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Journal of Chromatography B, 859 (2007) 201–207
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Determination of acetaldehyde and acetone emanating from human skin using a passive flux sampler—HPLC system

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Received 11 May 2007; accepted 22 September 2007
Available online 5 October 2007

Abstract

The authors have developed a new passive flux sampler (PFS), which was a simple device to determine emission fluxes of potential biomarkers such as acetaldehyde and acetone emanating from the surface of the human skin. The sampler was placed on the skin surface to create a headspace. Within the space, gases emanating from skin moved toward the trapping filter (DNPH impregnated filter) by molecular diffusion and the trapped carbonyls were subsequently determined by HPLC. The PFS was practically applied to volunteers. The emission flux varies with sampling positions, probably depending on the different emanation routes. Personal emission flux also showed great variations between individuals.

Keywords: Skin gas; Acetaldehyde; Acetone; Emission flux; Passive sampler; HPLC

1. Introduction

Trace gases emanating from human skin has invited considerable attention during recent years as a mosquito attractant [1,2], a specific odour of aged people [3], a potential non-invasive biomarker of individual physical or physiological status [4–7] and in relation to indoor air quality of living environments. The gaseous substances are mostly synthesized by internal metabolism or bacterial activity on the surface of skin. The reaction products of metabolism generally rise to the skin surface with perspiration and/or travel directly from blood through the skin, because there is a network of blood capillaries under the skin [4].

However, determination of emissions of such volatile organic/inorganic compounds has been rarely reported due to difficulty of sampling and their very low concentrations. Therefore, previous studies required complicated instruments for sampling, pre-concentration and highly sensitive analysis, which should be operated by professionals. According to Naitoh et al. [4], a homemade sampling probe with a continuous nitrogen gas flow was directly attached to the skin of a forearm. The carrier gas was then introduced either to a cold trap system for the determination of acetone by gas chromatography with flame ionization detector (GC-FID) or to an aluminum bag for analysis of hydrogen by GC. Nose et al. [5] used a polyfluorovinyl bag for covering hand under a continuous helium flow and introduced the carrier gas to a cold trap system coupled with GC-FID for the determination of methane, ethylene and ethane. A real time trace gas sensing method was reported by Moeskops et al. [8]. A small quartz cuvette was placed on the skin surface to create headspace from which a carrier gas transports the skin emissions to a laser-based photoacoustic detection system and proton-transfer reaction mass spectrometry for determinations of ethylene, propanal and acetaldehyde. Semi-quantitative analysis was also demonstrated by Zhang et al. [7] for characterizing the emanations from human arm skin. A sampling jar with nitrogen gas flow system, into which a hand of volunteer was inserted, was used for sampling device. The emanations were trapped by a solid phase micro-extraction (SPME) technique and then detected by GC–mass spectrometry (GC–MS).

From the above, it is clear that although work is needed to more closely identify metabolic sources of medically useful skin gases, and that the determination of skin gas has many technical problems, the prize of easily and cheaply being able to screen large numbers of people for various disorders is potentially

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doi:10.1016/j.jchromb.2007.09.033

very great indeed. Good examples of target substances might be acetaldehyde and acetone. Acetaldehyde is well known as metabolite of alcohol and its concentration in breath has been used as a biomarker of drinking and alcoholism. Acetone is a member of ketone bodies which are products of the metabolic reaction of fatty acids when there is low blood glucose. Acetone in breath has attracted much attention, because it is markedly influenced by the period of fasting, starvation or even type of diet [9]. Moreover, elevated acetone concentration in breath is produced in patients with uncontrolled diabetes mellitus [10]. However, the acetone-like smell also comes from skin. Naitoh et al. found the acetone concentration in breath had a significant correlation with acetone content in the human skin gas [4]. Therefore, measurement of acetone from skin could be very important in the diagnosis and treatment of diabetes [11].

