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Application of thermal desorption to the biological monitoring of organic compounds in exhaled breath☆

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ABSTRACT

We have developed a thermal desorption–gas chromatographic method for the analysis of organic compounds in exhaled breath air, to be used in the biological monitoring of environmental exposure. The exhaled breath sampler is based on the concentration of compounds present in alveolar air in a solid sorbent material. Isoflurane (1-chloro-2,2,2-trifluoroethyl-difluoromethyl-ether), an inhaled anaesthetic used widely in surgery, and styrene, used in boat construction and the manufacture of fibreglass-reinforced plastics, are partially eliminated from the body in exhaled breath, samples of which can therefore be used to monitor biological exposure to these two organic compounds. Recoveries were tested in controlled atmospheres of isoflurane or styrene, with Chromosorb 106 or Tenax, respectively, as the adsorbent. We also investigated the influence of relative humidity, an important factor in breath sampling, on adsorption.

INTRODUCTION

The development of industrial hygiene programme as a complement to environmental assessment of chemical contaminants with biological monitoring has awakened enormous interest in the search for biological indices of occupational exposure. Among the methods available for biological monitoring, exhaled breath offers certain advantages, because it is non-invasive in nature, and hence well accepted by workers. Moreover, the compounds of interest are analysed directly, thus minimizing the chances of spurious results caused by factors from outside the atmosphere in the workplace [1,2].

Several types of exhaled breath samplers have been used for breath analysis. Mixed expired air comprises alveolar air diluted by atmospheric air retained in the dead space of the respiratory tract (mouth, nose, pharynx, trachea and bronchi), whereas alveolar air (end-exhaled air) normally makes up two-thirds of the tidal volume.

Alveolar air sampling permits valid estimates of partial solvent pressure in arterial blood, particularly in the case of non-polar solvents, given the equilibrium between alveolar gas and arterial blood [3–5]. A number of methods have been developed to sample alveolar air from a single exhalation for chromatographic analysis [6–9], however these may be affected by water vapour condensation and excessive sample dilution. Instrumental methods have also been used for the direct analysis of exhaled air by gas chromatography [10], photoionization detection [11] or mass spectrometry [12,13]. These proce-

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dures may be cumbersome and are usually costly.

The aim of this work was to apply the thermal desorption technique to a portable system for end-exhaled breath sampling [14], based on the concentration of solvents in alveolar air captured in a solid adsorbent for chromatographic analysis. The advantages of thermal desorption prior to gas chromatography are considerable in samples collected 16 h post exposure, on the following morning before occupational re-exposure. The system was validated for isoflurane (1-chloro-2,2,2-trifluoroethyl-difluoromethyl-ether), a surgical anaesthetic, and for styrene, a basic component used in the manufacture of reinforced fibreglass plastics, in controlled atmospheres and in human exposures in the field.

EXPERIMENTAL

Sampler design

The prototype of the exhaled breath sampler designed and built in our laboratory consists of a Haldane–Priestley tube modified to concentrate aliquots of exhaled air from one or more exhalations. The system (Fig. 1) is based on an inversion of the polarity of the motor that powers the syringe, so that the plunger alternately draws in exhaled air and expels the sample through the adsorbent cartridge, a mechanism controlled by automatic changes in the position of the three-way valve. The aluminium tube, 1 m long by 26 mm in diameter, is fitted with a disposable mouthpiece, and equipped with a valve that is opened when air is exhaled through the system, and which closes at the end of exhalation. The tube is insulated with a 15-mm-thick electrically heated jacket that maintains the internal temperature at 40–45°C. The three-way PTFE valve is connected to (I) the end of the Haldane–Priestley tube at a point approximately 5 cm from the mouthpiece with a copper connector, (II) the 50-ml capacity gas syringe, which is filled and emptied by a rack system, and (III) the adsorbent tube. In the first step of the procedure, the three-way valve control mechanism (Fig. 1) opens channels I and II while exhaled air is being aspirated from the aluminium tube, and closes channel III. In the second step, channels II and III are open to pass the exhaled air through the adsorbent tube, while channel I is kept closed. The servomotor-driven syringe draws in air from the aluminium tube and expels it through the adsorbent. The direction of the motor is reversed automatically by a pair of end-of-run switches, which also control the position of the three-way valve. A counter records the number of complete runs of the aspiration/expulsion cycle, and the sequence can be repeated automatically or step by step. The prototype, which weighs approximately 5 kg, is built so that the 1-m-long aluminium tube can be separated into two sections for transport. The sampler requires an external electrical power source.

