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Determination of polysulphides in blood by gas chromatography and gas chromatography–mass spectrometry

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ABSTRACT

A sensitive and simple method to determine polysulphides in human blood, using an extractive alkylation technique and gas chromatography, has been devised. Polysulphides were alkylated with pentafluorobenzyl bromide, and then converted into bis(pentafluorobenzyl)disulphide by desulphuration with potassium cyanide. The disulphide was analysed qualitatively by mass fragmentography and quantitatively by gas chromatography with electron-capture detection. The lower limit of detection was 0.005 μmol/ml. Field testing in a suicide case confirmed the validity of the method.

INTRODUCTION

Polysulphides present in sulphur-containing bathing agents, lime-sulphur used for fungicide and insecticide, industrial waste water and sludge, etc., are often toxic and can be fatal as hydrogen sulphide is released.

Several methods [1–7] for the analyses of polysulphides have been reported, mainly titration after sulphitolysis [1,3,7] and cyanolysis [5]. Although these methods are in routine use, the sensitivity is poor and the techniques are non-specific and tedious.

We previously reported analytical methods for sulphide [8,9] and the metabolite of sulphide [10] in biological materials. This paper describes a sensitive and simple method for the determination of polysulphides in human blood, by conversion into a stable disulphide, bis(pentafluorobenzyl)disulphide (BPFBD), followed by gas chromatography (GC) with electron-capture detection (ECD) and gas chromatography–mass spectrometry (GC–MS).

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EXPERIMENTAL

Reagents

Oxygen-free water was used throughout this study and was prepared by bubbling nitrogen into distilled water for 15 min.

A solution of internal standard (I.S.) was prepared by dissolving 1,3,5-tribromobenzene (TBB) in ethyl acetate to give a concentration of 10 μM. TBB was purchased from Wako Pure Chemical Industries (Osaka, Japan).

A standard stock solution of polysulphide was prepared in the following manner. Crystalline sodium sulphide (1.2 g) and powdered sulphur (0.48 g) were dissolved in oxygen-free water (40 ml) in a 50-ml flask, and the solution was shaken for *ca.* 1 h at room temperature. The solution was diluted with oxygen-free water to 50 ml to give a concentration of 100 μmol/ml sodium tetrasulphide (Na_2S_4) with a reddish brown colour. The solution was covered with liquid paraffin to prevent oxidation. This stock solution was directly pipetted out at the time of experiments and diluted to the required concentration.

An alkylating agent, pentafluorobenzyl bromide (PFBBr; Tokyo Kasei Kogyo, Tokyo, Japan), was dissolved in toluene. Tetradecyldimethylbenzylammonium chloride (TDMBA; Tokyo Kasei Kogyo) was used as the counter ion, and potassium cyanide as the desulphurizing agent.

All reagents were of analytical grade.

Preparation of samples

Samples to be tested were prepared by adding the standard solution of polysulphide to the oxygen-free water and to blood, which was collected from healthy volunteers.

Alkylation procedure

Polysulphides (n greater than 2) were alkylated with the alkylating agent, PFBBr, to yield bis(pentafluorobenzyl)polysulphides. Monosulphide was alkylated to bis(pentafluorobenzyl)sulphide (BPFBS):

$$2R\text{–}Br + Na_2S_n \rightarrow R\text{–}S_n\text{–}R + 2NaBr \qquad R = \text{pentafluorobenzyl};\ n = 1\text{–}5$$

A 0.5-ml volume of 20 mM PFBBr solution in toluene and 2.0 ml of ethyl acetate containing 10 μM I.S. (TBB) were put into a 10-ml glass-stoppered test-tube, with 0.8 ml of 5 mM TDMBA solution in the oxygen-free water saturated with sodium tetraborate. To this mixture was added 0.2 ml of the test sample, and the preparation was stirred vigorously for 1 min at room temperature. About 0.1 g of potassium dihydrogenphosphate (KH_2PO_4) was added as a buffer, the mixture was stirred vigorously for 10 s and then centrifuged at 1400 g for 15 min. The organic phase was placed in another test-tube, a 0.1-μl aliquot of this organic phase was injected into the GC–ECD apparatus and a 0.5-μl aliquot into the

GC–MS apparatus for the determination of the BPFBS concentration in the sample. The remaining part of the organic phase was used for the second step, desulphuration.

Desulphuration procedure

Desulphuration takes place according to the following reaction:

$$R\text{–}S_n\text{–}R + (n-2)KCN \longrightarrow R\text{–}S_2\text{–}R + (n-2)KSCN$$

R = pentafluorobenzyl; n = 3–5

Bis(pentafluorobenzyl)polysulphides with three to five sulphur atoms are desulphurated by potassium cyanide to the alkylated disulphide, bis(pentafluorobenzyl)disulphide (BPFBD).

