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Liquid membrane work-up of blood plasma samples applied to gas chromatographic determination of aliphatic amines

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ABSTRACT

A technique for sample work-up and enrichment using a supported liquid membrane in an automated flow system, connected to a gas chromatograph, was used for the determination of aliphatic amines in human blood plasma. The amines studied were N,N-dimethylethylamine, triethylamine, N-methylmorpholine, cyclohexylamine and N,N-dimethylcyclohexylamine. An efficient clean-up of the complex plasma matrix was achieved, resulting in identical blank chromatograms for plasma samples and aqueous solutions. Different parameters influencing the efficiency and selectivity of the extraction procedure were experimentally studied and theoretically explained. The detection limit depends on the extraction flow-rate and the available sample volume. With 1 ml of sample and a flow-rate giving an extraction time of 16 min, the detection limit was *ca.* 5 ppb (5 μg/l); with 4 ml of sample and a lower flow-rate, sub-ppb detection limits could be reached in *ca.* 3 h. Linear calibration curves up to 500 ppb were obtained. Blood plasma samples from volunteers exposed to N,N-dimethylethylamine in air were analysed, and the results compared favourably with independent measurements by another method.

INTRODUCTION

Gas chromatographic (GC) determination of trace compounds in blood plasma can usually be undertaken only with elaborate sample preparation procedures, involving precipitation, extraction and concentration steps, as recently reviewed [1]. There is a need for the development of new sample preparation methods, especially ones that can easily be automated.

Recently, extraction using a supported liquid membrane in a flow system has been described [2] and used for various applications, such as determination of amines in urine [3], of herbicides in water [4,5] and of acids in manure [6]. The equipment can be connected directly to analytical instruments, thus permitting automation of the sample preparation procedure. It has also been used off-line for time-integrating environmental field sampling of herbicides [7]. The method can be directly used for either acidic or basic compounds, and with suitable complexing agents also for other types of analyte.

The most important feature of liquid membrane work-up for plasma samples is that it offers a highly selective extraction and efficient enrichment of the analytes from the matrix in one step.

Determination of trace levels of aliphatic amines in body fluids is of interest for the evaluation of metabolic patterns and for biological monitoring after occupational exposure [8]. It is advantageous to perform these studies with plasma samples instead of with urine samples, because the presence of excess endogenous amines, dilution variations and the risk of microbial degradation are avoided. Typical concentrations in plasma after occupational exposure are 40–200 ppb for triethylamine [8] and *ca.* 15 ppb for dimethylethylamine [9]. In these studies, amines were determined in plasma by GC after extrac-

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tion with di-*n*-butyl ether [8,10] or benzene [11].

This paper describes the application of the liquid membrane sample preparation technique to the determination of some aliphatic amines in blood plasma. The amines chosen are of interest in studies of occupational exposure.

EXPERIMENTAL

Equipment

The experimental set-up was earlier described by Audunsson [3] and is shown in Fig. 1. Two peristaltic pumps (Minipuls 2, Gilson Medical Electronics, Villers-le-Bel, France) with PVC tubing (Elkay Products, Shrewsbury, MA, USA) were used to control the donor and acceptor flow-rates independently. The various parts of the flow system were connected with 0.5 and 0.3 mm I.D. PTFE tubing and Altex screw fittings. The junctions were made of PTFE where the channels meet at a 60° angle. The sample loop (usually 2.5 ml) was filled by a peristaltic pump (Ismatec, Zürich, Switzerland), and the sample was introduced into the donor stream via a pneumatically actuated six-way Kel-F slider valve (I) (Cheminert; Laboratory Data Control, UK). The switching valves for exchanging the donor stream and the washing solution (III) and for bypassing the membrane separator (IV) were both pneumatically actuated four-way Kel-F slider valves (Cheminert).

The sample was mixed with sulphuric acid and EDTA in a knitted tubing (II), 13 cm (six knots) × 0.5 mm I.D., made of ethylvinyl acetate (Micro-Line tubing, Cole-Parmer, Chicago, IL, USA).

Before injection into the gas chromatograph, the treated sample was mixed with sodium hydroxide in a single-bead string reactor (V) (10 cm × 0.8 mm I.D. tubing with 40–60 mesh glass beads).