In this study, the aim is to demonstrate the application of a novel sampler to skin gas determinations of medically significant species—acetaldehyde and acetone. We have developed a new type of passive diffusion sampler, which is a simple device to determine the emission flux of volatile carbonyl compounds emanating from the surface of the human skin, based on the concept of passive flux sampler (PFS) [12–15]. The new sampler was then applied to *in vivo* sampling of human skin gases of volunteers, and the emission fluxes of acetaldehyde and acetone were determined using high performance liquid chromatography (HPLC).

2. Experimental

2.1. Reagents

Reagent grade acetaldehyde, acetone, 2,4-dinitrophenylhydrazine (DNPH), and phosphoric acid; and HPLC grade acetonitrile were obtained from Kanto chemicals. 16-Aldehydes-DNPH mixture standard solution (10 μg mL^{-1} in acetonitrile) was purchased from Wako Pure Chemical Industries and used as the analytical standard.

2.2. Passive flux sampler (PFS)

Passive sampling device for collecting human skin gases was developed based on the concept of the PFS. This type of passive sampler was originally developed for the determination of emission flux of hazardous chemicals from building materials: volatile organic compounds (VOCs) from interior finishing materials [12], phthalates from plastic materials [10], formaldehyde from plywood [11] and hydrogen chloride from damaged polyvinylchloride [12]. The device simply consisted of Petri dish made of stainless steel, trapping filter, polytetrafluoroethylene (PTFE) O-ring and backup plate (Fig. 1). The passive sampler was placed on the skin surface to create a headspace. Through the open face of the sampler, gases emanating from skin move toward the trapping filter within the headspace by molecular diffusion and the gas molecules were then collected on the filter. The trapping filter was prepared by dipping a commercially available cellulose filter paper (Advantec, Tokyo, Japan, No. 51A, Ø36 mm, 0.18 mm *t*) into 0.2% DNPH–1% phosphoric acid in acetonitrile solution and subsequently drying in a vacuum desiccator. Before use, the open end of the sampler was covered with a cap of the stainless steel Petri dish, sealed by a piece of Parafilm^{TM}, enveloped in an aluminum bag and stored in a refrigerator.

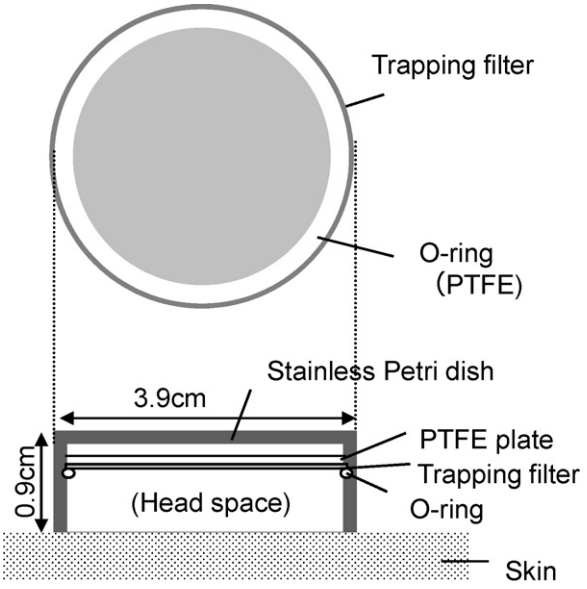

Fig. 1. Schematic view of the passive flux sampler for human skin gas.

Since the PFS employs molecular diffusion process and hence does not require power supply and other services, the volunteers easily handle the simple device by themselves. This is a great advantage when simultaneous and multiple sampling of human skin gas is carried out.

2.3. Sampling and HPLC analysis

The passive sampler was softly fixed on the surface of forearm and/or palm (Fig. 2) using an elastic band. The carbonyl compounds emanating from skin surface were collected as DNPH derivatives and subsequently determined by HPLC after extraction with 10 mL of acetonitrile. In recent years, this DNPH-HPLC method has been established as the most widely used standard procedure for the determination of aldehydes and ketones in air sample [16–18]. Sampling was conducted for 1 h in a given atmosphere (indoor environmental conditions such as temperature, humidity and air stream were not controlled). At the same time, the sampler was put on the Petri dish for 1 h at room temperature, to collect carbonyl compounds in the internal space of the PFS as background. The emission flux of carbonyl compounds, E (mg cm^{-2} h^{-1}) was obtained by

$$E = \frac{W}{St} \quad (1)$$

where W (ng) is a collection amount of analyte by the PFS, S (cm^2) a cross-section of the exposed human skin (7.79 cm^2) and t (h) is a sampling duration.

