efficiency (number of moles collected in the acceptor phase divided by number of moles originally in the extracted sample), K is the distribution coefficient of the analyte between the organic membrane phase and the donor phase, k_M is the mass transfer coefficient in the membrane phase, D_D is the diffusion coefficient of the analyte in the donor phase, ϕ is equal to $F_D/(L \cdot w)$. F_D is the volume flow-rate of the donor phase and L, w, and h_D are the length, width and depth of the donor channel, respectively.

The extraction efficiency increases when F_D is decreased, *i.e.*, when the enrichment time of the analyte increases, other parameters being the same. With the ASTED instrument, the lowest flow-rate that can be obtained is 180 μl/min, leading to a maximum enrichment time of only *ca.* 5.5 min for a 1-ml sample, which for the polar analytes considered is insufficient to give high recoveries. However, the construction of the instrument permits the sample to pass by the membrane several times, in a "push–pull" mode. Thereby the total enrichment time can easily be extended up to *e.g.* 15–20 min, significantly increasing the efficiency without exceeding the time for a reasonable HPLC analysis cycle.

The extraction efficiency after n passes, E_n, is given by [15]:

$$E_n = 1 - (1 - E_1)^n \quad (2)$$

A plot of $\log(1 - E_n)$ *vs.* n should thus be a straight line passing through the origin. As is seen in Fig. 4, the agreement with eqn. 2 is better for higher than for lower flow-rates. This is probably due to the fact that the broadening of the sample plug is larger at low flow-rates, leading to some losses during the subsequent passages. The volume set for the syringe ("3" in Fig. 2) in the push–pull operation was the same as that of the sample aliquot.

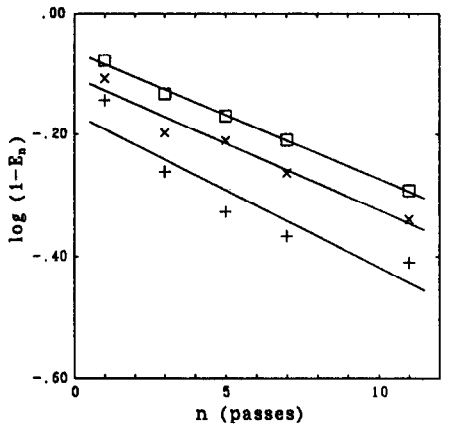


Fig. 4. Plots of $\log(1 - E_n)$ of Amperozide *vs.* the number of passes (n) through the extraction unit at different donor flow-rates. Donor pH = 9.0, acceptor pH = 2.3, membrane liquid was 5% TOPO in di-*n*-hexyl ether. + = 0.18 ml/min; × = 0.36 ml/min; ■ = 0.75 ml/min.

In Fig. 5, the extraction efficiency of Amperozide (I) is plotted *vs.* total enrichment time at three different donor flow-rates (F_D). This experiment suggests that the extraction efficiency of Amperozide, at a constant enrichment time, increases only slightly with the flow-rate. Theoretically, this implies that the mass transfer in this system is mainly limited by the mass transfer in the membrane [8,15]. It appears to be somewhat better to use a higher flow-rate and compensate for the decreased enrichment time with an increased number of passages.

The results show that reasonable extraction efficiencies (in the order of 50%) can be obtained even for polar substances within *ca.* 15 min. With an acceptor volume of *ca.* 10 μl and a sample volume of *ca.* 1 ml, a considerable enrichment (*ca.* 50 times) of the analyte is achieved.

3.3. Composition of the membrane liquid

When the mass transfer in the membrane phase is controlling the overall mass transfer, it is important that the distribution coefficient K for the analyte compound is as large as possible. For polar compounds, as the ones investigated, a polar liquid in the membrane is desirable. Since polar liquids tend to be at least somewhat soluble in water, a compromise between membrane stability and efficiency must be made.

In Table 1, experimental extraction efficiencies are given for the three model compounds with different membrane liquids under otherwise identical conditions.