The membrane separator was machined from blocks of titanium by cutting two grooves in the form of a spiral with a radius increase of 2 mm per cycle on the opposite faces of the two blocks (see Fig. 2). Each groove was 0.25 mm deep, 1.5 mm wide and had a length of 150 mm, resulting in a calculated volume of *ca.* 56 μl. The membrane was clamped tightly between the surfaces of the blocks by six screws. An O-ring encircling the grooves outside the membrane provided an additional seal. The liquid membrane support was Fluoropore FG (average pore size 0.2 μm, porosity 0.70, total thickness 175 μm, of which 115 μm is polyethylene backing; Millipore, Bedford, MA, USA).

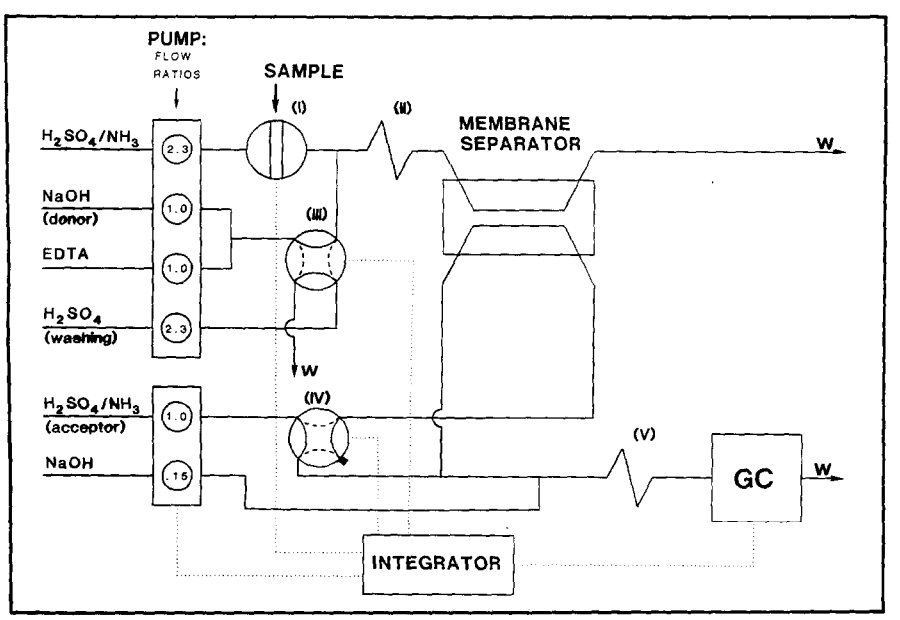

Fig. 1. Experimental set-up (for details, see text).

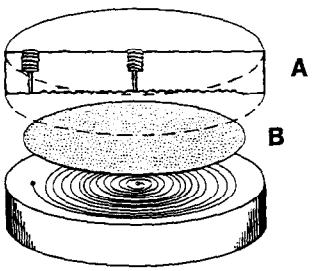

Fig. 2. Membrane separator unit composed of two machined titanium blocks (A) and a PTFE membrane (B), impregnated with stationary liquid.

The liquid membrane was prepared by immersing the membrane in the chosen solvent for 15 min. After installation in the separator, excess solvent on the membrane surface was removed by pressing water through both channels. We never observed physical rupture of the membrane, but after extended use breakthrough occurred between the two aqueous phases owing to disappearance of the organic liquid. This was indicated by flow-rate changes and verified by the observation of high pH values in the acceptor stream.

A gas chromatograph (Model 3700, Varian, Walnut Creek, CA, USA) equipped with a Varian 8070 automatic sampling interface with a 10-μl syringe was used for the measurements. The injected volume was adjusted to 2 or 4 μl. A glass column (190 cm × 3 mm I.D.) was used, packed with *ca.* 10 g of 28% Pennwalt 223 with 4% potassium hydroxide on Gas-Chrom R (Alltech Assoc., Deerfield, IL, USA)[a].

A nitrogen-sensitive detector (TSD, Varian) was used, and typical parameter settings were: bias voltage, −4 V; bead current, 500 scale divisions; detector temperature, 250°C; injector temperature, 290°C; air flow-rate, 170 ml/min; hydrogen flow-rate, 2 ml/min. Nitrogen was used as carrier gas at a flow-rate of 26 ml/min.

An integrator (Model CI-10, LDC/Milton Roy, Riviera Beach, FL, USA) was used both for data acquisition and for controlling the time sequence of the operation of valves, pumps and sampling interface.

[a] Pennwalt 223 is no longer available, but a replacement (Alltech 223) is offered, which is claimed to be identical.