Adsorbent cartridges

The adsorbent cartridge consists of a standard metallic tube for an ATD 50 thermal desorption system (Perkin-Elmer, Beaconsfield, UK) measuring 89 mm long by 6.4 mm in diameter. The cartridge was packed with 200 mg of 20–40-

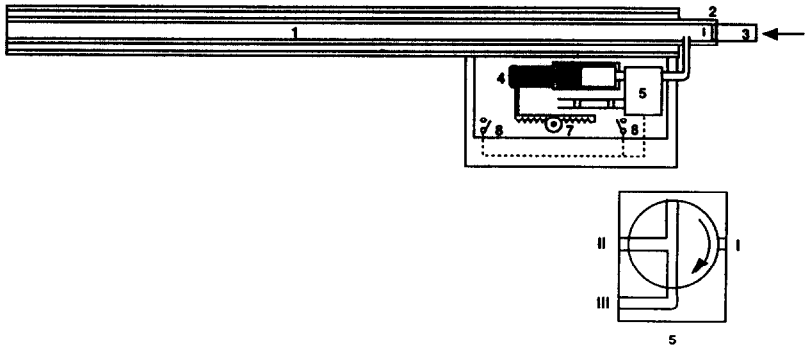

Fig. 1. Schematic diagram of the exhaled breath sampler system. 1 = Insulated aluminium tube; 2 = back-flow valve; 3 = disposable mouthpiece; 4 = syringe; 5 = three-way valve; 6 = adsorbent cartridge; 7 = servomotor; 8 = end-of-run switches.

mesh Chromosorb 106 (SKC, PA, USA) for isoflurane, or with 150 mg of 20–40-mesh (0.84–0.42 mm) Tenax TA (SKC) for styrene, and was sealed at the end of the procedure for transport and storage.

Instrumental analysis

All samples of exhaled breath air were desorbed with a Perkin-Elmer Model ATD 50 automatic thermal desorption system, directly connected to a Perkin-Elmer Model 8700 gas chromatograph by a heated transfer line. Thermal desorption was done in two stages. First, the tube was heated with the flowing carrier gas, which transfers the desorbed vapours from the sample tube to a cooled trap. When the entire sample had been collected, the trap was rapidly heated to desorb the volatile materials and to rapidly inject the sample into the GC column via a heated transfer line. The cooled trap was packed with Tenax. The operating conditions are summarized in Table I.

Validation in controlled atmosphere

The system was validated in controlled atmospheres containing isoflurane (Abbot Laboratories, Madrid, Spain) and styrene (Merck, Darmstadt, Germany) and designed to simulate the concentrations of these compounds in human breath. Fig. 2 illustrates the system used to generate the controlled atmosphere. The coefficient of variation, as determined by on-line chromatography, was less than 4%. Validation assays were performed under varying conditions of relative humidity and concentration. Six sam-

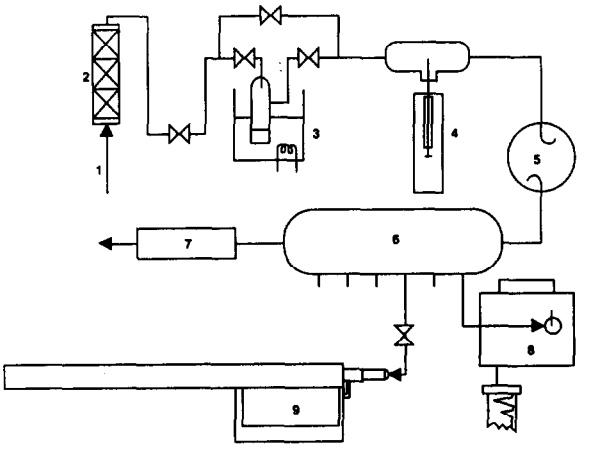

Fig. 2. Scheme of the system used to generate the controlled atmosphere. 1 = Air intake; 2 = filter; 3 = humidifier; 4 = automatic injector; 5 = mixing chamber; 6 = sampling chamber; 7 = humidity sensor; 8 = gas chromatograph; 9 = exhaled air sampler system.

