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SIMULTANEOUS GAS CHROMATOGRAPHIC DETERMINATION OF IODIDE, NITRITE, SULPHIDE AND THIOCYANATE ANIONS BY DERIVATIZATION WITH PENTAFLUOROBENZYL BROMIDE

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SUMMARY

A gas chromatographic method is described for the simultaneous determination of iodide, nitrite, sulphide and thiocyanate anions as their pentafluorobenzyl derivatives. The method is based on transfer of the anions from the aqueous solution by *n*-hexadecyltrimethylammonium bromide into the dichloromethane organic phase, where derivatization with pentafluorobenzyl bromide occurs. Several parameters affecting the transfer and/or derivatization of the anions were investigated, including the phase-transfer catalyst, concentration of acid and base added, organic solvent, reaction time and the amount of derivatizing agent needed. Individual and simultaneous determinations of the anions could be achieved at sub-μmol levels initially in the aqueous phase, by use of a flame ionization detector.

INTRODUCTION

Direct gas chromatography (GC) is used for the separation and the determination of thermally stable or volatile organic and inorganic compounds. In order to extend its application to thermolabile and polar compounds, derivatization has been widely employed, based on the chemical modification of compounds with a variety of reagents mainly for better chromatographic properties and for higher responses to a specific detector.

Pentafluorobenzyl bromide (PFBBr) has been used for the derivatization of polar organic compounds[1,2], including carboxylic acid, phenols, mercaptans and sulphonamides, to enhance their responses to electron-capture detection (ECD); PFBBr was originally introduced by us for the derivatization of inorganic anions[3–7] for individual analysis of such anions.

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Simultaneous GC determination of several inorganic anions is definitely more difficult than the determination of a single anion, due to the lack of suitable derivatizing reagents common to anions with different chemical and physical properties; thus, there is the problem of optimization of the derivatization conditions for several anions. Therefore, only a few methods have been reported for the simultaneous determination of several anions, including the silylation of oxyanions[8,9], and alkylation of various anions with dialkyl sulphate[10,11], *n*-butyl iodide[12], *n*-decyl methanesulphonate[13], *n*-butyl *p*-toluenesulphonate[14,15] or by the thermal decomposition of tetraalkylammonium halide[16]. In general, these methods have inherent limits and are not appropriate for trace analysis because the silyl or alkyl moieties incorporated into the anion derivatives are not highly responsive to specific detection such as ECD, thermionic detection (TID) or flame photometric detection (FPD). Exceptional is the case where the anion moiety of the derivative has inherent sensitivity to the specific detector, *e.g.*, a sulphide derivative to FPD, an halide derivative to ECD or a cyanide or thiocyanate derivative to TID. In this situation, however, simultaneous determination of these anions is still inaccessible because only a single detector usually can be used. Therefore, the simultaneous determination of anions based on their derivatization with pentafluorophenyl-type reagents such as PFBBr and pentafluorobenzyl *p*-toluenesulphonate[17,18] is very attractive, because the pentafluorophenyl moiety of the resulting derivatives is highly responsive to ECD.

In this paper, we have report the application of PFBBr to the simultaneous derivatization and GC determination of various anions of biological interest. The simultaneous determination of iodide, nitrite, sulphide and thiocyanate anions is possible as their pentafluorobenzyl derivatives based on an extractive alkylation mechanism, using *n*-hexadecyltrimethylammonium bromide as the phase-transfer catalyst.

EXPERIMENTAL

GC conditions

A Shimadzu GC-8A gas chromatograph equipped with dual flame ionization detection (FID) (Kyoto, Japan) was used. The separation column and the temperature employed varied with the anions in individual and simultaneous determinations; details are given in Table I. Nitrogen was used as the carrier gas at a flow-rate of 40 ml/min. A Shimadzu R-111 and Shimadzu Chromatopac C-E1B were used as the recorder and integrator, respectively.