Operation of the system

The acidic sample solution is filled into a sample loop by a peristaltic pump and the sample is introduced into the donor stream by switching valve I. The sample is mixed with sodium hydroxide (to ensure that the amines are uncharged) and with EDTA (to keep the metals in the plasma in solution) in a knitted tubing II. The donor stream passes along the donor channel on one side of the hydrophobic liquid membrane, and only those substances that are uncharged can penetrate the membrane. On the other side of the membrane the solution is acidic and kept static. The amines and other basic substances are protonated and are irreversely trapped on the acceptor side. After the sample has passed the membrane, valve III is switched and the donor side is washed with dilute sulphuric acid, which removes uncharged substances that are distributed between all three phases. Valve IV is then switched, and the accumulated and washed sample plug is mixed with sodium hydroxide in a single-bead string reactor (V) and then transferred to the sampling interface and injected into the gas chromatograph.

To prevent bubble formation, the solutions (except for sodium hydroxide and sample) were degassed with helium.

The time needed for a complete enrichment experiment is determined by the volume of the sample loop and the corresponding flow-rate, which is *ca.* 53% of the total flow-rate through the donor channel, referred to below.

If a low flow-rate is necessary because of small sample volumes and low concentrations, several samples can be enriched simultaneously in separate membrane devices coupled to a single gas chromatograph.

Chemicals

The amines studied are listed in Table I. Stock solutions (0.1%) of the amines were prepared in 50 m*M* sulphuric acid. The stock solutions were stable for at least one year.

The organic solvents used were dihexyl ether (Sigma, St Louis, MO, USA) and *n*-undecane (E. Merck, Darmstadt, Germany). Ammonium sulphate and sulphuric acid were purchased from E. Merck and sodium hydroxide from EKA (Bohus, Sweden). The water was purified with a Milli-Q/RO-4 unit (Millipore).

TABLE I

INVESTIGATED AMINES

Name	Abbreviation	Source[a]	pK_a[b]	CAS number
N,N-Dimethylethylamine	DMEA	Janssen	10.0	598-56-1
Triethylamine	TEA	Fluka	11.0	121-44-8
N-Methylmorpholine	NMM	Fluka	7.4	109-02-4
Cyclohexylamine	CHA	Schuchardt	10.7	108-91-8
N,N-Dimethylcyclohexylamine	DMCHA	Aldrich	10.5	98-94-2

[a] Janssen Chimica, Beerse, Belgium; Fluka, Buchs, Switzerland; Schuchardt, Munich, Germany; Aldrich Chemie, Steinheim, Germany.
[b] Ref. 12.

Plasma samples

Venous blood was collected in heparinized tubes. After cooling and centrifugation, 2 ml of plasma were acidified with 0.5 ml of 1.0 *M* hydrochloric acid and stored at 4°C. The samples were then diluted 1:1 with water to decrease the viscosity and analysed on the same day. The total dilution of the samples amounts to 2.5 times and a sample spiked with 40 ppb (μg/l) of amines thus corresponds to an original concentration of 100 ppb. When not otherwise stated, amine concentrations in plasma refer to the diluted sample.

RESULTS AND DISCUSSION

Chromatograms

Fig. 3a shows a chromatogram of an acid solution of the amines DMEA, TEA, NMM, CHA and DMCHA (see Table I for abbreviations) enriched via the liquid membrane after optimization of various parameters as discussed below. Fig. 3b shows a chromatogram of the corresponding blank.

Chromatograms of a plasma sample spiked with the same amines and of a plasma blank are shown in Fig. 4a and b, respectively.

In these figures, it is clear that the acid blank and the plasma blank are virtually identical, showing that a very selective clean-up of the complex plasma matrix is achieved. The response of the amines is slightly lower in plasma than in acid, which is due to a viscosity effect. The appearance of some ghost peaks at the start of the chromatograms is related to the GC procedure, and not to the liquid membrane system. These aspects are further discussed below.

Immobilized liquid and acceptor pH

In ref. 3, where amines were determined in human urine, *n*-undecane was used as membrane solvent. Human urine contains relatively high concentrations of ammonia and water-soluble amines as monomethylamine, dimethylamine and monoethylamine, whereas amines occurring in urine after occupational exposure in general are tertiary, *e.g.* TEA, and therefore less water-soluble. The non-polar *n*-undecane gives a low enrichment factor for very water-soluble compounds, providing an increased selectivity for the less hydrophilic amines of interest.