About 0.1 ml of the alkylated polysulphide solution (the organic phase) was put into a 10-ml test-tube with 0.4 ml of potassium cyanide in aqueous solution. The optimum concentration of potassium cyanide was checked in advance, and 5.0 m*M* for blood and 2.5 m*M* for the water sample were used. The solution was stirred vigorously for 40 s at room temperature, and then 2 ml of *n*-hexane were added to stabilize the product. The mixture was again stirred vigorously for 20 s, and then centrifuged at 1400 *g* for 15 min. The organic phase with BPFBD was placed in another test-tube, a 1-μl aliquot of the solution was injected into the GC–ECD apparatus, and a 3-μl aliquot into the GC–MS apparatus.

The procedure is summarized in Fig. 1.

Apparatus

The apparatus used was a Shimadzu Model GC-14AE gas chromatograph, equipped with a ^{63}Ni electron-capture detector, connected to a Shimadzu Model C-R5A Chromatopac computerized recorder and a Shimadzu Model QP-1000 gas chromatograph–mass spectrometer.

GC conditions

The column was a glass tube (2.1 m × 3 mm I.D.) packed with 5% Apiezon grease L on Uniport HP (60–80 mesh). The temperatures of the column, the injection port and the detector were kept at 220, 270 and 270°C, respectively. Nitrogen was used as the carrier gas at a flow-rate of 30 ml/min.

GC–MS conditions

The column was a glass tube (2.1 m × 3 mm I.D.) packed with 5% Apiezon grease L on Uniport HP (60–80 mesh). The temperature of the column was kept at 220°C, the temperatures of the injection port, the separator and the ion source were kept at 250°C, respectively. Helium was used as the carrier gas, at a flow-rate of 30 ml/min. The ionization energy was 70 eV.

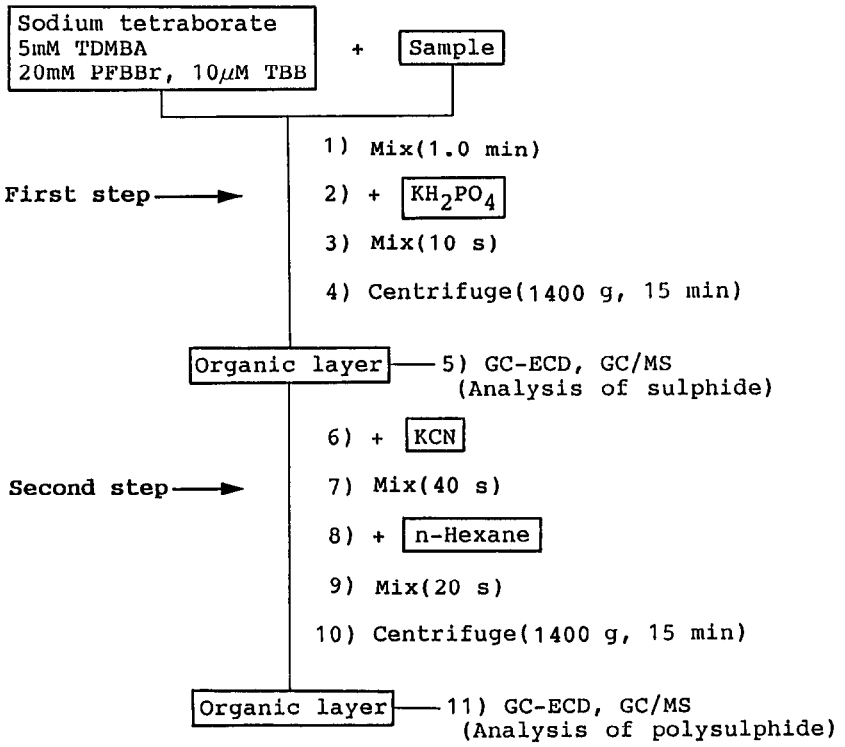

Fig. 1. Procedure for the extractive alkylation and desulphuration.

RESULTS

Analysis by GC

A gas chromatogram of an extract from blood containing 0.5 μmol/ml of the reaction product of polysulphide is shown in Fig. 2. Sharp and symmetrical peaks of the I.S. and the reaction product can be seen, with retention times of 2.8 and 4.6 min, respectively. There were no interfering peaks.