Materials

α-Bromo-2,3,4,5,6-pentafluorotoluene (PFBBr) (Aldrich, Milwaukee, WI, U.S.A.), bibenzyl and *p*-dibromobenzene (Wako, Osaka, Japan), *p*-bromoiodobenzene and 2,3,5,6-tetrachloronitrobenzene (Tokyo Kasei, Tokyo, Japan), tetra-*n*-butylammonium hydrogensulphate (TBAHS), tetra-*n*-pentylammonium bromide (TPAB) and tetra-*n*-hexylammonium bromide (THAB) (Aldrich), benzalkonium chloride [dimethyl *n*-tetradecylbenzylammonium chloride (BAC)] and dimethylbenzylphenylammonium chloride (DMBPAC) (Tokyo Kasei), *n*-hexadecyltrimethylammonium bromide (HDTMAB) (Sigma, St. Louis, MO, U.S.A.), 10% OV-17 on Uniport HP (60–80 mesh) (Gasukuro, Tokyo, Japan) and 4% OV-101 + 6% OV-210

TABLE I

GC CONDITIONS

Anion	*Column stationary phase*	*Temperature (°C)*		
		Column	*Detector*	*Injector*
I^-	10% OV-17*	140	280	280
NO_2^-	4% OV-101 + 6% OV-210**	170	280	280
S^{2-}	10% OV-17	200	280	280
SCN^-	4% OV-101 + 6% OV-210	200	280	280
Simultaneous determination	4% OV-101 + 6% OV-210	Programmed (see Fig. 4)	280	280

* Coated on Uniport HP (60–80 mesh) and packed in a stainless-steel column (2.0 m × 3 mm I.D.).

** Coated on Chromosorb W HP (60–80 mesh) and packed in a stainless-steel column (3.0 m × 3 mm I.D.).

on Chromosorb W HP (60–80 mesh) (Shimadzu) were used without further treatment. Potassium thiocyanate (E. Merck, Darmstadt, F.R.G.), potassium cyanide, sodium iodide, sodium nitrite and sodium sulphide (Wako), dichloromethane and other reagents were of analytical reagent grade. Solutions of inorganic anions and the phase-transfer catalyst (PTC) wsere prepared by dissolving these chemicals in distilled and deionized water.

Derivatization procedures

A. Procedure for individual determination. As shown in Table II, a 0.1-ml volume of various PTC aqueous solutions was added to a 10-ml glass-stoppered test-tube containing 0.1 ml of 10^{-3} *M* aqueous potassium hydroxide solution and 0.1 ml of an aqueous solution of each anion reference standard. Then, 0.4 ml of the internal standard (I.S.) in dichloromethane and 0.1 ml of a PFBBr solution containing various amounts of neat PFBBr in dichloromethane were added. The reaction mixture was shaken mechanically at 30°C for a fixed time. At the end of the reaction period, 3.0 ml of water were added and the reaction vessel was shaken slightly. After the separation of the organic phase, an aliquot of the dichloromethane layer was injected for GC determination.

B. Procedure for simultaneous determination. Also as indicated in Table II, 0.1 ml of 0.1 *M* HDTMAB was added to a test-tube containing 0.1 ml of $10^{-3}M$ potassium hydroxide solution and 0.1 ml of a solution of the anions. Then 0.2 ml of I.S. (2,3,5,6-tetrachloronitrobenzene) solution and 0.4 ml of PFBBr solution were added. The reaction mixture was shaken mechanically at 30°C for 1.5 h. Then it was treated with a suitable amount of water (3.0 ml) and centrifuged at 1500 *g* for 15 min for the phase separation. An aliquot of the organic dichloromethane layer was subjected to GC analysis.

RESULTS AND DISCUSSION

Extractive pentafluorobenzylation of biologically important inorganic anions

TABLE II

DERIVATIZATION CONDITIONS

Deionized and distilled water was used to prepare the anions, potassium hydroxide and various phase-transfer catalyst solutions; dichloromethane, the organic solvent, was used to prepare the PFBBr and various internal standard solutions.