The HPLC system consists of Hitachi L-2130 pump with Hitachi L-2400 UV–vis detector. The following condition was used: column, 4.6 mm × 150 mm, 5 μm, Inertsil ODS-80A

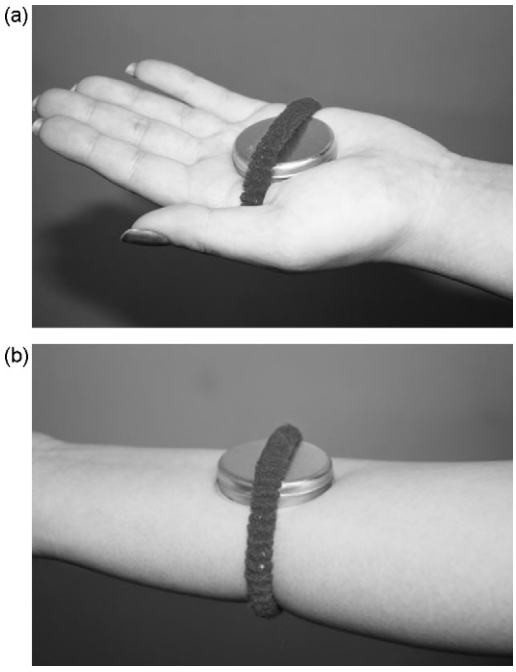

Fig. 2. Sampling of human skin gas from palm (upper) and forearm (lower).

(GL Sciences); eluent, acetonitrile:distilled water in the ratio of 1:1 (v/v) at 1.0 mL min^{-1} (isocratic); detection, 360 nm; injection volume, 20 μL; injection mode, manual. Dilution series of DNPH-carbonyl compounds in acetonitrile solution, 0.01, 0.025, 0.05 and 0.1 μg mL^{-1}, were prepared from 16-Aldehydes-DNPH mixture standard solution and used for calibration.

2.4. Sampler performance

Recovery rates of the trapped carbonyl compounds were determined by the preparation of spiked filters. An aqueous solution comprising 0.10 mL of 10 μg mL^{-1} of acetaldehyde and 0.010 mL of 10 μg mL^{-1} acetone was dropped and spread on the DNPH impregnated filter. Then, the PFS was capped immediately and stood for 1 h to allow reaction of the carbonyl compounds with the DNPH. The filters were subsequently analyzed as described above. The administrated PFSs were also stored in refrigerator (4 °C) for 24 and 48 h and change of the recovery rate was examined. Three parallel experiments were done ($n = 3$).

Reproducibility of the passive sampling method was assessed by simultaneous exposure of five samplers to a vapour of acetaldehyde and acetone. The PFSs were placed on a Petri dish where 0.10 mL of 1000 μg mL^{-1} aqueous solution of acetaldehyde and acetone was dropped. The samplings were made for 1 h. The filters were then analyzed as described above.

2.5. Measurement of carbonyl compounds from human skin

Measurement of dermal emission fluxes of carbonyl compounds were carried out using the newly developed PFS, to investigate:

1) effect of diffusion length on the dermal emission flux;
2) variation of the emission flux by sampling position;
3) distributions of personal emission fluxes of acetaldehyde and acetone of volunteers.

In all cases the sampling was simultaneous and lasted for 1 h.

The emission flux was determined by changing the diffusion length which is a distance between the skin surface and trapping filter within the PFS. The test volunteer was a female (22 years old), and a pair of samplers was simultaneously exposed to the left forearm. The diffusion lengths of the two samplers were set at 0.45 and 0.75 cm.

