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Simultaneous gas chromatographic determination of cyanide, iodide, nitrite, sulphide and thiocyanate anions by derivatization with pentafluorobenzyl bromide and using a kryptand as phase-transfer catalyst

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SUMMARY

An improved gas chromatographic method has been established for the simultaneous determination of cyanide, iodide, nitrite, sulphide and thiocyanate anions as their volatile pentafluorobenzyl derivatives. The method is based on kryptate formation using Kryptofix 222 B polymer as complexing agent and phase-transfer catalyst to transfer the anions from aqueous alkaline solution to the dichloromethane organic phase for derivatization with pentafluorobenzyl bromide to be effected. Structural identification and correction of the cyanide derivative was studied. The parameters affecting the partition and/or derivatization of the anions were investigated, including the concentrations of acid and base in the aqueous phase. Individual and simultaneous determinations of the anions are attainable at sub-micromole levels with flame ionization detection.

INTRODUCTION

In a previous paper¹, a gas chromatographic (GC) method was described for the determination of anions by derivatization with pentafluorobenzyl bromide (PFBBR) and using *n*-hexadecyltrimethylammonium bromide as phase-transfer catalyst (PTC). The disadvantages of using quaternary ammonium PTC without additional treatment include the formation of emulsions from derivatization based on biphasic reactions and the unavoidable introduction of various amounts of PTC onto the separation column. This could result in baseline drift and, worse, contamination of the highly sensitive detector.

To solve these problems, we applied a polymeric and bonded kryptand instead of quaternary ammonium compounds as PTC for the simultaneous determination of anions including cyanide, iodide, nitrite, sulphide and thiocyanate at sub-micromole levels. This led to emulsion-free reactions, easy layer separation and injection of the sample without PTC as to it is in the form of a bonded solid particle. The problems of baseline drift, detector contamination and emulsion formation were overcome. Several parameters that affecting the partition and derivatization of the anions were studied and are discussed in this paper.

EXPERIMENTAL

GC conditions

A Shimadzu (Kyoto, Japan) GC-8A gas chromatograph equipped with dual flame ionization detectors was used. The separation columns and the temperatures for individual and simultaneous determinations of inorganic anions are indicated in Table I. Nitrogen was used as the carrier gas at a flow-rate of 40 ml/min. A Shimadzu R-111 recorder and Shimadzu Chromatopac C-E1B integrator were used.

Materials

Kryptofix 222 B polymer (250–300 μm) (E. Merck, Darmstadt, F.R.G.) α -bromo-2,3,4,5,6-pentafluorotoluene (PFBBr) (Aldrich, Milwaukee, WI, U.S.A.), bibenzyl and *p*-dibromobenzene (Wako, Osaka, Japan), *p*-bromiodobenzene and 2,3,5,6-tetrachloronitrobenzene (Tokyo Kasei, Tokyo, Japan), 10% OV-17 on Uniport HP (60–80 mesh) (Gasukuro, Tokyo, Japan), 4% OV-101 + 6% OV-210 on Chromosorb W HP (60–80 mesh) (Shimadzu) and 5% silicone DC QF-1 Chromosorb W AW DMCS (60–80 mesh) (Wako) were used without further treatment. Potassium hydroxide, potassium nitrate, potassium nitrite and potassium thiocyanate (E. Merck), potassium cyanide, potassium iodide and sodium sulphide (Wako), dichloro-

TABLE I
GC CONDITIONS

Detector and injector temperatures, 280°C.

Anion	Stationary phase	Column temperature (°C)
CN ⁻	4% OV-101 + 6% OV-210 ^a	170
NO ₂ ⁻	4% OV-101 + 6% OV-210	160
SCN ⁻	4% OV-101 + 6% OV-210	200
I ⁻	10% OV-17 ^b	140
S ²⁻	10% OV-17	200
Simultaneous determination	5% silicone DC QF-1 ^c	Programmed (see Fig. 2)

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

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

^c Coated on Chromosorb W AW DMCS (60–80 mesh) and packed in a stainless-steel column (5.0 m \times 3 mm I.D.).

methane and other reagents were of analytical-reagent grade. Solutions of inorganic anions were prepared by dissolving appropriate compounds in distilled, deionized water.

Derivatization procedures

Procedure for individual determination. As shown in Table II, a 0.2-ml volume of a solution of various inorganic anions was added to a 10-ml glass-stoppered test-tube containing a suitable amount of Kryptofix 222 B polymer and 0.1 ml of $2 \cdot 10^{-3}$ M potassium hydroxide solution, then 0.4 ml of the internal standard (I.S.) in dichloromethane and 0.1 ml of PFBBr solution containing various amounts of neat PFBBr in dichloromethane was added. The reaction mixture was shaken mechanically at 30°C for a fixed time, then an aliquot of the dichloromethane layer was injected for GC analysis with flame ionization detection (FID).

