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PENTAFLUOROBENZYL *p*-TOLUENESULPHONATE AS A NEW DERIVATIZING REAGENT FOR ELECTRON-CAPTURE GAS CHROMATOGRAPHIC DETERMINATION OF TRACE INORGANIC ANIONS

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SUMMARY

Inorganic anions in water were reacted with pentafluorobenzyl *p*-toluenesulphonate in dichloromethane, using tetra-*n*-amylammonium chloride as a phase-transfer catalyst. Bromide, iodide, thiocyanate and nitrite at relatively low concentrations can be pentafluorobenzylated, and the resulting derivatives can be detected by electron-capture gas chromatography. The interferences of some anions were examined. The recovery test of bromide, iodide or thiocyanate spikes and an intercomparison study for nitrite were performed. This gas chromatographic method has been successfully applied to the determination of bromide and iodide in kelp extract, and thiocyanate and/or nitrite in human urine or saliva.

INTRODUCTION

To date, gas chromatography (GC) has been widely used for separation and analyses of volatile compounds. Furthermore, by using derivatization, non-volatile compounds can be determined by GC. Derivatization has been employed not only to volatilize the analyte but also to introduce a detector-oriented tag into the molecule. Electron-capture detection (ECD) has been studied in conjunction with derivatization to introduce a detector-oriented tag, due to its high sensitivity, and several derivatizing reagents have been developed for GC–ECD[1]. The determination of inorganic anions by GC with derivatization has not been extensively studied[2], compared to that for inorganic cations. This is due to the failure to develop a suitable method applicable to the derivatization of various anions.

MacGee and Allen[3] reported a method for determining halide ions, which were converted into their tetraalkylammonium salts on an ion-exchange resin and then into the corresponding alkyl halides by thermal decomposition in the injection port

of the gas chromatograph. Moore[4] has modified the method by using *n*-butyl *p*-toluenesulphonate as the alkylating reagent. Faigle and Klockow[5] have also developed the method using *n*-decyl methanesulphonate: in this case, cyanide, thiocyanate and nitrate can be determined, as well as halides. These methods are complicated and time-consuming, because the inorganic anions must first be isolated or dried, and then derivatized in the water-free organic solvent. We have improved Moore's method: aqueous inorganic anions were extracted into the organic layer as their tetraalkylammonium salts and then converted into their *n*-butyl derivatives, using *n*-butyl *p*-toluenesulphonate as the derivatizing reagent[6]. The method is not very sensitive because the use of flame ionization detection (FID) could not be avoided, owing to the introduction of the hydrocarbon moiety from the derivatizing reagent. Therefore, in order to use ECD, which is much more sensitive than FID, we have attempted pentafluorobenzylation of inorganic anions instead of *n*-butylation as in the above method. A new derivatizing reagent, pentafluorobenzyl *p*-toluenesulphonate (TsO-PFB) has been synthesized, and its applicability to the simultaneous derivatization of inorganic anions has been studied by GC–FID[7]. As a result, bromide, iodide, cyanide, thiocyanate, nitrite, nitrate and sulphide can be derivatized and detected simultaneously. However, this technique is an ECD-oriented derivatization designed to enhance sensitivity. In this work, we have investigated the determination of trace amounts of inorganic anions by pentafluorobenzylation with TsO-PFB, using GC–ECD.

EXPERIMENTAL

Apparatus

A Shimadzu GC-4BM gas chromatograph equipped with a [63]Ni ECD unit (Kyoto, Japan) was used. A stainless-steel column (4 m × 3 mm I.D.) was packed with 5% OV-225 (2.5 m) and 5% OV-210 (1.5 m), both of which were coated on 60-80 mesh Uniport HP, obtained from Gasukuro Kogyo (Tokyo, Japan). Nitrogen was used as the carrier gas at a constant flow-rate of 30 ml/min. The detector and injection port temperatures were maintained at 200°C, and the column temperature was kept at 130°C for the simultaneous determination of bromide and iodide, and at 190°C for that of thiocyanate and nitrite. A Shimadzu Chromatopac C-R3A data processor was used as the recorder and integrator.