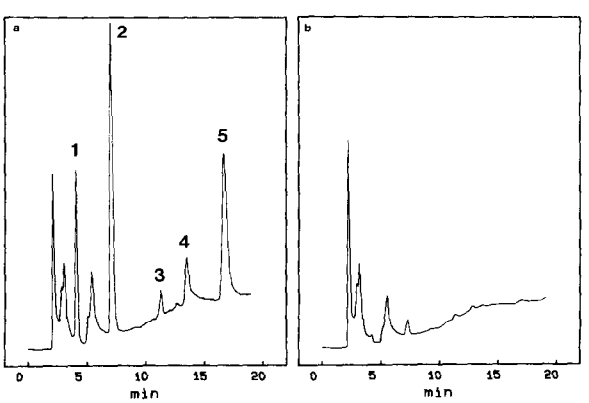

Fig. 3. (a) Chromatogram of a solution of the investigated amines (peaks: 1 = DMEA; 2 = TEA; 3 = NMM; 4 = CHA; 5 = DMCHA; 25 ppb of DMEA, 50 ppb of the others) in 50 m*M* H_2SO_4. The 2.5-ml sample was enriched by the liquid membrane technique. For abbreviations of amines see Table I. (b) Chromatogram of a blank, processed in the same way. Conditions: donor phase flow-rate, 0.30 ml/min; donor stream solutes, 0.58 *M* NaOH, 6 m*M* EDTA; liquid membrane, 50% dihexyl ether in *n*-undecane; acceptor phase, 52 m*M* H_2SO_4, 17 m*M* NH_3; washing step, 3 min with 25 m*M* H_2SO_4; attenuation $4 \cdot 10^{-12}$ A f.s.; temperature programming, 90°C (1 min), 10°C/min to 185°C (10 min).

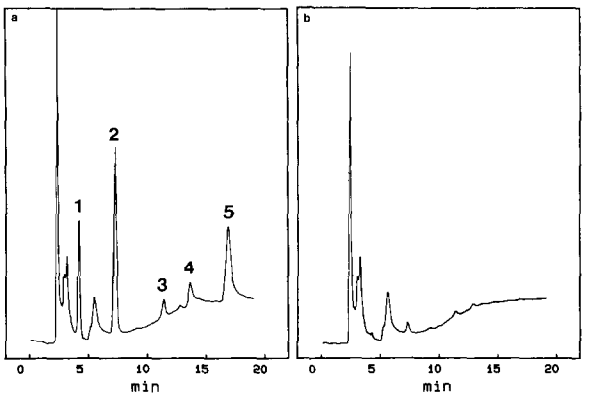

Fig. 4. (a) Chromatogram of acidified blood plasma, diluted 1:1 with water, and spiked with 40 ppb each of the investigated amines (20 ppb of DMEA) (see Fig. 3). (b) Chromatogram of a blank, processed in the same way. Conditions as in Fig. 3.

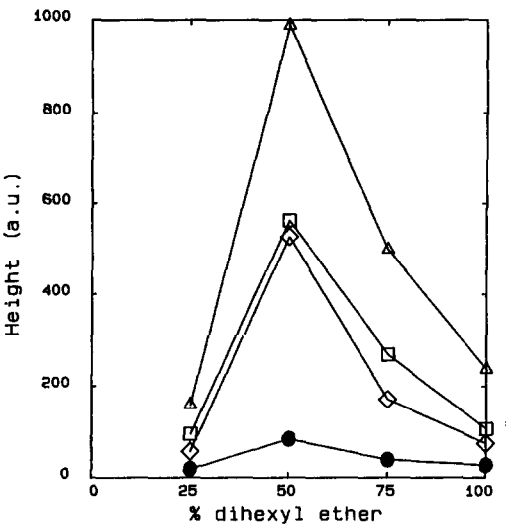

Fig. 5. Peak height (arbitrary units) for the amines DMEA (□), TEA (△), NMM (●) and CHA (◇) after enrichment of 2.5 ml of a 1-ppm solution (0.5 ppm of DMEA) in 50 m*M* H_2SO_4. The liquid membrane contained different concentrations of dihexyl ether in *n*-undecane. Other conditions were similar to those in Fig. 3, except for the acceptor, which consisted of 0.1 *M* phosphate buffer (pH 3.5).