To examine variation of the dermal emission flux by sampling position and distribution of personal emission fluxes, three sets of experiments were carried out involving (a) four samplers deployed on the left palm, left forearm, right palm and right forearm of one volunteer (female, 22 years old). Diffusion length was set at 0.75 cm. The measurements were conducted once a day for 5 days ($n = 5$). (b) Additional measurements were conducted on six volunteers, consisting of two males (age 21) and four females (age 21–23). The sampling positions were set at left forearm, left palm, left upper part of arm, chest, midriff, left calf, back and left bottom of foot. The six samplers were simultaneously deployed at each sampling position (single sampler at one position). (c) Measurements were conducted on 60 volunteers to assess the distribution of personal emission fluxes of acetaldehyde and acetone. Subjects were students of Tokai University (46 males and 14 females). Their ages ranged from 19 to 23 years old (average 20.9 years old). The sampling was carried out 13:30–14:30, 15 December 2004. One sampler was put on the surface of the forearm which in each case was not the volunteer's dominant side. A simple questionnaire, asking age, sex, and condition on the day, was also administered at the same time.

After sampling, the passive samplers were analyzed described above and emission fluxes were obtained by Eq. (1).

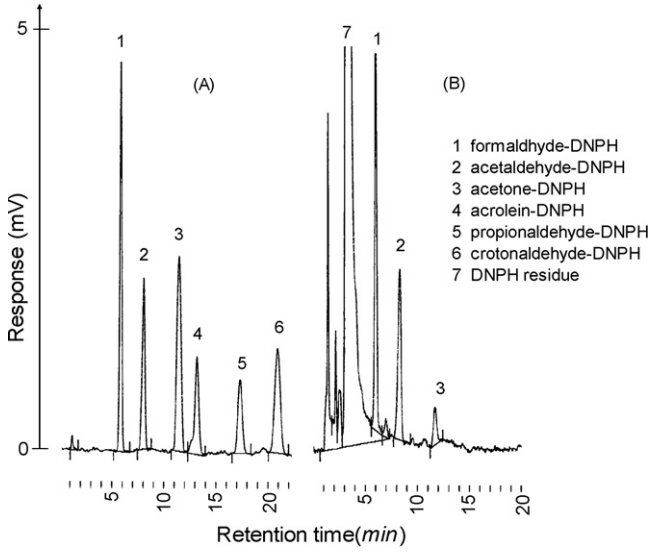

Fig. 3. Typical HPLC chromatograms of (A) standard solution (1 μg mL^{-1} of carbonyls-DNPH) and (B) emanations from skin surface collected at forearm.

This study was conducted under approval of Ethics Committee on Human Studies, Tokai University.

3. Results and discussion

3.1. Sampler performance

HPLC analysis of the DNPH impregnated filter yields the number of moles of DNPH-carbonyl derivatives and hence the number of moles of carbonyl compounds collected on the trapping filter. In the typical HPLC chromatograms of a standard solution and sample as shown in Fig. 3, peaks of DNPH-carbonyl derivatives were well separated. Acetaldehyde reacts with DNPH and gives possible two geometric isomers of hydrazone: *E* and *Z*. However, only one peak was found as a sum of both isomers under this analytical condition. Relative standard deviations (R.S.D.) of peak areas for repeated injections of 0.1 μg mL^{-1} of standard solution were 4.3% for acetaldehyde and 3.4% for acetone ($n=5$). R.S.D. of retention times were 0.67% for acetaldehyde and 0.64% for acetone ($n=5$).

Significant contamination was detected in the blank filters of the PFS, mostly due to contamination from the laboratory air, impregnation process of the DNPH solution to the cellulose filter, and drying process of the dipped filter and contamination in the cellulose filter. To avoid them, the trapping filter was prepared by dipping method instead of pipetting the DNPH solution into the cellulose filter, in a separate room without any uses of carbonyl compounds and dried in a vacuum desiccator by removing desiccant (silica gel) which was a possible source of carbonyls. As a result, the blank level was reduced to 0.10 ± 0.002 μg of acetaldehyde and 0.011 ± 0.0006 μg of acetone ($n=3$). The blank readings were subtracted from sample readings of HPLC chromatogram, because of the small R.S.D.s of the blank reading (2.2% for acetaldehyde and 5.3% for acetone).