Procedure for simultaneous determination. Also as indicated in Table II, 0.2 ml of a solution of five anions was added to a 10 ml glass-stoppered test-tube containing 15 mg of Kryptofix 222 B polymer and 0.1 ml of $2 \cdot 10^{-3}$ M potassium hydroxide solution, then, 0.3 ml of I.S. (2,3,5,6-tetrachloronitrobenzene) solution and 0.2 ml of PFBBr solution were added. The reaction mixture was shaken mechanically at 30°C for 2.0 h, then an aliquot of the dichloromethane layer was subjected to GC-FID.

RESULTS AND DISCUSSION

The analytical derivatization of biologically important inorganic anions with PFBBr in a heterogeneous reaction system using immobilized PTC was studied. The PTC used is a polymeric and bound kryptand, Kryptofix 222 B polymer; its basic functional unit is similar to 4,7,13,16,21,24-hexaoxy-1,10-diazabicyclo[8.8.8]hexacosane (Kryptofix 222), which is highly selective for complexing potassium ion from the aqueous phase and formation of related kryptates with various anions. Therefore, the anion in the kryptate could partition to the organic phase, where derivatization with a suitable reagent proceeds. Based on this mechanism, only a few methods have been reported for the individual derivatization of thiocyanate anion with benzyl chloride² or ethyl iodide³. We have investigated the conditions for the individual and simultaneous derivatization of several biologically active anions with PFBBr, using Kryptofix 222 B polymer as PTC.

Derivatization conditions

The anions and the amounts used for study of the optimum conditions for derivatization were as follows: cyanide (3.85 μmol), iodide (1.58 μmol), nitrite (4.35 μmol), sulphide (1.56 μmol) and thiocyanate (3.45 μmol). The parameters that affect the formation of the anion derivatives were evaluated by procedures similar to those described previously¹, except that the PTC used was Kryptofix 222 B polymer.

The effects of the addition of 0.1 ml of base or acid at various concentrations to the reaction system as indicated under *Derivatization procedures* are illustrated in Fig. 1. The results indicate that the simultaneous determination of the anions is most favourable when using 0.1 ml of aqueous base at a concentration of *ca.* $2 \cdot 10^{-3}$ M; for individual determinations, a wider concentration range of aqueous base or acid can be adopted.

TABLE II
DERIVATIZATION CONDITIONS

Anion	Amount of anion (μmol)	Kryptofix 222 B polymer (mg)	Reaction time (h)	Neal PFBBr (μl)	Internal standard (mM)
For individual determinations:					
CN ⁻	3.85	10	1.0	2	<i>p</i> -Bromiodobenzene (4.2)
NO ₂ ⁻	4.35	12	1.5	4	<i>p</i> -Bromiodobenzene (5.3)
SCN ⁻	3.45	8	0.5	2	Bibenzyl (13.7)
I ⁻	1.58	8	0.5	2	<i>p</i> -Dibromobenzene (3.4)
S ²⁻	1.56	8	0.5	4	2,3,5,6-Tetrachloronitrobenzene (9.6)
For simultaneous determinations ^a		15	2.0	8	2,3,5,6-Tetrachloronitrobenzene (9.6)

^a The amounts of anions used for simultaneous study were as follows: CN⁻, 3.85; NO₂⁻, 4.35; SCN⁻, 3.45; I⁻, 1.58; and S²⁻, 1.56 μmol , in 0.2 ml of $2 \cdot 10^{-3}$ M KOH solution.

As shown in Fig. 1, the partition of the anions (iodide, thiocyanate and nitrate) from the aqueous to the dichloromethane phase for derivatization is also favourable in neutral or dilute acidic media. This could be due to the available anions in the aqueous media forming various kryptates with the complexed PTC (the potassium ion here for complexation is from the sample solution of potassium salts of the anions); the ready availability of the above-mentioned anions in diluted acidic media, especially iodide and thiocyanate, could probably be explained in part by their physical and chemical properties, as it is reported⁴ that HI and HSCN are freely soluble in water, revealing the good dissociation characteristics of these acids. However, with cyanide and sulphide, the HCN and H_2S formed from acidic media are volatile and difficult to dissociate, which unfavourably affects their transfer for derivatization. Discontinuation of the study of cyanide derivatized in acidic media was partly due to the high toxicity of the volatile HCN. In addition, the low derivatization yields of the anions at high concentrations of base or acid are generally assumed from the competition of excess OH^- with the anions and the protonation of the anions, respectively, which unfavourably results in poor phase transfer and derivatization.