TABLE I

EXPERIMENTAL CONDITIONS FOR THERMAL DESORPTION AND GAS CHROMATOGRAPHY

FID = Flame ionization detection.

	Isoflurane	Styrene
Thermal desorption parameters		
Carrier gas	N_2 (68.95 kPa, 10 p.s.i.)	N_2 (68.95 kPa, 10 p.s.i)
Desorption	Two stages	Two stages
Oven temperature	240°C	200°C
Cold trap		
Lower temperature	−30°C	−30°C
Upper temperature	240°C	300°C
Heated transfer line temperature	100°C	120°C
Gas chromatographic parameters		
Stationary phase	FFAP[a]	FFAP[a]
Film thickness	0.3 μm	0.3 μm
Column length	25 m	25 m
Column inside diameter	0.2 mm	0.2 mm
Detector temperature	70°C	120°C
Detection	FID	FID

[a] Hewlett-Packard, Palo Alto, CA, USA.

TABLE II

EXPERIMENTAL CONDITIONS USED TO VALIDATE THE EXHALED BREATH SAMPLER IN A CONTROLLED ATMOSPHERE OF ISOFLURANE

Uptake volume: 0.6 l.

Relative humidity (%)	Concentration (mg/m^3)	Recovery (C_c/C_a)[a]
98	24.8	0.986 ± 0.033 (6)
98	5.0	1.023 ± 0.013 (6)
40	24.8	1.007 ± 0.022 (6)
40	5.0	1.009 ± 0.029 (6)
50	15.2	0.998 ($n = 2$)[b]

[a] Mean ± standard deviation, with number of samples in parentheses.
[b] Samples analysed 15 days after collection.

ples were collected under each of the conditions tested, which are summarized in Tables II and III. The total sample volume was always 0.6 l, divided into three aliquots of 0.2 l each. An air stream was passed through the tube at a rate of 4 l/min for 2 min before each sample run. In an additional control assay, clean air was passed through the tube and a blank sample collected under each of the experimental conditions. Two additional samples taken at concentrations near the higher end of the concentration range were stored for 15 days at room temperature before analysis to compare these results with those from

TABLE III

EXPERIMENTAL CONDITIONS USED TO VALIDATE THE EXHALED BREATH SAMPLER IN A CONTROLLED ATMOSPHERE OF STYRENE

Uptake volume: 0.6 l.

Relative humidity (%)	Concentration (mg/m^3)	Recovery (C_c/C_a)[a]
98	26.6	1.019 ± 0.047 (6)
98	3.8	0.995 ± 0.026 (6)
40	26.6	1.004 ± 0.033 (6)
40	3.8	0.975 ± 0.033 (6)
50	21.0	0.989 ($n = 2$)[b]

[a] Mean ± standard deviation, with number of samples in parentheses.
[b] Samples analysed 15 days after collection.

other samples at high concentrations, which were analysed immediately.

Experimental studies in human exposures

The system was assayed in subjects habitually exposed to these compounds in the workplace. In both cases we measured the exposure concentrations with personal diffusive samplers attached to three exposed subjects. For isoflurane, Perkin-Elmer-type diffusive tubes packed with Chromosorb 106 were used; for styrene, 3M-3500 diffusive samplers (3M, MN, USA) were used.

For isoflurane, the same subject was exposed on two consecutive days to levels of 55 mg/m^3 for 2.8 h (day 1) and 70 mg/m^3 for 6 h (day 2). Exposures took place during the subjects' habitual occupation in an operating room of a large medical centre.

For styrene, two factory workers were exposed to 360 or 243 mg/m^3 for 6 h on the same day.

In all subjects, exhaled breath samples were collected immediately after the end of the shift, every 15 min for the next 1.5 h and 16 h later, before beginning the next shift. The uptake volume collected in all cases was 1 l of exhaled air from five exhalations (0.2 l each).