Limit of detection (LOD) of the sampler was defined as threefold standard deviation of multiple blank samplers and obtained from Eq. (1) for 1 h sampling duration following the analytical procedure described above. The LOD of emission flux of acetaldehyde resulted in 0.85 ng cm^{-2} h^{-1} and that of acetone was 0.48 ng cm^{-2} h^{-1}.

Recovery rate of the trapped carbonyl compounds were investigated by administrating known amount of carbonyl compounds to the DNPH impregnated filters. The recovery rates resulted in 101 ± 0.50% for acetaldehyde and 98 ± 0.15% for acetone ($n=3$). No significant differences in the recovery rates were found in the samples stored in refrigerator for 24 and 48 h. Reproducibility of the passive sampling method was assessed by simultaneous exposure of five samplers to vapours of acetaldehyde and acetone generated from standard solutions. R.S.D. of the collected amounts of carbonyl compounds were 8.3% for 0.27 μg of acetaldehyde and 13% for 0.38 μg of acetone. Based on the excellent recovery rate and repeatability, we then applied the PFS to passive sampling of human skin gas.

Table 1
Collection amount of acetaldehyde at the forearm of one volunteer by the PFS setting the diffusion length at 0.45 and 0.75 cm

	$W_{0.45}$ (μg)	$W_{0.75}$ (μg)	$W_{0.45}/W_{0.75}$
1	0.010	0.010	1.0
2	0.021	0.018	1.2
3	0.094	0.086	1.1
4	0.115	0.113	1.0

3.2. Effect of diffusion length on the dermal emission flux

Sampling rate of the human skin gas is potentially affected by both mass transfer from bulk skin to air phase and molecular diffusion of gas inside the air layer (boundary layer) of the sampler. The slower of the two determines the overall sampling rate [13]. The rate-determining step can be distinguished by changing the diffusion length which is a distance between human skin surface and trapping filter. When the molecular diffusion in the air layer is a rate-determining step, the emission flux (or collected amounts of the filter) changes with the diffusion length according to Fick's law. On the other hand, the thickness of boundary layer of materials in indoor environment without mechanical ventilation is 1.4–1.6 cm under 0.08 m s^{-1} of wind speed [19]. When we set the diffusion length shorter than the thickness, the mass transfer rate dominates the overall flux.

Then, the emission flux from a forearm was determined by changing the diffusion length of the PFS. The diffusion lengths of the two samplers were set at 0.45 and 0.75 cm, much shorter than the thickness of boundary layer. Table 1 shows the collection amount of acetaldehyde from human skin at each diffusion length. Acetone from this volunteer was mostly below LOD. As can be seen, the collection amount of acetaldehyde did not

Table 2
Emission fluxes of acetaldehyde and acetone from palms and forearms of one volunteer (ng cm^{-2} h^{-1})

	Left palm	Left forearm	Right palm	Right forearm
Acetaldehyde				
1	17	5.8	9.6	5.4
2	13	ND	8.1	ND
3	13	ND	ND	ND
4	6.2	ND	ND	ND
5	9.6	ND	11	ND
Mean	12	5.8	9.6	5.4
S.D.	4.0	–	1.5	–
Median	13	ND	8.1	ND
Acetone				
1	4.4	2.9	ND	7.9
2	1.2	2.8	3.0	2.7
3	ND	2.6	ND	2.0
4	ND	1.5	ND	ND
5	ND	ND	ND	ND
Mean	2.8	2.4	3.0	4.2
S.D.	2.2	0.6	–	3.2
Median	ND	2.6	ND	2.0

Mean and standard deviation (S.D.) were calculated on the basis of measured data only. ND: not detected.