Studies of the effects of organic solvents (dichloromethane, acetophenone, cyclohexanone, 1-pentanol, 2-octanol and methyl isobutyl ketone), the reaction time at 30°C for dichloromethane and at 50°C for the other solvents, and the amount of PFBBR on the derivatization of the anions at a constant aqueous base level ($2 \cdot 10^{-3} \text{ M}$, 0.1 ml) led the adoption of dichloromethane as the best solvent for derivatization at 30°C ; other optimum parameters including reaction time and the amount of PFBBR needed to reach an equilibrium reaction are summarized in Table II for individual and simultaneous determinations.

The amount of Kryptofix 222 B polymer used is also indicated in Table II; an attempt to reduce the reaction time for nitrite, which is assumed to be strongly solvated in water, by increasing the amount of the PTC failed, probably because the amount (12 mg) of immobilized and insoluble PTC used already overloaded the interface between dichloromethane and the aqueous phase under present conditions. Therefore, by the conventional mechanism of increasing the soluble PTC concentration to increase

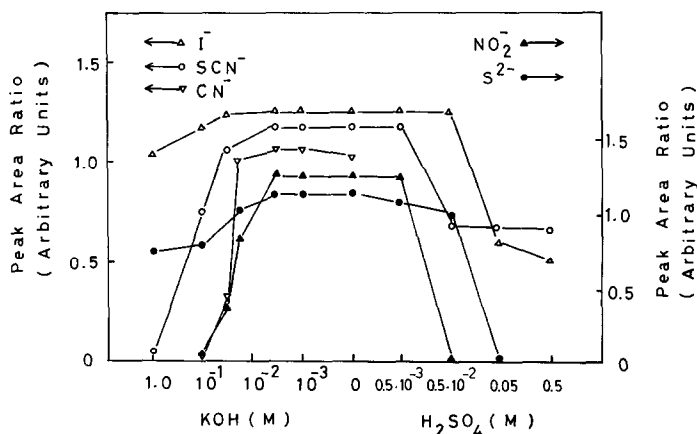


Fig. 1. Effect of added base or acid on the formation of the derivatives. \circ , SCN^- ; \triangle , I^- ; \blacktriangle , NO_2^- ; \bullet , S^{2-} ; ∇ , CN^- ; each point represents the average of three measurements.

ion-pair formation for better partition of anions to the organic layer for derivatization does not work here for the immobilized and insoluble PTC.

Based on the optimum conditions established, the derivatization procedures for the individual and simultaneous determinations of the anions were formulated as described under Experimental.

Quantitative determinations

To evaluate the quantitative applicability of the methods, six different concentrations of each anion down to submicromole levels were studied for individual and simultaneous determinations. The results in Table III indicate good linearity for quantification over the range tested, reflected in the high correlation coefficient ($r > 0.998$) derived from both determinations. The lower determination limits are similar to those obtained by the previous method¹. Simultaneous determination of anions including nitrate is impractical because the equilibrium for the derivatization of nitrate cannot be attained in 24 h, probably owing to its high water solvation resulting in poor phase partition and derivatization.

Gas chromatography and structural identification of the derivatives

A typical gas chromatogram is presented in Fig. 2 and demonstrates good resolution of the anion derivatives using simultaneous determination with temperature-programmed elution. No drift of the baseline was observed in this method, but drift did occur in the previous study¹ using tetraalkylammonium compound as PTC for individual and simultaneous determinations of anions, owing to contamination of the column; therefore, additional treatment of the reacted solution with water to remove the PTC was necessary in the derivatization procedures in the previous work. The structures of the peaks equivalent to the derivatives of iodide (peak 1), nitrite (2), thiocyanate (4) and sulphide (6) are identical with pentafluorobenzyl iodide, α -nitro-2,3,4,5,6-pentafluorotoluene, pentafluorobenzyl thiocyanate and bis(pentafluorobenzyl)sulphide, respectively, reported in the previous paper¹ with similar identification techniques.