Reagents

Commercial grade tetra-*n*-amylammonium chloride (TAAC) was obtained from Wako (Osaka, Japan). The derivatizing reagent, TsO-PFB, was prepared from *p*-toluenesulphonyl chloride and pentafluorobenzyl alcohol by a modification of the literature method[8], and recrystallized from ethanol. It was identified by mass spectrometry and infrared spectroscopy. The inorganic anions were used as their sodium or potassium salts, of analytical-reagent grade. Dichloromethane and deionized water were distilled before use for analysis. All other chemicals were also of analytical-reagent grade.

Procedure

The procedure for the determination of bromide, iodide, thiocyanate and ni-

trite was as follows. A brown-coloured test tube with a screw cap (*ca.* 10 ml) was used as the reaction vessel, in order to protect the contents from the ligth. To 1.0 ml of sample solution were added 0.10 ml of an aqueous solution of TAAC ($2.0 \cdot 10^{-2}$ *M*), 1.0 ml of a dichloromethane solution of TsO-PFB ($2.5 \cdot 10^{-3}$ *M*), and 0.10 ml of a buffer solution (pH 7) containing disodium hydrogenphosphate (0.1674 *M*) and citric acid (0.01765 *M*). Then, the vessel was sealed tightly with the screw cap and shaken mechanically for 30 min at room temperature. After shaking, 0.10 ml of the dichloromethane layer was separated from the aqueous layer. In the analyses of human urine or saliva, the organic and aqueous layers often became muddy and inseparable, so the organic layer was separated from the aqueous layer after ultrasonic irradiation. The dichloromethane solution was evaporated carefully under a nitrogen stream for 2 min at room temperature, and then 1.0 ml of *tert.*-butyl methyl ether solution was added to the residue. The *tert.*-butyl methyl ether solution contained 1,2,4-trichlorobenzene ($2.0 \cdot 10^{-4}$ *M*) or 1,2,3,4-tetrachlorobenzene ($1.0 \cdot 10^{-4}$ *M*) as an internal standard for the determination of bromide and iodide or thiocyanate and nitrite, respectively. An aliquot (0.10 μl) of the *tert.*-butyl methyl ether solution was injected into the gas chromatograph.

Samples

Kelp. "Wakame" seaweed grown in Japanese waters were washed with water and then ashed in a crucible. About 0.3 g of the resulting ash (*i.e.* kelp) was weighed accurately, and bromide and iodide in the kelp were extracted with water (8 ml) by shaking for 2 h at 60°C. Then, the solution was used for analysis after filtration and 50-fold dilution with water for bromide determination or 10-fold dilution for iodide determination.

Human urine and saliva. The samples were offered from the young investigators in our laboratory. Immediately after collection, urine and saliva were diluted 2- and 10-fold, respectively, with water and used for analysis. In the determination of nitrite in saliva, saturated aqueous silver sulphate solution was added to the sample in order to prevent the interference of chloride.

RESULTS AND DISCUSSION

Pentafluorobenzylation of inorganic anions with TsO-PFB

As previously reported[7], relatively high concentrations ($> ca.$ 50 μg/ml) of bromide, iodide, cyanide, thiocyanate, nitrite, nitrate and sulphide can be pentafluorobenzylated with TSO-PFB, and their PFB derivatives determined by GC–FID. Therefore, pentafluorobenzylation of the above anions was studied at lower concentrations ($< ca.$ 5 μg/ml), and their PFB derivatives were detected by GC–ECD. At lower concentrations, only four anions (bromide, iodide, thiocyanate and nitrite) gave peaks for their PFB derivatives (*i.e.* pentafluorobenzyl bromide, pentafluorobenzyl iodide, pentafluorobenzyl thiocyanate and α-nitro-2,3,4,5,6-pentafluorotoluene, respectively). The others did not give GC peaks corresponding to their PFB derivatives, even when derivatization was performed under varying reaction conditions. Therefore, further work was concentrated on the above four anions.