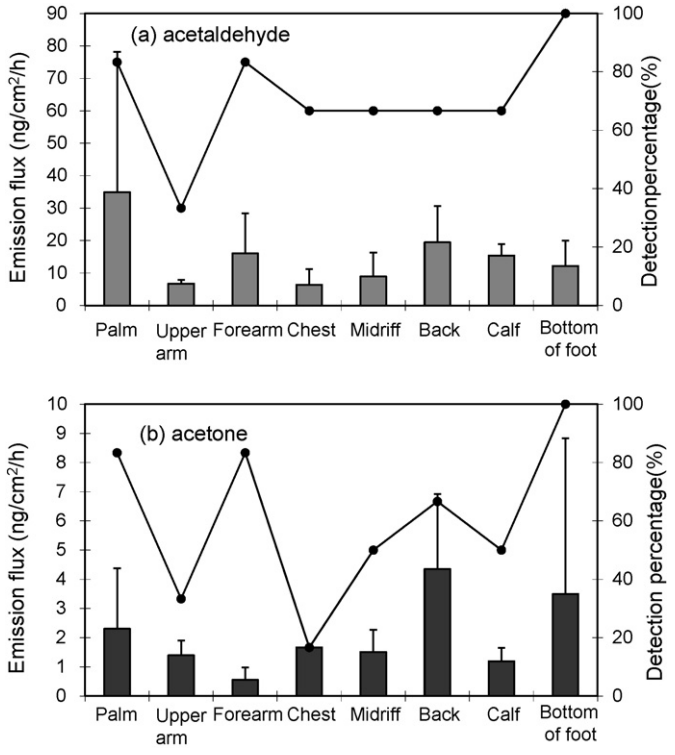

Fig. 4. Comparison of detection percentage (line graph) and emission fluxes of acetaldehyde and acetone (bar) between sampling positions. Samplings were conducted simultaneously for six volunteers. Bars show mean concentrations with standard deviations of emission fluxes of acetaldehyde and acetone and line graphs show detection percentage.

change by the difference of the diffusion length of the PFS. Therefore, the mass transfer rate of the skin gas was a rate-determining process when the diffusion length of PFS was up to 0.75 cm, and Eq. (1) can be practically used for determining the dermal emission flux.

The headspace, created by setting the diffusion length at 0.75 cm, was also useful to avoid direct contact of skin to the trapping filter. Therefore, the PFS can be easily handled by non-trained personnel.

3.3. Variation of the dermal emission flux by sampling position

The effect of sampling position on the dermal emission fluxes of carbonyl compounds was investigated in a series of experiments by deploying PFSs on:

(a) *The palms and forearms of one volunteer*. Table 2 shows the dermal emission fluxes of acetaldehyde and acetone at each sampling position. The backgrounds in the internal space of the PFS were not detected in any cases. Acetaldehyde was detectable in most cases from the palms. However, the emission fluxes from the forearms were mostly not detected by the PFS method. On the other hand, acetone was more detectable from the forearms rather than the palms. This was probably because of different emission routes of these different carbonyl compounds. Acetaldehyde is well known as a product of the metabolic reaction of alcohol and a component of sweat, and since there is a denser network of sweat glands on the palm than the forearm, detection of acetaldehyde may be easier at the palms. Acetone on the other hand is a product of the metabolic reaction of fatty acids which are necessary to produce energy in case of low concentration of glucose in the blood. The ketone in blood capillaries emerges as a component of sweat and also rises directly to the skin from the blood capillaries because of its highly volatility [4], therefore, the emission flux of acetone may also depend on the distribution of hypodermic blood capillaries.