It should be noted that the structure of the cyanide derivative equivalent to peak 3 in Fig. 2 is identical with pentafluorobenzyl cyanide as obtained previously⁵ by mass spectrometry, but it is different from the derivative of cyanide^{6,7} obtained by its derivatization in a homogeneous system (cyanide in 0.015 *M* aqueous KOH reacted with PFBBr in acetone). The derivative from the homogeneous reaction was identified as 2,3-bis(pentafluorophenyl)propionitrile by electron-impact mass spectrometry (20 eV), with a molecular ion at m/z 387, and infrared spectrometry, with a characteristic cyano absorption at 2250 cm^{-1} , and by acceptable results of elemental analysis. The derivative of cyanide from the homogeneous reaction could be speculated from the following mechanism: the initial nucleophilic substitution between CN^- and PFBBr ($\text{C}_6\text{F}_5\text{CH}_2\text{Br}$) results in the formation of $\text{C}_6\text{F}_5\text{CH}_2\text{CN}$, which then deprotonates one of its active methylene hydrogens in strongly alkaline homogeneous media; the resulting anion species, $\text{C}_6\text{F}_5\text{CHCN}^-$ as a new nucleophile further attacks the PFBBr and proceeds via a second nucleophilic substitution to the formation of the final derivative, 2,3-bis(pentafluorophenyl)propionitrile. Therefore, a correction should be made to previous reports^{6,7} of the cyanide derivative being incorrectly depicted as pentafluorobenzyl cyanide from homogeneous derivatization; the retention time of

TABLE III

REGRESSION ANALYSES FOR THE DETERMINATION OF ANIONS

y = Peak-area ratio of the anion derivative to the internal standard; x = amount of anions (μmol).

Anion	Amount (range) (μmol)	Regression equation	Correlation coefficient (r)
<i>Individual determinations</i>			
CN^-	0.0385–3.85	$y = 0.7358x - 0.00995$	0.999
I^-	0.0158–1.58	$y = 2.121x + 0.00504$	0.999
NO_2^-	0.0217–4.35	$y = 0.6302x - 0.00253$	0.998
S^{2-}	0.0156–1.56	$y = 1.654x + 0.00984$	0.998
SCN^-	0.00345–3.45	$y = 0.8352x - 0.00956$	0.999
<i>Simultaneous determinations</i>			
CN^-	0.0385–3.85	$y = 0.9802x + 0.0270$	0.999
I^-	0.0158–1.58	$y = 1.727x + 0.0128$	0.999
NO_2^-	0.0435–4.35	$y = 0.7222x + 0.0141$	0.998
S^{2-}	0.0156–1.56	$y = 2.688x + 0.0177$	0.998
SCN^-	0.0172–3.45	$y = 1.607x + 0.0142$	0.999

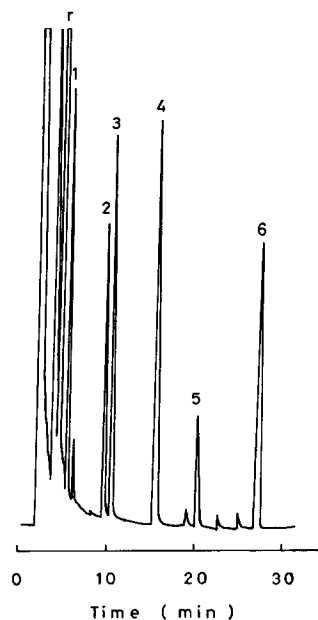


Fig. 2. Gas chromatogram for the simultaneous determination of five inorganic anions in 0.2 ml of solution containing 1.58 μmol I^- , 4.35 μmol NO_2^- , 3.85 μmol CN^- , 3.45 μmol SCN^- and 1.56 μmol S^{2-} . Peaks: 1 = derivative of I^- ; 2 = derivative of NO_2^- ; 3 = derivative of CN^- ; 4 = derivative of SCN^- ; 5 = tetrachloronitrobenzene (I.S.); 6 = derivative of S^{2-} ; and r = PFBBr reagent blank. Conditions: stainless-steel column, 5.0 m \times 3 mm I.D., 5% silicone DC QF-1 on Chromosorb W AW DMCS (60–80 mesh); nitrogen flow-rate, 25 ml/min; column temperature, initially 150°C, programmed at 2°C/min to 200°C, then held there for 5 min; flame ionization detection.

2,3-bis(pentafluorophenyl)propionitrile is significantly longer than that of pentafluorobenzyl cyanide obtained from the present method.

CONCLUSION

A study of the determination of inorganic anions at sub-micromole levels by derivatization GC using Kryptofix 222 B polymer as immobilized PTC resulted in simple procedures for the individual and simultaneous determinations of cyanide, iodide, nitrite, sulphide and thiocyanate anions. One of the advantages is that the PTC used for derivatization is immobilized as solid particles; also, the introduction of the sample solution of anion derivatives for GC determination will never contaminate the column and the detector by the PTC. This is especially important for the trace analysis of inorganic anions in biological samples by GC with a highly sensitive detector such as an electron-capture detector. Development of the method for the trace determination of nitrite and thiocyanate anions in biological specimens is in progress.

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