The amount of shorter amines and ammonia is very much lower in plasma than in urine. Thus the selectivity of the chosen solvent mixture is not as important as a high transfer rate of the amines to be determined. Therefore, different amounts of the more polar solvent dihexyl ether were added to *n*-undecane in order to facilitate the transport of the water-soluble substances, especially NMM, through the membrane. A 1:1 (v/v) mixture of *n*-undecane and dihexyl ether was found to give the highest transfer rate for DMEA, TEA, NMM and CHA (see Fig. 5).

With this mixture, the stability of the liquid membrane was satisfactory: the average lifetime before breakthrough was two to three weeks, after which it was usually possible to reimpregnate the membrane with new liquid, but with a shorter subsequent lifetime. One membrane preparation survived 52 plasma runs. Another one lasted for 146 runs, of which 20 were plasma.

Two different values of the pH in the acceptor phase were studied, 3.5 and 5.5, in 0.1 *M* phosphate buffer. Both values are well below the pK_a for the least basic amine, NMM, (which is 7.4) but nevertheless slightly higher recoveries were obtained at the lower pH (3.5). This indicates that it may not be enough to have an acceptor pH just below the theoretical pK_a of the amines, as has been previously discussed [3].

Thus a 50% (v/v) mixture of *n*-undecane and dihexyl ether and an acceptor pH not exceeding 3.5 was chosen for the subsequent measurements.

Donor flow composition

The donor flow consisted of a mixture of the sample, sodium hydroxide to make the amines unprotonated and EDTA to ensure that the metals in plasma did not precipitate at the high pH values used. Increasing the concentration of sodium hydroxide and EDTA led to increased ionic strength and viscosity. Assuming steady-state kinetics, instant protonation, and that the transfer rate depends only on the mass transfer in the donor and the membrane phases, the transfer rate is determined [2] by the overall mass transfer coefficient *K*:

$$\frac{1}{K} = \frac{1}{k_A} + \frac{1}{k_B K_P} \qquad (1)$$

where k_A and k_B are the mass transfer coefficients in the donor phase and in the membrane, respectively, and K_P is the partition coefficient.

Increasing the salt concentration increases K_P (salting-out effect) but decreases k_A (viscosity effect). If K_P is low, the mass transfer is mainly controlled by $k_B K_P$. Therefore the transfer rate of water-soluble amines will be increased by a high

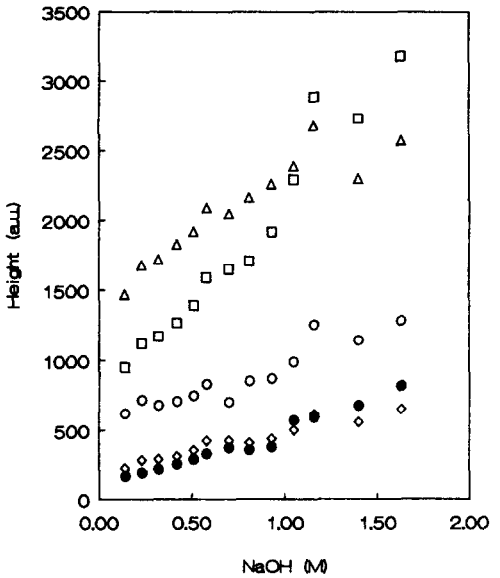

Fig. 6. Peak height (arbitrary units) for the amines DMEA (□), TEA (△), NMM (●), CHA (◇) and DMCHA (○) after enrichment of 2.5 ml of 50-ppb (25 ppb of DMEA) solutions in blood plasma, diluted 1:1 with water. The acceptor contained different concentrations of NaOH and 5.8 m*M* EDTA. Other conditions were similar to those in Fig. 3.

ionic strength. However, if K_P is initially high, the mass transfer is mainly controlled by k_A, so the transfer rate of less water-soluble amines will be decreased by a high salt concentration.

To examine this influence on the response of the five amines in plasma, the sodium hydroxide concentration in the donor phase was varied between 0.14 and 1.63 *M* (see Fig. 6). The concentration of EDTA was 5.8 m*M*, high enough to cover the expected concentration of metals in plasma.

For all amines, the response increased with increasing sodium hydroxide concentration regardless of the higher viscosity, although the viscosity of plasma is 1.4–1.8 times higher than that of water [13]. The response at the highest sodium hydroxide concentration was between 1.8 and 4.8 times higher than the response at the lowest concentration, and NMM showed the greatest increase, which is expected as this compound is the most water-soluble.