Anion	*Potassium hydroxide (M)*	*Phase transfer catalyst (M)*	*Reaction time (min)*	*Neat PFBBr (μl)*	*Internal standard (mM)*
I^-	10^{-3}	HDTMAB (0.05)	30	2	*p*-Dibromobenzene (3.4)
NO_2^-	10^{-3}	TPAB (0.1)	60	2	*p*-Bromoiodobenzene (5.3)
S^{2-}	10^{-1}	HDTMAB (0.05)	60	4	2,3,5,6-Tetrachloronitrobenzene (9.6)
SCN^-	10^{-3}	TBAHS (1.0)	30	2	Bibenzyl (13.7)
Simultaneous determination	10^{-3}	HDTMAB (0.1)	90	8	2,3,5,6-Tetrachloronitrobenzene (9.6)

such as cyanide, iodide, nitrate, nitrite, sulphide and thiocyanate has been investigated under various conditions. The results indicate that iodide, nitrite, sulphide and thiocyanate can be derivatized under the conditions indicated in Table II, but cyanide and nitrate failed to derivatize, due probably to the high solvation of these anions in water which results in poor transfer of the anions to the organic phase for derivatization. Therefore, the derivatization of iodide (1.58 μmol), nitrite (4.35 μmol), sulphide (1.56 μmol) and thiocyanate (3.45 μmol) anions was optimized. The evaluation of the effects of several parameters was based on the peak-area ratio of each anion derivative to the respective I.S.

Effect of phase-transfer catalysts

The effect of quaternary ammonium compounds at various concentrations corresponding to their water solubilities, including 0.05 *M* HDTMAB, 0.1 *M* TPAB, 1.0 *M* TBAHS, 0.1 *M* BAC, 0.1 *M* THAB and 0.2 *M* DMBPAC, on the transfer of various anions in alkaline or acidic aqueous solutions to the dichloromethane phase for derivatization was briefly examined. As shown in Table II, HDTMAB and TBAHS were equally good for iodide, either can be used as PTC. TPAB and BAC had similar effects for nitrite, but TPAB was chosen for individual determination of nitrite because a simpler chromatogram can be obtained. HDTMAB gave about 70% of the peak-area ratio obtained with TPAB and is a compromise choice for simultaneous determination of anions including nitrite. HDTMAB was judged to be the best PTC for sulphide, TBAHS for thiocyanate anion and HDTMAB the next best choice with an effect of about 90% that of TBAHS. When TBAHS was used for the transfer of nitrite, good derivatization only results in a narrow potassium hydroxide concentration range 1.20–1.25 *M*, which prohibits the use of TBAHS as PTC for all of the anions as shown in Fig. 1. Therefore, HDTMAB was selected as optimum PTC for the simultaneous determination of the four anions. After the reaction, water was added as stated in the derivatization procedure, to decrease the amount of ion pair in the dichloromethane phase; otherwise, a gradual drifting of the baseline of the chromatogram occurred, presumably due to the introduction into the column of the organic phase contaminated with hardly volatile substances.

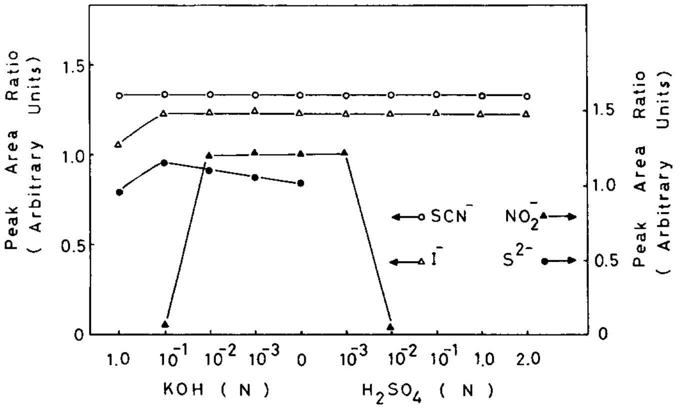

Fig. 1. Effect of added base or acid on the formation of the derivatives. ○ = SCN^-; △ = I^-; ▲ = NO_2^-; ● = S^{2-}; each point indicates the average of three measurements.