Dichloromethane was the most suitable organic solvent for pentafluorobenzylation. As this solvent is apparently unfavourable for ECD, various organic sol-

vents immiscible with water were tried in order to find a suitable solvent for penta-fluorobenzylation followed by ECD. Among the solvents *tert.*-butyl methyl ether, methyl acetate, ethyl acetate, benzene, toluene, ethylbenzene, 1-heptanol, 1-octanol, 1-decanol, methyl isopropyl ketone, methyl isobutyl ketone and methyl isoamyl ketone, *tert.*-butyl methyl ether gave the highest derivatization yield for each inorganic anion: only *ca.* 10% of the yield with dichloromethane. In this work, therefore, dichloromethane was used for the PFB derivatization reaction and then *tert.*-butyl methyl ether was used for ECD, as described in the procedure in the Experimental section.

Determination of bromide and iodide

As the gas chromatograph used was equipped with a single ECD unit, GC determination with column temperature-programming is very difficult. Therefore, the determination of bromide and iodide was performed at a different column temperature from that for the determination of thiocyanate and nitrite.

Ten standard reference samples containing bromide and iodide were determined over the concentration ranges 0.16–1.6 and 0.38–3.8 $\mu g/ml$, respectively, to evaluate the quantitative application of the method for the determination of both anions. Each calibration curve was constructed by plotting the concentration of analyte (x) *vs.* the peak-area ratio of its PFB derivative to internal standard (y). Linear regression equations, $y = 0.3113x + 0.0086$ for bromide and $y = 0.1091x + 0.0022$ for iodide, were obtained with correlation coefficients of 0.9967 and 0.9971, respec-

TABLE I

INTERFERENCE STUDY FOR THE DETERMINATION OF Br^- AND I^-

Concentrations of Br^- and I^- are 1.0 and 3.0 $\mu g/ml$, respectively.

Anion	Added as	Concentration ($\mu g/ml$)	Relative peak area[*]	
			Br^-	I^-
Standard			100.0 ± 1.1	100.0 ± 4.7
F^-	KF	100	98.5 ± 2.8	100.5 ± 2.7
Cl^-	KCl	100	101.6 ± 3.7	103.7 ± 0.7
Br^-	KBr	100	—	105.2 ± 0.9
I^-	KI	10	97.6 ± 2.0	—
CN^-	KCN	100	95.8 ± 1.9	100.7 ± 3.5
SCN^-	KSCN	50	100.9 ± 1.5	100.6 ± 2.6
S^{2-}	Na₂S	10	99.7 ± 4.8	—
		50	**	101.5 ± 3.2
NO_2^-	NaNO₂	10	97.9 ± 3.0	104.2 ± 2.6
NO_3^-	NaNO₃	10	96.3 ± 3.3	—
		50	**	101.5 ± 3.2
CO_3^{2-}	Na₂CO₃	50	100.6 ± 2.9	—
		100	**	97.8 ± 2.6
SO_3^{2-}	Na₂SO₃	100	99.1 ± 1.6	100.3 ± 2.6
SO_4^{2-}	Na₂SO₄	50	101.3 ± 3.6	103.6 ± 2.1
$H_2PO_4^-$	NaH₂PO₄	100	97.5 ± 1.9	104.0 ± 2.7
HPO_4^{2-}	Na₂HPO₄	100	101.5 ± 3.1	101.5 ± 2.0

[*] Mean ± S.D. of five replicate analyses.
** Interference was observed.

TABLE II

RESULTS OF ANALYSES OF KELP EXTRACT WITH Br⁻ AND I⁻ RECOVERY TESTS

Amount added (µg/ml)		Amount found (µg/ml)		Recovery (%)	
Br⁻	I⁻	Br⁻	I⁻	Br⁻	I⁻
0.00	0.00	(0.30 ± 0.012)*	(1.17 ± 0.026)*	–	–
0.30	0.50	0.61	1.65	103.3	96.0
0.60	1.00	0.93	2.10	105.0	93.0
0.90	1.50	1.18	2.64	97.8	98.0
1.20	2.00	1.48	3.20	98.3	101.5

* Mean ± S.D. of five replicate analyses.

tively. This indicates good applicability of the method to quantitation of bromide and iodide. The interferences of some anions with the determination of bromide and iodide are shown in Table I. The anions selected are those usually found in environmental and biological samples. These anions do not appear to interfere with this determination, except for sulphide (50 µg/ml), nitrate (50 µg/ml) and carbonate (100 µg/ml). However, the interferences were not observed at 10 µg/ml sulphide and nitrate and at 50 µg/ml carbonate.