The calibration curve was obtained by plotting the peak-area ratio of the reaction product to the I.S. against the concentration of polysulphide, using GC. The curve for the blood sample was linear in the concentration range from 0.2 to at least 2 μmol/ml, with a correlation coefficient of 0.998. A lime-sulphur agent (the calcium polysulphide content is 27.5%) purchased from a market was tested in the same manner, and a very similar linear calibration curve was obtained.

The gross recoveries of polysulphide from blood were 60–70%, in the concentration range 0.2–2 μmol/ml, but did not reach 50% in the range below 0.2 μmol/ml, Analysis of five samples containing 0.5 μmol/ml polysulphide yielded a coefficient of variation of 4.94% for the intra-assay precision and 6.31% for the

Fig. 2. Gas chromatogram of the internal standard TBB (a) and the reaction product of polysulphide (b), present in blood at a concentration of 0.5 μmol/ml.

inter-assay precision. A measured polysulphide concentration (mean ± S.D.) of 0.47 ± 0.02 μmol/ml was obtained in the intra-assay, and 0.48 ± 0.03 μmol/ml in inter-assay.

The lower limit of detection, based on a concentration giving a signal three times the average noise, was *ca.* 0.005 μmol/ml in blood samples, which was considered to be sufficiently good to compensate for the relatively poor recovery of polysulphide at low concentrations.

Analysis by GC–MS

The peaks of TBB and the reaction product of polysulphide were analysed by GC–MS: their mass spectra are shown in Fig. 3. The molecular ion of TBB appeared at *m/z* 314 and 316, and the fragment ion at *m/z* 235 (M − Br). The molecular ion of the reaction product of polysulphide was observed at *m/z* 426 (M^+), the base peak at *m/z* 181 ($M - C_6F_5CH_2SS$) and a small fragment ion at *m/z* 213 ($M - C_6F_5CH_2S$). The mass spectral pattern indicated that the reaction product was bis(pentafluorobenzyl)disulphide (BPFBD).

Mass fragmentography was carried out by monitoring two ions of the I.S., *m/z* 314 and 235, and two ions of BPFBD, *m/z* 426 and 181. All peaks were clearly separated, with no interference. The lower limit of detection of polysulphide in mass fragmentography was much the same as that in GC. Mass fragmentography proved useful to identify the polysulphide product, BPFBD, in unknown samples, based on the sensitivity and selectivity.

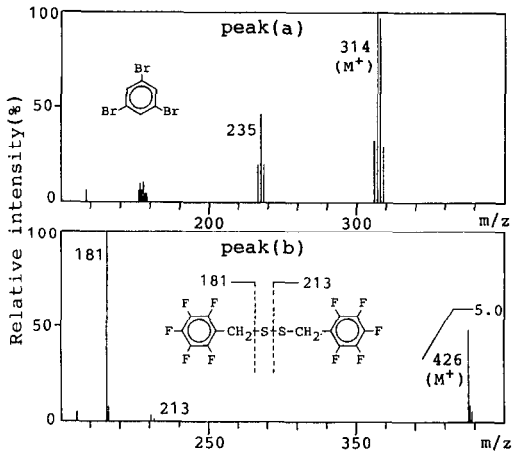

Fig. 3. Mass spectra of (a) the internal standard, TBB, and (b) the reaction product of polysulphide.

Application

We examined a blood sample collected from an individual who had died 23 h after drinking a polysulphide solution (a bathing agent containing lime and sulphur). The amount of polysulphide in this sample was 1.4 μmol/ml.

DISCUSSION

Under acid conditions, polysulphides are unstable and readily decompose to sulphur and hydrogen sulphide. Although monosulphide can be analysed by GC or GC–MS as BPFBS after alkylation, the calibration curve of each bis(pentafluorobenzyl)polysulphide is complicated and tedious to plot over a wide range of concentrations. These problems can be overcome using our technique, *i.e.* the two-step procedure of alkylation and desulphuration. Polysulphides were alkylated in the first step to a mixture of bis(pentafluorobenzyl)polysulphides, with two to five sulphur atoms, then unified to BPFBD by desulphuration, so that the polysulphides could be simultaneously analysed by GC and GC–MS. Hence, this technique makes feasible the analysis of monosulphide and polysulphides as a series. The other advantages are that the entire process is rapid, alkylation and desulphuration are readily carried out at room temperature, and the sensitivity is good, with a lower limit of detection of 0.005 μmol/ml.

Toxicological examination in forensic investigations verified the practicability of the procedure.

CONCLUSION

A sensitive and simple method for the analysis of polysulphides in human blood was devised. This method is suitable for the analysis of polysulphides in cases of related toxicity.

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