To obtain the highest donor concentration of sodium hydroxide (1.63 *M*) a 7.0 *M* solution must be used, owing to the dilution in the flow system. Using such a high concentration tends to reduce the lifetime of the liquid membrane and the reproducibility, apart from being messy. A lower concentration is thus recommended under "normal" conditions and, for most of the measurements, a donor flow concentration of sodium hydroxide of 0.58 *M* was used.

To decrease the viscosity of the sample, the plasma was diluted 1:1 with water. As seen in Table II, the response of the amines is somewhat lower in a plasma–water mixture than in 50 m*M* sulphuric acid. A similar effect has previously been noticed during the determination of drugs in plasma, and was explained by association of the drug with the plasma proteins [1]. This is, however, probably not the case here as the most water-soluble amine, NMM, is hardly affected, whereas the transfer rate in plasma of the more hydrophobic DMCHA is lowered to 78%. The reason for this is instead explained by eqn. 1 as the change of viscosity, and thus of k_A, influences K mainly for compounds with high K_P values.

TABLE II

MATRIX INFLUENCES

Matrix	Peak height[a] (arbitrary units)				
	DMEA	TEA	NMM	CHA	DMCHA
Plasma	5936	5994	1569	1799	2011
50 m*M* H_2SO_4	6635	7214	1606	2132	2567
Quotient[b]	0.89	0.83	0.98	0.84	0.78

[a] Concentration: 25 ppb of DMEA, 50 ppb of other amines.

[b] (Peak height in plasma)/(peak height in acid).

Donor flow-rate

When the sample size is limited, it is better to use low flow-rates, which gives higher enrichment per sample volume [2]. This is the case when analysing plasma samples, where the sample volume usually is limited to 10 ml or less. The influence of the flow-rate on the recovery of the five amines was investigated by enriching 2.5 ml of spiked plasma samples at two different flow-rates, 0.10 and 0.35 ml/min.

As can be seen in Table III, a lower donor flow-rate leads to markedly increased enrichment, owing to a longer contact time. This effect is less pronounced for less polar substances. For these compounds the transfer rate is mainly governed by k_A, which is proportional to the square root of the flow-rate [2]. Therefore, the transfer rate increase due to longer contact times at lower flow-rates is offset by a decrease in k_A. The time for enrichment, however, increases from 14 to 47 min with the lower donor flow-rate.

Plug profile

When the sample enters the donor channel, the extraction process of the solutes starts and their concentrations in the donor channel decrease as the sample moves towards the outlet. A substance with a high transfer rate is extracted rapidly into the liquid membrane, so the highest proportion is found in the beginning of the acceptor channel. For a substance which is extracted more slowly, *e.g.* NMM, the concentration profile on the acceptor side is more spread out. After enrichment the acceptor plug is pumped to the sampling interface. The final peak-width is somewhat increased by diffusion during the enrichment and by dispersion during the transport. As only a minor part of the acceptor volume is injected into the gas chromatograph (typically 2 μl of *ca.* 60 μl), it is very important that the part of the plug profile with the highest concentration is injected. This also implies that it is necessary to maintain a constant acceptor flow-rate and a reproducible timing.

To examine the position of the peak concentration maxima for the different amines when the enriched sample enters the flow-through syringe in the sampling interface, the transfer time was varied between 1.0 and 1.9 min. This is the time between switching valve IV, which starts the transfer of the plug from the acceptor channel, and injecting the plug into the gas chromatograph. The donor flow-rate was 0.3 ml/min.

As can be seen in Fig. 7, the concentration maxima are very close to each other and the peak shapes are very similar. Only NMM, the most polar of the examined amines, has its maximum slightly later than the other amines. At lower flow-rates the difference can be greater, which can be seen in a similar experiment with TEA and monoethylamine [3].

Memory and carry-over effects

One of the greatest problems in the trace determination of amines is their tendency to be adsorbed on the surfaces of glass, metals and polymers, which may lead to memory effects in the chromatographic system [14] or carry-over problems in flow systems. One way to minimize the problem is to keep the amines in ionized form for

TABLE III

INFLUENCE OF DONOR PHASE FLOW-RATE

Flow-rate (ml/min)	Peak height[a] (arbitrary units)				
	DMEA	TEA	NMM	CHA	DMCHA
0.35	563	833	88	77	257
0.10	1066	1472	216	232	544
Quotient[b]	1.9	1.8	2.5	3.0	2.1

[a] Concentration: 25 ppb of DMEA, 50 ppb of other amines.