(b) *The left forearm*, *left palm*, *left upper part of arm*, *chest*, *midriff*, *left calf*, *back and left bottom of foot of six volunteers*. Fig. 4 shows the results. Mean concentrations and standard deviations were calculated using measured data only. Whilst higher detection percentages of acetaldehyde was found at the bottom of foot (100%), palm (83%) and forearm (83%), the lowest was found at upper part of arm (33%), where sweat glands were sparsely distributed. Similarly, higher detection rates were found at bottom of foot (100%), palm (83%) and forearm (83%) for acetone. Lower percentages were found at upper part of arm (33%) and chest (17%), where blood capillaries were distributed under muscles or bones. On the other hand, differences between emission flux levels by sampling position were not clear for

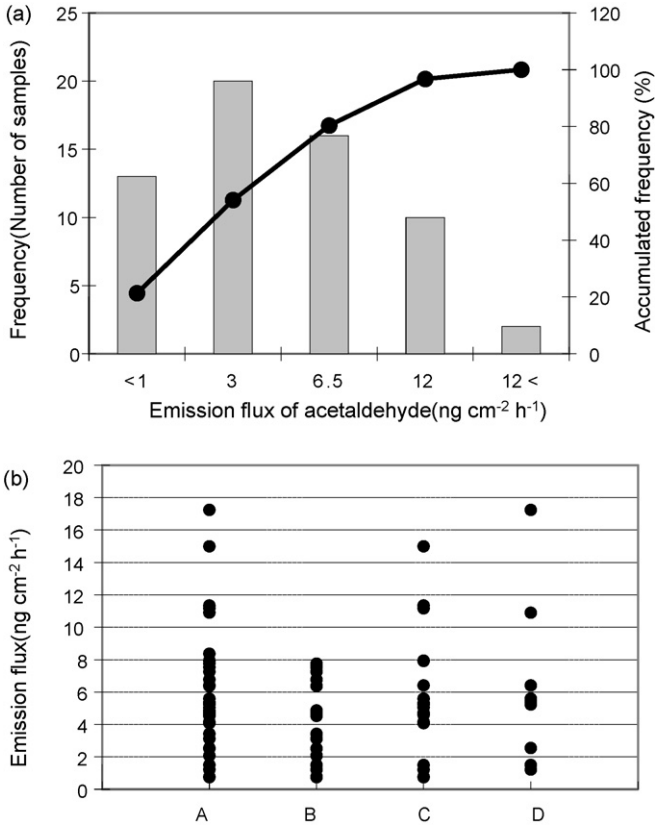

Fig. 5. Distribution of emission flux of acetaldehyde from skin surface of 60 volunteers, measured by the PFS. (a) Histogram and accumulated frequency, (b) comparative plots based on the volunteer's condition; A, all volunteers; B, volunteers answered in good condition; C, volunteers who had lost asleep last night; D, volunteers who caught a slight cold on the day of the sampling.

both compounds. ANOVA tests using these data sets could not detect any significant effects of sampling positions on the dermal emission fluxes of both compounds ($P = 0.05$), probably due to great variations among volunteers. Therefore, distribution of emission flux was investigated for 60 volunteers.

(c) *Forearms of 60 volunteers*. Fig. 5a shows a histogram and accumulated frequency of emission flux of acetaldehyde from one forearm of 60 volunteers. The flux ranged widely from below LOD to 17 ng cm^{-2} h^{-1} with 2.0 ng cm^{-2} h^{-1} at 50% of the accumulated frequency. Arithmetic mean ± standard deviation was 3.8 ± 3.7 ng cm^{-2} h^{-1}, when a half of the LOD was used for the samples whose readings were below LOD. Several higher values were found in the volunteers who lost sleep the night before and had a slight cold on the day of the sampling. Therefore, effects of physical or physiological status on the emission flux should be investigated by further well-designed clinical tests.

As above, Fig. 6a shows a histogram and accumulated frequency of emission flux of acetone from one forearm of 60 volunteers. The flux ranged from below LOD to 6.3 ng cm^{-2} h^{-1}. Since the results on 63% of the volunteers showed below LOD, the 50% of the accumulated frequency resulted in below LOD. Arithmetic mean ± standard deviation was 1.7 ± 4.7 ng cm^{-2} h^{-1}, when a half of the LOD was used for the samples whose readings were below LOD. In case of removing the below LOD, the arithmetic mean ± standard deviation resulted in 4.3 ± 7.2 ng cm^{-2} h^{-1}. This may be because this survey was carried out just after lunch, as lower levels of ketones are found in plasmas when blood glucose levels are higher (*e.g.* just after meals). Therefore, great variations of emission flux of acetone would be expected during the day. As with acetaldehyde, relatively higher values were found in volunteers who had lost sleep the night before and seemed to have colds.