In order to investigate the applicability of the method to the analyses of real samples, bromide and iodide in kelp extract were determined by the present method. Recovery tests for bromide and iodide added to the samples were performed. The results are given in Table II, and Fig. 1 shows the gas chromatograms obtained on analysis of the kelp extract. For both anions, the recoveries are all >90% and the relative standard deviations of the method are <5%.
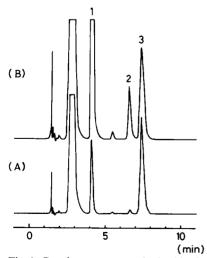

Fig. 1. Gas chromatograms obtained in the determination of bromide (A) and iodide (B) in kelp extract. Peaks: 1 = pentafluorobenzyl bromide; 2 = pentafluorobenzyl iodide; 3 = 1,2,4-trichlorobenzene (internal standard).

Determination of thiocyanate and nitrite

The calibration curves for thiocyanate and nitrite were constructed in the same manner as those for bromide and iodide. Good linear relationships were obtained; linear regression equations are $y = 0.296x - 0.0185$ and $y = 0.0839x - 0.00453$, and correlation coefficients are 0.9988 and 0.9995 for thiocyanate (0.5–5.0 μg/ml) and nitrite (1.0–10.0 μg/ml), respectively. The interferences of some anions with the determination of thiocyanate and nitrite were also examined, and the results are given in Table III. None of the anions at the concentrations examined interfere with the present method. The method was applied to the determination of thiocyanate in human urine samples, and recovery tests were also performed, as in Table IV. The recoveries are all > 90%. Thiocyanate and nitrite in human saliva were also determined by the present method. When nitrite co-exists with > 10 μg/ml thiocyanate, the analytical value for nitrite is lower than its actual value. This is presumably due to the preferential reaction of thiocyanate with TsO-PFB. Unfortunately, human saliva often contains > 10 μg/ml thiocyanate. Therefore, in the determination of nitrite, silver sulphate was added to each saliva sample solution in order to precipitate thiocyanate and to stop it from interfering. In this case, nitrite could not be determined simultaneously with thiocyanate. Fig. 2 shows the gas chromatogram of the analysis, and the results are given in Table V, together with the values for nitrite concentration determined by the widely used colorimetric method[9]. The nitrite concentrations for each sample obtained by the two methods are in good agreement.

TABLE III

INTERFERENCE STUDY FOR THE DETERMINATION OF SCN^- AND NO_2^-

Concentrations of SCN^- and NO_2^- are 5.0 μg/ml.

Anion	Added as	Amount added (μg/ml)	Relative peak area[*]	
			SCN^-	NO_2^-
Standard			100.0 ± 3.0	100.0 ± 5.0
F^-	KF	100	102.5 ± 4.2	97.4 ± 2.0
Cl^-	KCl	1000	97.9 ± 2.9	95.6 ± 4.3
Br^-	KBr	10	100.9 ± 4.1	103.0 ± 1.4
I^-	KI	1	104.2 ± 4.9	97.2 ± 2.8
CN^-	KCN	10	104.6 ± 5.8	98.5 ± 1.8
SCN^-	KSCN	10	–	97.8 ± 2.7
S^{2-}	Na_2S	10	101.1 ± 1.5	97.5 ± 1.8
NO_2^-	$NaNO_2$	100	99.6 ± 2.0	–
NO_3^-	$NaNO_3$	50	98.3 ± 2.5	97.5 ± 3.2
CO_3^{2-}	Na_2CO_3	100	100.9 ± 3.2	102.5 ± 5.9
SO_3^{2-}	Na_2SO_3	100	103.8 ± 1.0	97.4 ± 2.0
SO_4^{2-}	Na_2SO_4	100	103.9 ± 5.0	96.3 ± 2.1
$H_2PO_4^-$	NaH_2PO_4	100	102.1 ± 3.3	102.5 ± 5.8
HPO_4^{2-}	Na_2HPO_4	100	103.7 ± 5.4	97.8 ± 2.4

[*] Mean ± S.D. of five and three replicate analyses for SCN^- and NO_2^-, respectively.