[b] (Peak height at 0.10 ml/min)/(peak height at 0.35 ml/min).

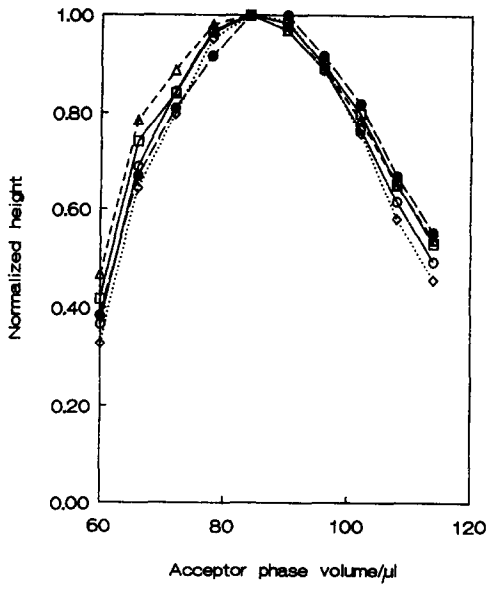

Fig. 7. Normalized peak height for the amines DMEA (□), TEA (△), NMM (●), CHA (◇) and DMCHA (○) after enrichment of 0.5 ml of 5-ppm solutions in 50 m*M* H_2SO_4. The transfer time (see text) was varied between 1.0 and 1.9 min, leading to different volumes of acceptor liquid passing the sampling interface before injection. Other conditions were similar to those in Fig. 3.

as long as possible. However, when passing the liquid membrane for enrichment, and also before injection into the gas chromatograph, the amines must be uncharged. To decrease the adsorption of uncharged amines, *ca.* 200 ppm of ammonia as ammonium sulphate was added to the donor and acceptor phases. To diminish the adsorption in the chromatographic system, a 2.5 *M* solution of sodium hydroxide was injected when necessary, as described in ref. 15.

After the sample has been enriched, dilute sulphuric acid as a washing solution is pumped through the donor side. Neutral compounds in the acceptor phase, which can equilibrate between the donor phase, the membrane and the acceptor phase, diffuse back to the donor phase and then to waste. Additionally, the acid washes out any remaining plasma and amines from the flow system.

Carry-over effects in the acceptor channel occur in sub-ppm determinations of amines if the channel is not sufficiently washed with pure acceptor solution.When the flow through the acceptor channel was stopped directly after the time needed to transport the sample plug to the sampling interface, *ca.* 20% of a 100-ppb amine sample remained and showed up in a subsequent blank chromatogram. Rinsing the acceptor channel with three additional volumes of the acceptor solution practically eliminated this problem. Thus a fraction of the amines does not reach the chromatographic column. It is therefore important that calibrations are made with amine concentrations similar to those in the samples.

Ghost peaks

In an experiment in which the washing time after enrichment of a plasma sample was varied between 0 and 10 min, it was shown that the ghost peaks seen in Figs. 3 and 4 were unaffected by the washing. This means that they do not originate from neutral compounds. The peaks probably originate from degradation products of the column, as they also show up after manual syringe injection of aqueous basic amine solutions directly into the gas chromatograph. Similar effects were previously observed with the same column material and similar conditions [14].

Quantification

Calibration curves, based on peak heights of triple injections (Table IV) of the five amines studied, were made in both 50 m*M* sulphuric acid and plasma samples. In sulphuric acid, seven different samples with a volume of 2.5 ml and amine concentrations in the range 10–500 ppb were processed with a donor flow-rate of 0.30 ml/min. The enrichment time was thus 16 min. In plasma, eight different concentrations in the range 5–500 ppb were processed in the same way. The intercepts did not differ significantly from zero at a 95% confidence level, except for CHA and DMCHA in plasma, probably owing to adsorption at low concentrations.

As has been discussed above and shown in Table II, the reponse is lower in plasma than in sulphuric acid. This is most pronounced in the case of DMCHA, while NMM hardly is affected. The experiments for plasma samples presented in Table IV were made two months later than those for acid samples, so the absolute values of the slopes are not directly comparable owing to the unavoidable change in the sensitivity of the nitrogen-sensitive detector.