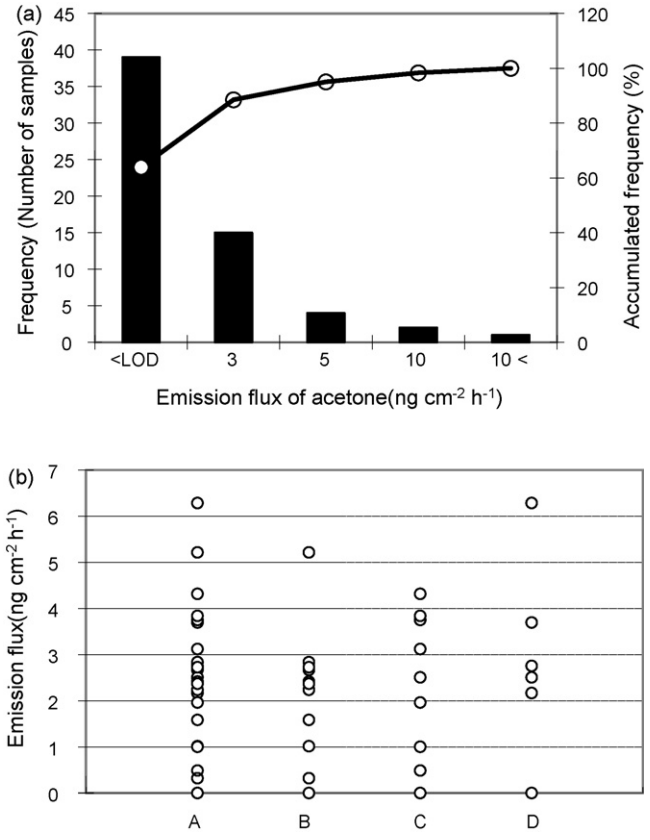

Fig. 6. Distribution of emission flux of acetone from skin surface of 60 volunteers, measured by the PFS. (a) Histogram and accumulated frequency, (b) comparative plots based on the volunteer's condition; A, all volunteers; B, volunteers answered in good condition; C, volunteers who had lost asleep last night; D, volunteers who caught a slight cold on the day of the sampling.

4. Conclusions

A new type of passive sampler, PFS was developed for the determination of emission fluxes of acetaldehyde and acetone emanating from the surface of human skin. Excellent recovery rate and repeatability were shown for the sampler. Collection amount of analyte was shown to be independent of diffusion length within the sampler, at least up to 0.75 cm, for the cross-section of exposed human skin (7.79 cm^2) and sampling time (1 h) employed.

The emission flux varied with sampling position, probably due to the different emanation routes of the gases for the different parts of the body sampled. Personal emission fluxes of acetaldehyde and acetone from the forearm of 60 volunteers widely ranged from below LOD to 17 ng cm^{-2} h^{-1} and from below LOD to 6.3 ng cm^{-2} h^{-1}, respectively.

The PFS was a simple device suitable for simultaneous and multiple sampling of human skin gas and quantitative demonstrations. This type of non-invasive measurement may have the possibility to be a simple and efficient tool for diagnosis of human conditions, when clinical significance of measurements of both compounds emanating from human skin is established.

Acknowledgements

Authors would like to thank Ms. Tomoko Kawashima and Ms. Yumiko Umehara, Tokai University for their great helps in experiments. Authors awfully thank Prof. Yukio Yanagisawa, Prof. Shinsuke Kato, Dr. Kazukiyo Kumagai, The University of Tokyo and Dr. Minoru Fujii, National Institute for Environmental Studies, Dr. Kazuhide Ito, Kyushu University, Mr. Mitsuru Machida, Preventive Clinical Laboratories, for their great advices on this study. A part of this work was supported by Japan Society for the Promotion of Science (JSPS) through Grants-in-Aid for Scientific Research (No. 17550084).

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