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METHYLATION OF INORGANIC ANIONS FOR GAS CHROMATOGRAPHIC DETERMINATION

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SUMMARY

Some inorganic anions were converted into their methyl derivatives by reaction with three methylating agents (dimethyl sulphate, methyl *p*-toluenesulphonate and trimethyl phosphate) at 70°C. The derivatives were extracted with dichloromethane or 1,2-dichloroethane, and determined by gas chromatography with a flame ionization detector. By this method, cyanide, thiocyanate, iodide, bromide and sulphide were determined at concentrations of 0.1–1.0 mg/ml.

INTRODUCTION

Some inorganic anions, such as cyanide (CN^-) and sulphide (S^{2-}), are among the important pollutants of the environment. Furthermore, thiocyanate (SCN^-), nitrite (NO_2^-), halides, etc., have biologically important rôles in living organisms. Therefore, the determination of inorganic anions in various environmental and biological matrices is of vital importance. We have investigated the determination of inorganic anions by gas chromatography (GC).

Some compounds cannot be directly determined by GC, and derivatization reactions must be used prior to analysis[1–4]. The application of GC with derivatization to the determination of inorganic anions is a new and as yet relatively unexplored field, because of the non-volatility of simple inorganic anions and the failure to find a universal derivative comparable to the volatile β-diketone chelates of metal cations. In spite of these difficulties, some methods have been developed for the GC analysis of inorganic anions[4–6].

The preparation of trimethylsilyl derivatives of anions has been studied extensively[4]. For example, trimethylsilyl derivatives of the ammonium salts of several common oxyanions (borate, carbonate, oxalate, phosphite, sulphate, arsenite, phosphate, vanadate and arsenate) were prepared and successfully analyzed by Butts[7] and Butts and Rainey[8].

In previous studies[9,10], we converted NO_2^- and CN^- into *p*-bromochlorobenzene and benzonitrile, respectively, according to reactions similar to the Sandmeyer reaction. These products were determined by GC with an electron-capture detector (ECD) and a flame ionization detector (FID), respectively.

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It is well known that some inorganic anions react with methylating reagents, such as dimethyl sulphate, methyl *p*-toluenesulphonate and trimethyl phosphate, to form their methyl derivatives[11]. In the present work, inorganic anions were methylated and the resulting methyl derivatives were determined by GC with a FID. The objective was to investigate the possibility of applying this GC method to the determination of inorganic anions in environmental or biological samples, by using more sensitive detectors than a FID [*e.g.*, ECD, flame thermionic detector (FTD), flame photometric detector (FPD)].

EXPERIMENTAL

Apparatus

A Yanagimoto G-180 gas chromatograph equipped with a dual flame ionization detector was used. The column and column temperature used were dependent on the methyl derivative of the inorganic anion; details are given in Table I. The column packing materials, Porapak Q (80–100 mesh) and T (80–100 mesh), were purchased from Waters Assoc. (Milford, MA, U.S.A.), and 5% PEG-HT on Uniport HP (60–80 mesh) was obtained from Gasukuro Kogyo (Tokyo, Japan). Nitrogen was used as the carrier gas at a constant flow-rate of 45 ml/min. The injection port temperature was maintained at 250°C. The peak areas were measured by a digital integrator (Shimadzu Chromatopac-E1A, Kyoto, Japan).

TABLE I

METHYLATION AND GAS CHROMATOGRAPHIC CONDITIONS

Stainless-steel columns (3 mm I.D.) were used. Column packing: Q = Porapak Q (1 m); T = Porapak T (1 m); PEG = PEG-HT (2 m).