TABLE IV

RESULTS OF ANALYSES OF HUMAN URINE WITH SCN⁻ RECOVERY TESTS

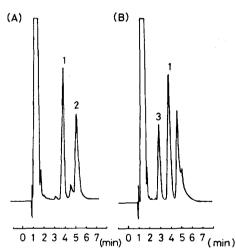| Urine No.	Amount added (µg/ml)	Amount found (µg/ml)	Recovery (%)
1	0.0	(1.5 ± 0.03)*	—
	2.0	3.3	93.1
	4.0	5.6	103.2
	6.0	7.5	99.7
	10	12.2	106.9
2	0.0	(0.9 ± 0.03)*	—
	2.0	3.0	102.0
	4.0	4.9	98.6
	6.0	7.1	102.0
	10.0	10.7	97.7
3	0.0	(1.4 ± 0.03)*	—
	2.0	3.4	99.0
	4.0	5.6	103.8
	6.0	7.5	101.7
	10.0	10.9	94.9

* Mean ± S.D. of five replicate analyses.

Fig. 2. Gas chromatograms obtained in the determination of thiocyanate (A) and nitrite (B) in human saliva. Peaks: 1 = 1,2,3,4-tetrachlorobenzene (internal standard); 2 = pentafluorobenzyl thiocyanate; 3 = α-nitro-2,3,4,5,6-pentafluorotoluene.

CONCLUSIONS

From the results mentioned above, the present method is applicable to the simultaneous determination of bromide and iodide or to that of thiocyanate and nitrite at low concentrations in real samples. As bromide, iodide, thiocyanate and nitrite at low concentrations are pentafluorobenzylated simultaneously, their simul-

TABLE V

RESULTS OF ANALYSES OF HUMAN SALIVA WITH INTERCOMPARISON STUDY FOR NO_2^- DETERMINATION

Saliva No.	Concentration of SCN^-* ($\mu g/ml$)	Concentration of NO_2^- ($\mu g/ml$)	
		GC*	Colorimetry**
1	6.71 ± 0.01	5.64 ± 0.03	5.64
2	7.41 ± 0.06	1.93 ± 0.01	1.98
3	30.43 ± 0.26	5.60 ± 0.14	5.59
4	12.77 ± 0.25	7.33 ± 0.10	7.22
5	7.80 ± 0.10	4.43 ± 0.23	4.61
6	14.95 ± 0.21	1.87 ± 0.32	1.81

* Mean ± S.D. of four replicate analyses.
** Colorimetric method using *n*-(1-naphthyl)-ethylenediamine and sulphanilic acid.

taneous determination may be performed by using the column temperature-programming technique.

Besides the above four anions, cyanide, nitrate and sulphide can also be pentafluorobenzylated at relatively high concentrations, as reported previously[7]. Cyanide, nitrate and sulphide at relatively low concentrations are not converted into their PFB derivatives because: none of these three anions at low concentrations are effectively extracted into the organic layer containing TsO-PFB; in the previous method using FID, 1.0 ml of a 0.1 M solution of TsO-PFB was used for the derivatization reaction, whereas in the present method 1.0 ml of a $2.5 \cdot 10^{-3}$ M was used. This means that there is a shortage of the reagent and the three anions cannot therefore be derivatized. The injection of larger amounts of TsO-PFB than that used in this work decreased the initial standing current of an ECD cell and the sensitivity of the detector. Moreover, it is difficult and time-consuming to remove TsO-PFB from the organic layer injected into the gas chromatograph. An attempt is now in progress to increase the concentration of TsO-PFB in the derivatization reaction without decreasing the detector sensitivity. It is of interest to try to derivatize anions with TsO-PFB in a homogeneous mixture using a water-miscible organic solvent, instead of the biphasic reaction system described here.

ACKNOWLEDGEMENT

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