TABLE IV

CALIBRATION CURVES

	Slope[a]	Intercept[a]	r	R.S.D.[b] (%)	LOD[c]
In sulphuric acid					
DMEA	42.6 ± 1.0	−5 ± 108	0.9997	8	5
TEA	36.5 ± 0.6	−32 ± 116	0.9999	8	2
NMM	2.7 ± 0.2	−13 ± 50	0.9969	8	5
CHA	8.6 ± 0.3	−59 ± 58	0.9996	6	10
DMCHA	21.0 ± 0.6	−68 ± 116	0.9997	6	5
In plasma					
DMEA	33.6 ± 0.8	−14 ± 78	0.9997	4	5
TEA	22.0 ± 0.5	−19 ± 99	0.9997	4	2
NMM	2.4 ± 0.3	47 ± 52	0.9938	3	5
CHA	4.3 ± 0.2	−47 ± 43	0.9987	16	10
DMCHA	8.5 ± 0.2	−52 ± 45	0.9996	14	5

[a] Arbitrary units, 95% confidence interval.

[b] Relative standard deviation at 100 ppb.

[c] Limit of detection, ppb, at donor phase flow-rate of 0.30 ml/min, with 2.5-ml sample.

The overall precision (relative standard deviation), based on triple injections at a concentration of 100 ppb was *ca.* 7% both in sulphuric acid and in plasma.

The detection limits were *ca.* 5 ppb in these experiments, corresponding to 12.5 ppb in an original sample (see Experimental). They can, however, be decreased if a lower donor flow-rate and a larger sample volume are used. With a flow-rate of 0.10 ml/min and 20 ml of sample (8 ml of plasma), the detection limits in the original plasma sample were *ca.* 0.05, 0.1, 0.5, 0.5 and 0.3 ppb for DMEA, TEA, NMM, CHA and DMCHA, respectively (Fig. 8). The total processing time is increased to *ca.* 6.5 h. At this concentration level a plasma blank gives rise to interfering peaks for NMM and CHA, but not for the other investigated amines.

Application

The liquid membrane technique was applied to plasma samples taken from volunteers esposed to DMEA in an exposure chamber, as described by Åkesson [8]. The concentration in the air was 40 mg/m^3, and the exposure lasted for 8 h. Blood samples were taken during and after the exposure, and analysed as described above (2.5 ml sample, donor flow-rate 0.1 ml/min). Table V lists the plasma concentrations of DMEA obtained. For comparison, the samples were also analysed by the Department of Occupational Medicine, using the method described by Lundh *et al.* [10]. Briefly, the amines were extracted from the plasma with *n*-butyl ether from basic solution and analysed by GC using standard addition. The detection limit for DMEA in plasma was 3 ppb.

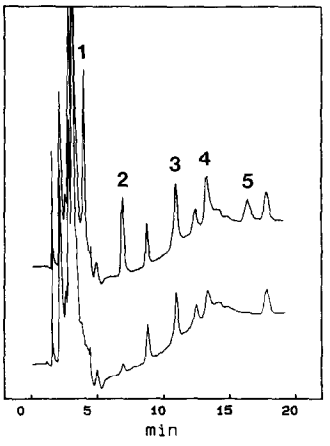

Fig. 8. Chromatogram of blood plasma, diluted 1:1 with water, and spiked with 1 ppb each (0.5 ppb of DMEA) of the investigated amines (see Fig. 3) and the corresponding blank. The donor flow-rate was 0.1 ml/min and the sample volume 20 ml. Other conditions as in Fig. 3.

TABLE V

CONCENTRATIONS OF DMEA IN PLASMA FROM EXPOSED PERSONS

Time[a] (h)	Subject	Concentration (ppb)	
		Membrane method	Extraction method[b]
4	A	113	113
9	B	52	44
10	A	42	41
11	A	24	24
12	B	15	12

[a] After start of exposure; the exposure lasted 8 h.
[b] As described in ref. 10.

The air concentration in the exposure experiment was about ten times higher than that found in foundries [9], where DMEA is used as a catalyst in the core-making process. The concentration in plasma from exposed workers was *ca.* 15 ppb.

CONCLUSION

The liquid membrane sample preparation method is suitable for determination of aliphatic amines in blood plasma. The clean-up procedure is very efficient: blank chromatograms from processed blood plasma are identical with blank chromatograms from aqueous solutions. The detection limit can be selected by changing the sample flow-rate and the volume. Sub-ppb detection limits can be reached at the expense of increased processing time. The process is performed in an automatic flow system with little manual intervention.

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