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Short Communication

Determination of dimethyl sulphide in blood and adipose tissue by headspace gas analysis

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ABSTRACT

A method for the headspace analysis of dimethyl sulphide in blood and adipose tissue has been established. Blood (0.2 ml) or adipose tissue (0.5 g) with added dimethyl sulphide was sealed in a 10-ml vial using PTFE sheet to prevent escape of dimethyl sulphide from the headspace. Equilibration was performed at 60°C for 4 h, and 20 μl of gaseous phase sampled from the headspace was subjected to gas chromatography (with flame photometric detection). Calibration curves were prepared for the two samples. Linearity was observed in the range from 5–10 μg to 2 mg.

INTRODUCTION

A man's body was examined postmortem in our laboratory. He had inhaled gaseous dimethyl sulphide (DMS), which had accumulated at the bottom of a tank in a paper-manufacturing plant. In order to determine the concentrations of DMS in the victim's blood and adipose tissue samples, we established a headspace gas analysis method for the compound in human samples.

EXPERIMENTAL

Chemicals and materials

DMS was supplied by Nakarai Chemicals (Kyoto, Japan). Polytetrafluoroethylene (PTFE) tape (0.05 mm thick) was purchased from Iuchi (Osaka, Japan). A

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50-μl microsyringe (50A-RN-GSG) was obtained from Scientific Glass Engineering (North Melbourne, Australia).

Blood and adipose tissue as a control were taken from another man's body, who was killed by strangulation, and whose age and postmortem interval were the same as those of the DMS victim: 25 years old and *ca.* one day, respectively.

Gas chromatography

The conditions for DMS chromatography were similar to those of the method of Kaji *et al.* [1], with some modifications. A gas chromatograph (GC-7AG, Shimadzu, Kyoto, Japan) was used, with a flame photometric detector. The glass column (3 m × 3 mm I.D.) was packed with 10% polyphenyl ether OS-124 on Shimalite TPA (60–80 mesh). The temperatures of column and detector were 60 and 120 °C, respectively. The pressures of the air and the hydrogen gas were adjusted to 1.2 and 0.7 kg/cm^2, respectively. Nitrogen was used as a carrier gas at a flow-rate of 100 ml/min.

Headspace method

Blood (0.2 ml) or adipose tissue (0.5 g) was put into a 10-ml glass vial, sealed with a rubber stopper and frozen until used. DMS dissolved in diethyl ether was chilled in an ice-bath, and 5 μl of different individual concentrations of the DMS solutions were injected with a microsyringe onto the surface of the frozen materials. The mouth of the vial was quickly covered with a piece of PTFE tape and sealed tightly with a rubber stopper and an aluminium cap using a hand crimper. The sample was heated at 60°C in an electric drier for a period of time, then allowed to stand at room temperature for 1 h. A 20-μl volume of the headspace gas was taken with a 50-μl microsyringe, and subjected to gas chromatography.

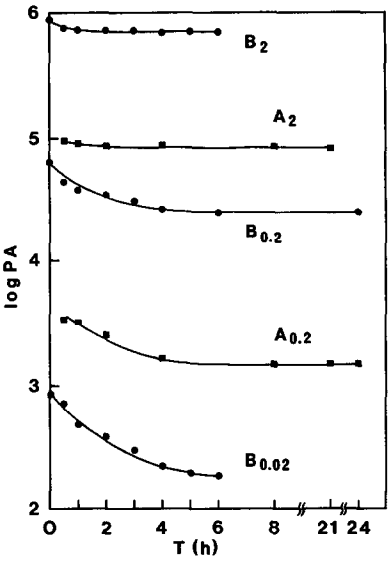
Experimental planning

In order to establish how long it takes for the DMS concentration to equilibrate between the headspace and the sample in the vial, the diethyl ether solution containing 0.02, 0.2 or 2 mg of DMS was added to 0.2 ml of blood (or 0.2 or 2 mg of DMS to 0.5 g of adipose tissue) and heated for various periods from 30 min to 24 h.

To prepare calibration curves individually for blood and adipose tissue, DMS solutions of various concentrations were added and heated for an adequate period of time, as decided by the above experiment.