RESULTS

Table II summarizes the experimental conditions of the validation tests in controlled atmospheres containing isoflurane and styrene. The influence of the humidity, concentration and storage were analysed from the recoveries obtained under each condition, calculated as $r = C_c/C_a$, where C_c represents the concentration in the adsorbent cartridge and C_a the mean concentration in the controlled atmosphere. The recovery values obtained with isoflurane and styrene approached unity, with relative standard deviation less than 12%. In blank samples taken after clean air was passed through the uptake system, no isoflurane or styrene was detected under any of the conditions tested, suggesting that no adsorption–desorption phenomena occurred in any of the components.

Profiles of isoflurane and styrene in exhaled breath samples collected after occupational exposures are shown in Figs. 3 and 4. Both

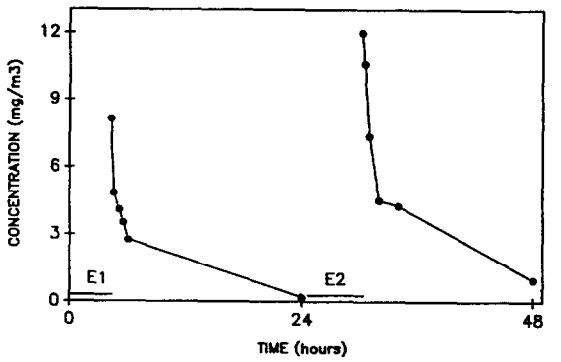

Fig. 3. Profiles of isoflurane in exhaled breath air after two consecutive daily exposures in the workplace. E1 = Environmental concentration = 58 mg/m^3, exposure time = 2.8 h. E2 = Environmental concentration = 70 mg/m^3, exposure time = 6 h.

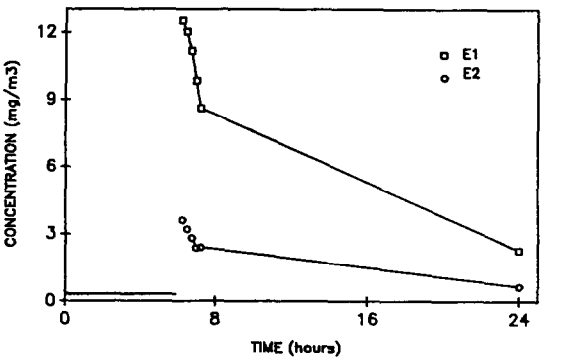

Fig. 4. Profiles of styrene in exhaled breath air of two workers after exposure in the workplace. E1 = Environmental concentration = 360 mg/m^3, exposure time = 6 h. E2 = Environmental concentration = 243 mg/m^3, exposure time = 6 h.

compounds were detectable in samples taken 16 h after exposure (0.19 and 0.98 mg/m^3 for isoflurane; 0.65 and 2.27 mg/m^3 for styrene).

DISCUSSION

The results of two-way analysis of variance (ANOVA) tests of the recoveries obtained in validation experiments in controlled atmospheres (Table IV) show no significant variations between the different conditions of humidity and concentration, thus the determinations were not affected by the elevated relative humidity of sampled air. These findings make our system similar in terms of reliability to those based on direct sampling of breath exhaled into glass tubes, as the fraction collected with our system is that which most closely represents the alveolar fraction. However, our sample system, in contrast to most of those described up to now, offers the further advantage of adsorption onto a solid substrate, which concentrates the sample and facilitates its transport and storage with minimal loss over a period of 15 days without requiring special measures of preservation (Tables II and III).

TABLE IV

RESULTS OF ANALYSIS OF VARIANCE OF THE RECOVERIES OBTAINED IN VALIDATION EXPERIMENTS IN CONTROLLED ATMOSPHERES

NS = Not significant at 5% level.