Effect of base or acid

The effect of the addition of 0.1 ml of base or acid at various concentrations to the reaction system with PTC, as indicated in Table II, was studied. As shown in Fig. 1, the transfer and derivatization of iodide or thiocyanate anion is optimal over a wide range of base or acid concentrations. For nitrite anion at low concentration, the addition of $\leq 10^{-2}$ *M* base or $\leq 0.5 \cdot 10^{-3}$ *M* acid is favourable. In the case of sulphide anion, the addition of 0.1 *M* base is most favourable, but the transfer and derivatization effect is gradually decreased at lower concentrations of base. This could be due in part to depression of the dissociation of sulphide anion at lower pH, which unfavourably affects its transfer for derivatization. For the simultaneous determination of the anions, HDTMAB is used as the common PTC instead of TBAHS or TPAB; therefore, the effect of the added acid or base with HDTMAB on the partition and derivatization of thiocyanate and nitrite anions was also studied. The results indicate that the range optimal for thiocyanate anion with TBAHS or nitrite anion with TPAB shown in Fig. 1 is also optimal for thiocyanate and nitrite anions with HDTMAB as the PTC. Consequently, 0.1 ml of 10^{-2}–10^{-3} *M* potassium hydroxide is added for simultaneous determination of these four anions.

Effects of organic solvent and reaction time

The effects of the organic solvent and reaction time were examined for the derivatization of each anion at a specific base concentration and PTC at 30°C, as shown in Table II. Among the water-immiscible organic solvents tested, including dichloromethane, acetophenone, cyclohexanone, 1-pentanol and 2-octanol, dichloromethane is the best for the derivatization of each anion with PFBBr. The time to reach equilibrium for the derivatization of thiocyanate, iodide, nitrite or sulphide anion was 10, 10, 60 and 30 min, respectively, as shown in Fig. 2. The rapid equilibria for thiocyanate and iodide could result partly from the rapid transfer and easy derivatization of both anions under the specified conditions. When HDTMAB was used as the common PTC for the four anions, 1.5 h are sufficient to reach equilibrium for the derivatization of iodide, sulphide and thiocyanate anions, but not for nitrite anion, probably due to the lower ability of HDTMAB to transfer the nitrite anion

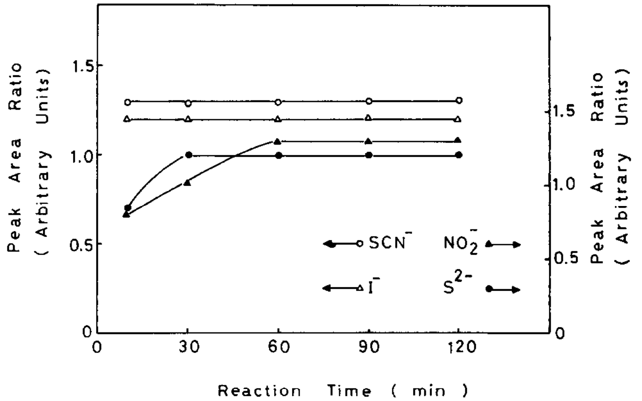

Fig. 2. Effect of the reaction time on the formation of the derivatives. Details as in Fig. 1.

to the organic phase; the derivatization yield of nitrite with HDTMAB as PTC is about 80% of that obtained with TPAB as PTC in 1.5 h.

Effect of amount of PFBBr

The volume of neat PFBBr required for the equilibrium derivatization of thiocyanate (3.45 μmol), iodide (1.58 μmol), nitrite (4.35 μmol) or sulphide (1.56 μmol) was 1, 1, 1 and 2 μl, respectively, as shown in Fig. 3. The higher molar ratio of PFBBr to sulphide needed 16.9:1, is due partly to the fact that the derivative of sulphide[3] contains two pentafluorobenzyl moieties. For individual and simultaneous determination of the anions, the amounts of PFBBr used are indicated in Table II.