RESULTS AND DISCUSSION

The DMS peak appeared sharply at 1.9 min after each injection. It appears from Fig. 1 that the added DMS first vapourizes in the headspace and then gradually dissolves in the blood or the adipose tissue to reach equilibrium. Fig. 1 also shows that the PTFE sheet prevents DMS gas from escaping outside the



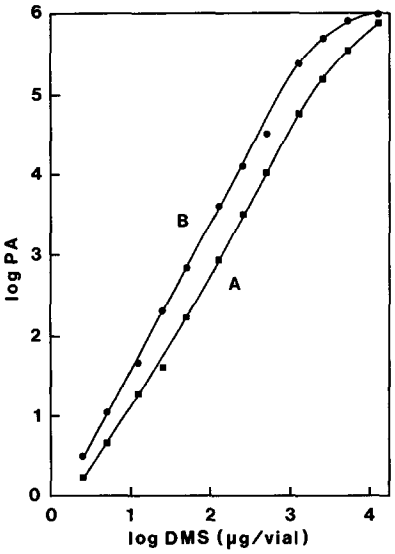


Fig. 1. Heating time required for equilibration. Amounts of 0.02, 0.2 or 2 mg of DMS were added to 0.2 ml of blood ($B_{0.02}$, $B_{0.2}$ and B_2); 0.2 or 2 mg DMS were added to 0.5 g of adipose tissue ($A_{0.2}$ and A_2). PA = Peak area; T = heating time.

Fig. 2. Calibration curves for DMS obtained by headspace gas analysis: 20 µl of gaseous phase were sampled after heating at 60°C for 4 h from the headspace of vials containing either 0.2 ml of blood (B) or 0.5 g of adipose tissue (A), plus diethyl ether solution containing various amounts of DMS.

headspace [2]. When no sheet was used, the DMS concentration in the headspace was much lower and kept on decreasing even after 24 h of heating (data not shown).

The ideal heating time (t) required for equilibration was determined to be 4 h, from the results shown in Fig. 1. This time variation corresponded to the amounts of DMS added to the vials: the more DMS, the shorter the time. When 0.2 ml of blood was used as a sample, t was longer than 6 h (0.02 mg of DMS added); t was 4 h for 0.2 mg and 30 min for 2 mg of DMS. When 0.5 g of adipose tissue was used, t was 4 h for 0.2 mg of DMS and 30 min for 2 mg. The adipose tissue became quite oily by the end of 4 h. This 4-h heating period would be applicable when real human samples containing DMS but no diethyl ether were subjected to the headspace method, because the time taken for dissolved DMS (b.p. 36.2 or 38°C) to vapourize from the samples into the headspace, and then to reach equilibrium, was thought to be shorter than the time taken for gaseous DMS in the headspace to dissolve into the samples and then to equilibrate.

When 0.5 mg of DMS was added to 0.5 g of blood and adipose tissue individually, the peak areas were $3.2 \cdot 10^4$ and $1.1 \cdot 10^4$, respectively. Because of this difference between the two materials, calibration curves were obtained by the

addition of various amounts of DMS to either 0.2 ml of blood or 0.5 g of adipose tissue. Linearity was observed in the range from 5–10 μg to 2 mg (Fig. 2). The limit of detection was *ca.* 2.5–5 μg for both 0.2 ml of blood and 0.5 g of adipose tissue. If 0.2 ml of blood subjected as a real sample to this headspace method gives a peak area of 10^3, for instance, the amount of DMS contained in the sample corresponds to *ca.* 50 μg (50 μg per 0.2 ml) from the calibration curve B in Fig. 2.

The blood and the adipose tissue of the gas victim mentioned at the beginning of this paper were analysed for DMS with the method described. They contained DMS at the concentrations of 0.20 and 0.04 mg/g wet weight, respectively. The blood level needed to cause coma by DMS inhalation was reported to be 0.43 mg/ml in rats [3]. This value is about twice as high as our victim's blood level, which would possibly be fatal. However, the victim's death should be explained more correctly by performing animal experiments. We will report on the experimental results and further discuss the fatality of the victim elsewhere [4].

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