Source of variation	Degrees of freedom	Sum of squares	Mean square	*F* Calculated	*F* Test
Isoflurane					
Relative humidity	1	$7.7042 \cdot 10^{-5}$	$7.7042 \cdot 10^{-5}$	0.107	NS
Concentration	1	$2.3404 \cdot 10^{-3}$	$2.3404 \cdot 10^{-3}$	3.257	NS
Interaction	1	$1.9260 \cdot 10^{-3}$	$1.9260 \cdot 10^{-3}$	2.680	NS
Error	20	0.0140	$7.1858 \cdot 10^{-4}$		
Styrene					
Relative humidity	1	$1.7340 \cdot 10^{-3}$	$1.7340 \cdot 10^{-3}$	1.321	NS
Concentration	1	$4.3202 \cdot 10^{-3}$	$4.3202 \cdot 10^{-3}$	3.292	NS
Interaction	1	$4.2667 \cdot 10^{-5}$	$4.2667 \cdot 10^{-5}$	0.032	NS
Error	20	0.0260	$1.3123 \cdot 10^{-3}$		

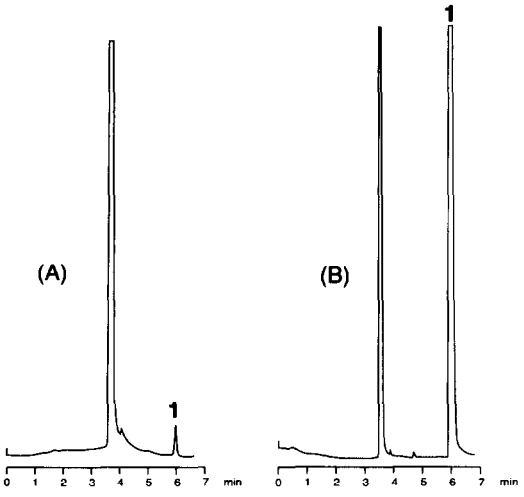

Fig. 5. Capillary gas chromatogram of samples taken from controlled atmospheres of styrene (1), using different desorption techniques. (A) Uptake volume of air: 500 ml. Adsorbent: activated charcoal. Desorption volume: 250 μl carbon disulphide. Injection volume: 2 μl. (B) Uptake volume of air: 50 ml. Adsorbent: Tenax TA. Thermal desorption.

The system designed by us permits repeated sampling of a single or of multiple successive exhalations with a single adsorbent cartridge, which provides concentrated samples.

Although the system enriches the sample on the adsorbent to concentrations appropriate for the analytical method proposed here, the use of adsorbents susceptible to thermal desorption can markedly improve the results, as the entire, undiluted sample is analysed. This contrasts with the need to dilute the sample when solvent desorption is used, as depicted in Fig. 5. This feature is of particular interest for measuring the very low concentration of organic compounds in exhaled breath some time after exposure has occurred, but before the next working day is begun. The value of such determinations in occupational toxicology lies in their ability to detect the accumulation of substances in fatty tissues [15]. Thermal desorption of samples collected in a solid adsorbent may therefore make it possible to detect and quantify low levels of compounds a considerable time after exposure has occurred, as shown in Figs. 6 and 7, which illustrate the findings in samples of exhaled breath collected 16 h after exposure to isoflurane and styrene, respectively.

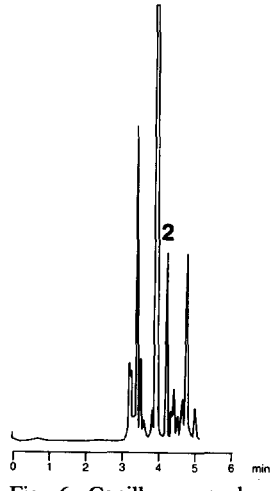

Fig. 6. Capillary gas chromatogram of an exhaled breath sample taken 16 h after exposure to 58 mg/m^3 isoflurane (2) for 2.8 h.

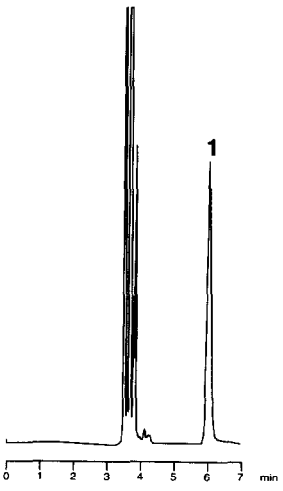

Fig. 7. Capillary gas chromatogram of an exhaled breath sample taken 16 h after exposure to 243 mg/m^3 styrene (1) for 6 h.

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