Obviously, there is a prominent influence of the nature of the membrane solvent on the recovery. The best pure solvent is di-*n*-hexyl ether. With the addition of 5% trioctyl phos-

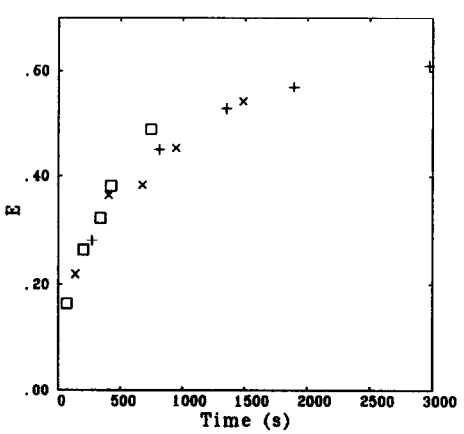


Fig. 5. Extraction efficiency (E) of Amperozide (I) *vs.* enrichment time at three different donor flow-rates. Conditions and symbols as in Fig. 4.

Table 1
Extraction efficiencies for compounds I, II and III with different membrane solvents

Solvent	Compound		
	I	II	III
n-Undecane	0.19	0.025	0.13
1-Undecanol	0.14	0.14	0.08
1-Undecanal	0.05	0.06	0.04
2-Undecanone	0.27	0.24	0.16
6-Undecanone	0.28	0.25	0.16
Di-*n*-hexyl ether	0.30	0.13	0.20
Di-*n*-hexyl ether + 5% TOPO	0.31	0.20	0.20
n-Undecane + 50% di-*n*-hexyl ether	0.22	0.07	0.13

F_D = 0.18 ml/min, n = 1, other conditions as in Fig. 4.

phine oxide (TOPO), the extraction efficiency for the most polar compound, the metabolite (II), is increased, and this combination was selected as the optimal one for the following experiments.

With this membrane liquid, the membrane lasted for *ca.* 80 samples.

3.4. Optimization of the donor pH

In order to examine the influence of pH of the donor phase on the extraction efficiency, it was varied between 5.0 and 12.0 by changing the sodium hydroxide concentration. As the pK_a of Amperozide is 6.8, any pH < 9 (*ca.* two pH units over pK_a), will lead to an incomplete deprotonation [15] and thus to a decreased extraction efficiency. At pH > 9, an increase in the sodium hydroxide concentration may increase the partition coefficient by salting out, thereby increasing the mass transfer in the membrane (*cf.* eqn. 1). Simultaneously, the viscosity of the donor solution will increase, decreasing the mass transfer in the donor phase. As the mass transfer in the membrane limits the overall mass transfer, the extraction efficiency is expected to increase slightly with increasing pH, also when pH > 9. As seen in Fig. 6, this is approximately the case for compounds I and II, while the pH dependence is less for the extraction of compound III.

The addition of ammonia is advantageous for suppression of adsorption (see memory effects). Therefore, the dependence of E on donor phase pH was investigated with the addition of ammonia as 3.8 m*M* ammonium sulphate. The results in Fig. 7 show that at pH > 9, a markedly decreased extraction efficiency is observed. This is probably due to extraction of significant amounts of NH_3, increasing the pH in the acceptor phase close to the membrane surface and preventing complete protonation of the amines.

To examine the influence of TOPO on the shape of the curve of E *vs.* pH, similar experiments were performed with only *di*-hexyl ether as the membrane liquid (and with ammonia included in the donor phase). In this case, the extraction efficiency of compound II, which is the most polar of the compounds, is diminished as the polarity of the membrane liquid is decreased (in agreement with Table 1). The shape of the curves is, however, more or less the same.

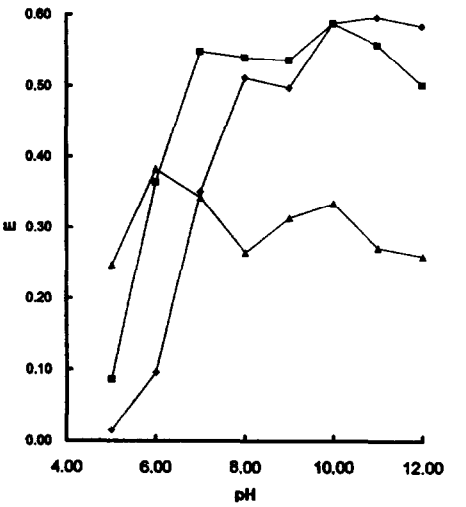


Fig. 6. Extraction efficiency for compounds I (■), II (◆) and III (▲) *vs.* donor pH. Donor: NaOH (different concentrations), EDTA (12.5 m*M*). Membrane liquid was 5% TOPO in di-hexyl ether. $F_D = 0.18$ ml/min, $n = 3$. Acceptor pH = 2.3.