Analytical calibration and gas chromatogram

Based on the optimum derivatization conditions, the derivatization procedures for individual and simultaneous determination of the anions were as stated in the Experimental. The quantitative applicability of the method to the individual determination of each anion was first tested. The results indicate good linearity for the determination of each anion at six different concentrations. The linear regression equations obtained were $y = 0.0238x + 0.00978$ with $r = 0.999$ for iodide anion over the range 0.5–200 μg, $y = 0.0167x - 0.0147$ with $r = 0.999$ for nitrite anion over the range 1–200 μg, $y = 0.0410x - 0.00234$ with $r = 0.999$ for sulphide anion over the range 0.1–50 μg and $y = 0.0229x + 0.00939$ with $r = 0.999$ for thiocyanate anion over the range 0.5–200 μg; where y is the peak-area ratio of the anion derivative to the I.S., x is the amount in μg of the anion and r is the correlation coefficient. The lower determination levels of the anions are better than those previously reported[4], based on GC with FID. Satisfactory results were also obtained for the simultaneous determination of the anions at six different concentrations, iodide and nitrite each in the range 2–200 μg, sulphide in the range 1–50 μg and thiocyanate in the range 1–200 μg; the linear regression equations were $y = 0.00857x - 0.00778$ with $r = 0.998$ for iodide, $y = 0.0136x - 0.00149$ with $r = 0.998$ for nitrite, $y = 0.0499x - 0.0476$ with $r = 0.999$ for sulphide and $y = 0.0233x + 0.0184$ with $r = 0.999$ for thiocyanate.

The lower determination levels indicate, the potential of the method for trace

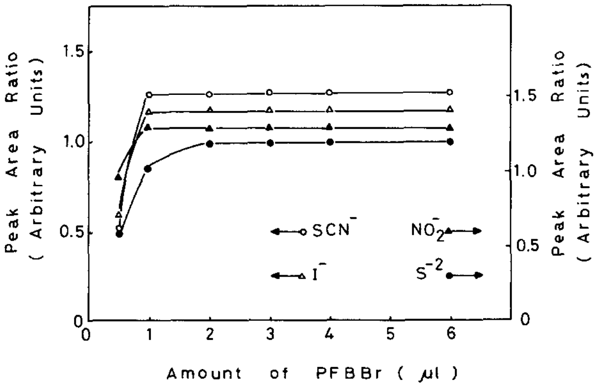

Fig. 3. Effect of the amount of PFBBr on the formation of the derivatives. Details as in Fig. 1.

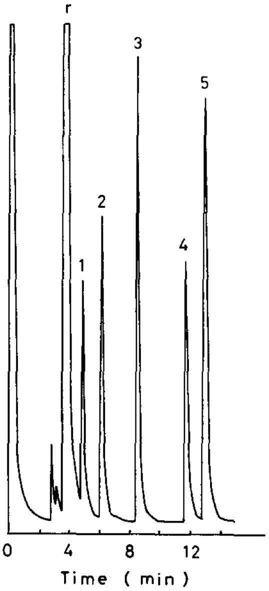

Fig. 4. Gas chromatogram for the simultaneous determination of four inorganic anions. Peaks: 1 = derivative of I^-; 2 = derivative of NO_2^-; 3 = derivative of SCN^-; 4 = 2,3,5,6-tetrachloronitrobenzene (I.S.); 5 = derivative of S^{2-}; r = reagent blank. Conditions: stainless-steel column, 3.0 m × 3 mm I.D., 4% OV-101 + 6% OV-210 on Chromosorb W HP (60–80 mesh); nitrogen flow-rate, 40 ml/min; column temperature, initially 110°C, then raised to 220°C at 10°/min, finally held for 4 min; sample injected, 3 μl; flame ionization detection.

analysis of the anions. A typical gas chromatogram, with well resolved and sharp peaks, for simultaneous GC determination of the anions with a temperature-programming technique is shown in Fig. 4. The structures of peaks 1, 2, 3 and 5, equivalent to the derivatives of iodide, nitrite, thiocyanate and sulphide anions, respectively, were identified by comparison of the retention times with those of the anion derivatives synthesized previously[3,4,6,17]. The results indicate that peaks 1, 2, 3 and 5 are due to pentafluorobenzyl iodide, α-nitro-2,3,4,5,6-pentafluorotoluene, pentafluorobenzyl thiocyanate and bis(pentafluorobenzyl) sulphide, respectively.

Simultaneous determination of inorganic anions including cyanide and nitrate in addition to the four anions is inaccessible with the present system using the stated quaternary ammonium compounds as PTC, probably due to the strong solvation of cyanide and nitrate anions with water. Another kind of PTC such as a cryptate or a crown ether is being investigated as PTC for derivatization with PFBBr; simultaneous determination of the pentafluorobenzyl derivatives of the anions at trace levels by GC with ECD is also under development.

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