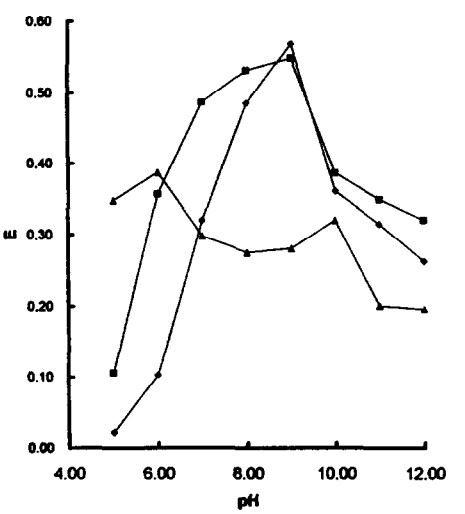


Fig. 7. Extraction efficiency *vs.* donor pH, as in Fig. 6, except that the donor phase additionally contains 3.8 m*M* ammonium sulphate.

3.5. Optimization of the acceptor pH

The pH of the acceptor phase was varied between 1.5 and 3.1. As can be seen in Fig. 8, all three compounds showed a maximum in extraction efficiency around pH 2.3. From earlier experiments [7] and theory [15], the recovery was expected to be independent of the acceptor pH as long as this is sufficiently low to ensure immediate protonation of the analytes. The same experiment was performed without TOPO. In this case, all three substances showed a similar maximum, only at a slightly higher pH (2.5).

From these comparisons, it seems that TOPO enhances the extraction of polar substances, but doesn't significantly influence the pH dependence of the extraction process.

3.6. Quantification

Calibration curves, based on peak heights, were made for all three compounds (Table 2). Seven aqueous samples with concentrations in the range 0–1000 ng/ml were processed in triplicate using a donor flow-rate of 0.18 ml/min, and one passage by the membrane, giving an enrichment time of *ca.* 5.5 min. All curves showed good linearity and the intercepts did not differ significantly from zero at a 95% confidence level. The mean recoveries were 35%, 34% and 20% for compounds **I**, **II** and **III**, respectively.

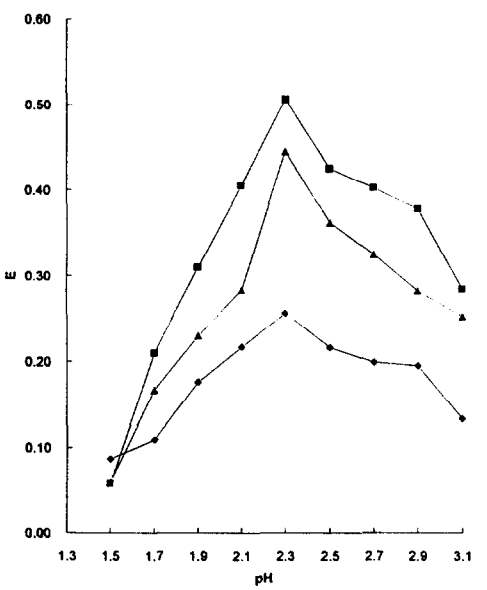


Fig. 8. Extraction efficiency *vs.* acceptor pH. Membrane liquid was 5% TOPO in di-*n*-hexyl ether. Donor pH = 9.0, other conditions were as in Fig. 6.

Table 2
Calibration curve parameters for the model compounds

Compound	Slope[a]	Intercept[a]	*r*
I	0.108 ± 0.004	0.05 ± 1.59	0.9996
II	0.121 ± 0.001	−0.22 ± 0.46	0.9999
III	0.098 ± 0.005	−1.45 ± 1.98	0.9992

[a] Arbitrary units; 95% confidence intervals.

3.7. Repeatability

The overall repeatability (relative standard deviation) was *ca.* 2% based on three analyses of a 0.5 μg/ml solution. Fifteen consecutive analyses of a 1 μg/ml solution were also made (see Memory effects, below). The peak heights of compound **III** showed a slight increase, which gave rise to a R.S.D. of 3.4%, but the other substances still showed a R.S.D. of *ca.* 2%. The first injection gave markedly smaller peak heights and is not included in the reproducibility calculations.

3.8. Detection limit

The detection limit with UV detection after one passage is about 20 ng/ml for all three substances. This corresponds to peak heights twice the baseline noise. For bioanalysis, much lower detection and determination limits are needed, which can be achieved with electrochemical detection [18].

3.9. Memory effects

Amines are in general apt to be adsorbed on surfaces of both polymers, glass and metals. One way to minimize this tendency is to keep the amines in ionized form. To decrease the ad-

sorption in the enrichment process when the amines are unprotonated, *ca.* 200 μg/ml of ammonia as ammonium sulphate (3.8 m*M*) was added to both the donor and acceptor phases as a competing adsorbate.

To evaluate the adsorption in the HPLC step, the substances were first injected directly on the column, without the ASTED equipment. All three substances exhibited no memory effects at all in a subsequent blank injection, except for compound **III**. For this substance, less than 1% of the first peak showed up in the subsequent blank. The experiment was performed with both a 0.5 and a 1.5 μg/ml solution.

To prevent adsorption in the sample vials waiting for analysis, the solutions were kept in glass vials at a pH around 4 and each sample was automatically mixed with the alkaline donor solution prior to the enrichment (see Operation). It was found that the recovery decreased with increasing number of mixing cycles, probably due to adsorption in the needle and the mixing coil ("9" in Fig. 2). The highest recovery was obtained when the donor solution was added and manually mixed with the sample before loading into the sample rack. This would, however, lead to the samples being stored in alkalized form in the sample rack, waiting for analysis during different periods of time.

This was evaluated with 15 identical samples which were injected consecutively. The substances (1 μg/ml) were dissolved in donor buffer (pH 9) and kept in glass vials during the 4-h experiment. There was a pronounced decrease of the peak heights during the experiment, for compound **I** 11%, for compound **II** 7% and for compound **III** 20%. When the substances were kept in a solution at pH 4 instead, and automatically mixed with pH 9 donor buffer immediately prior to the extraction, no decrease in peak height was observed. The experiment shows that it is important to keep the amines ionized as long as possible and that the mixing with alkali should be made immediately before each analysis, and with one mixing cycle.

With earlier set-ups [11] the alkalization was made in a flow system immediately before the membrane. It is a disadvantage with the ASTED equipment that the samples must be alkalized already in the vials so the amines are transferred through the needle and tubing in neutral form. The resulting memory effects can be controlled by washing as described below, but the overall recovery will be adversely affected. The standard materials in the equipment is stainless steel for the needle, EPF for the coiled tubing (9), PTFE for other tubing and PVDF for the membrane unit. Some experiments have been made with other materials, but without striking improvements.

The memory effects in the tubing and the membrane unit are dependent on the extent of washing. With the ASTED equipment three different washing procedures can be used: "wash", washing of the donor channel, "rinse", washing of the needle, and "regeneration", washing of both the donor and the acceptor channel simultaneously. By changing the different washing times, it was found that washing the acceptor channel had greater influence on the memory effects than washing the donor channel. With the different washing volumes chosen, wash: 1 ml, rinse: 2 ml and regeneration: 3 ml, the memory effects for compounds **I**, **II** and **III** were 2%, 0% and 10%, respectively.

3.10. Discussion

Compared to the previously developed methods for membrane enrichment, the approach presented here has two main advantages; a large number of samples, up to 60, can be automatically processed and the recovery of polar analytes, which normally is low, can be improved without seriously extending the analysis time by passing the same sample plug by the membrane several times.

The technique is especially interesting for bioanalysis of plasma samples. Often only small sample volumes are available (less than 1 ml) and the concentrations of the analytes are often so low that efficient enrichment is needed. The membrane workup results in a considerable enrichment and, additionally, a solution free from macromolecules, which is favourable for the final HPLC separation.

The extent of enrichment is determined by the ratio between the sample volume and the acceptor volume and by the analyte recovery. Here we have shown that with the procedure used, the recovery can be increased by extending the residence time. If this residence time exceeds the time needed for the final HPLC step, the time for the total analysis will be prolonged, otherwise, one sample can be extracted while the previous one is chromatographed. The choice may be to pay in analysis time to obtain sufficient recovery.

In this work we have used an UV detector. With the effective sample clean up in the enrichment step, an electrochemical detection approach seems promising. The model compounds, as is usually the case in bioanalysis, are electrochemically active [18], which makes the approach especially interesting in this area. Work along these lines is in progress.

Here we have shown how the system can be used for basic compounds. With small changes in the system set-up, *i.e.* by changing the composition of the donor and acceptor buffers and of the membrane liquid [8], other substances, such as organic acids or permanent ions, can be processed.

4. Acknowledgements

We want to thank Anna-Karin Nilsson, Ann Andersson and Henrik Björk for skilful experimental work, Martin Hjelmtvedt, Lambda Instruments, for putting the ASTED instrument at our disposal and the Crafoord Foundation for financial support.

5. References

[1] S.K. Poole, T.A. Dean, J.W. Oudsema and C.F. Poole, *Anal. Chim. Acta*, 236 (1990) 3.
[2] R.D. McDowall, *J. Chromatogr.*, 492 (1989) 3.
[3] M. Valcárcel and M.D. Luque de Castro, *Non-chromatographic Continuous Separation Techniques*, The Royal Society of Chemistry, Cambridge, 1991.
[4] G.D. Clark, D.A. Whitman, G.D. Christian and J. Ruzicka, *Crit. Rev. Anal. Chem.*, 21 (1990) 357.
[5] M.W.F. Nielen, R.W. Frei and U.A.Th. Brinkman, in R.W. Frei and K. Zech (Editors), *Selective Sample Handling and Detection in High-performance Liquid Chromatography*, Elsevier, Amsterdam, 1988, Ch.1, p. 5.
[6] R.D. McDowall, J.C. Pearce and G.S. Murkitt, *Trends Anal. Chem.*, 8 (1989) 134.
[7] G. Audunsson, *Anal. Chem.*, 58 (1986) 2714.
[8] J.Å. Jönsson and L. Mathiasson, *Trends Anal. Chem.*, 11 (1992) 106.
[9] N.C. van de Merbel, J.J. Hageman and U.A.Th. Brinkman, *J. Chromatogr.*, 634 (1993) 1.
[10] G.A. Audunsson, *Anal. Chem.*, 60 (1988) 1340.
[11] B. Lindegård, J.Å. Jönsson and L. Mathiasson, *J. Chromatogr.*, 573 (1992) 191.
[12] G. Nilvé, G. Audunsson and J.Å. Jönsson, *J. Chromatogr.*, 471 (1989) 151.
[13] L. Mathiasson, M. Knutsson, G. Bremle and L. Mårtensson, *Swedish J. Agric. Res.*, 21 (1991) 147.
[14] L. Grönberg, Y. Shen and J.Å. Jönsson, *J. Chromatogr. A*, 655 (1994) 207.
[15] J.Å. Jönsson, P. Lövkvist, G. Audunsson and G. Nilvé, *Anal. Chim. Acta*, 227 (1993) 9.
[16] L. Mathiasson, G. Nilvé and B. Ulén, *Int. J. Environ. Anal. Chem.*, 45 (1991) 117.
[17] M. Knutsson, G. Nilvé, L. Mathiasson and J.Å. Jönsson, *J. Agric. Food Chem.*, 40 (1992) 2413.
[18] A.-M. Olsson, C.G. Johansson and L. Gorton, Poster presented at the *9th International Symposium on Column Liquid Chromatography, Edinburgh, 1985.*