Anion	*Methyl derivative*	*Extraction solvent*	*Column*	*Column temp. (°C)*
CN^-	CH_3CN	CH_2ClCH_2Cl	Q	140
SCN^-	CH_3SCN	CH_2Cl_2	Q	190
I^-	CH_3I	CH_2ClCH_2Cl	T	150
Br^-	CH_3Br	CH_2ClCH_2Cl	Q	140
NO_2^-	CH_3NO_2	CH_2Cl_2	PEG	80
$HCOO^-$	CH_3OCHO	CH_2ClCH_2Cl	Q	140
CH_3COO^-	CH_3OCOCH_3	CH_2ClCH_2Cl	Q	175
S^{2-}	CH_3SCH_3	CH_2ClCH_2Cl	Q	170

Materials

All reagents were of analytical reagent grade and were used without further purification unless otherwise stated. Dimethyl sulphate, methyl *p*-toluenesulphonate and trimethyl phosphate were commercial grade reagents (Tokyo Kasei Kogyo, Tokyo, Japan). Sodium nitrite was dried in an oven at 110°C for 1 h before it was accurately weighed. Deionized water was distilled before use for the analysis. Solutions of the inorganic anions were prepared by dissolving their potassium or sodium salts in water.

Procedure

To 1.0 ml of an aqueous solution of inorganic anion in a reaction vessel was added 0.1 ml of a methylating reagent (dimethyl sulphate, methyl *p*-toluenesulphonate or trimethyl phosphate). In the determination of CN^- and S^{2-}, 0.5 ml of an aqueous solution of KOH (concentration as in Table III) were added before the addition of the methylating reagent. Then the vessel was shaken for a fixed time (see Table III) in an incubator at 70°C. At the end of the reaction period, 1.0 ml of dichloromethane or 1,2-dichloroethane (see Table I) was added to the cooled reaction solution. Then the methyl derivative was extracted by shaking for 10 min at room temperature, and the organic layer was separated from the aqueous layer. An aliquot (1.0 µl) of the organic layer was injected into the gas chromatograph and the methyl derivative was determined with a FID.

RESULTS AND DISCUSSION

Derivatization yield

The derivatization yield for each inorganic anion was estimated for reactions of each methylating reagent with no addition of either alkaline or acid solution. The peak area of the methyl derivative produced from each anion at 0.1 *M* was compared with that of a standard solution. The latter contained the methyl derivative at a concentration of 0.1 *M* which corresponded to a derivatization yield of 100%. The derivatization yields are shown in Table II as the average values from five replicate determination.

TABLE II

DERIVATIZATION YIELDS FOR INORGANIC ANIONS

Number of data: 5. PTS-Me = Methyl *p*-toluenesulphonate.

Anion	*Methylating reagent*		
	$(CH_3)_2SO_4$	*PTS-Me*	$(CH_3)_3PO_4$
CN^-	44.8 ± 1.2	46.7 ± 1.1	53.6 ± 1.1
SCN^-	104.3 ± 2.1	66.4 ± 1.9	16.1 ± 0.7
I^-	73.2 ± 1.8	63.1 ± 1.0	12.9 ± 0.3
Br^-	*ca.* 65	*ca.* 20	*ca.* 2
NO_2^-	20.3 ± 0.8	7.8 ± 0.2	9.2 ± 0.3
$HCOO^-$	11.6 ± 0.8	7.2 ± 0.2	2.6 ± 0.1
CH_3COO^-	11.0 ± 0.5	8.8 ± 0.2	3.1 ± 0.2
S^{2-}	0.4 ± 0.1	9.0 ± 0.6	44.3 ± 1.1

Thiocyanate was converted quantitatively into methyl thiocyanate by dimethyl sulphate. The derivatization yield of CN^- into acetonitrile by every methylating reagent was about 50% as shown in Table II; a large amount of the converted acetonitrile was not extracted with 1,2-dichloroethane from the aqueous reaction mixture. The actual yields are much higher (see Table III). The derivatization yields for bromide (Br^-) were not very accurate because it is very difficult to prepare 0.1 *M* methyl bromide solution in 1,2-dichloroethane due to the high volatility of methyl bromide (b.p. 4.5°C). Methylation of iodide (I^-) by dimethyl sulphate or methyl *p*-toluenesulphonate, and that of Br^- by dimethyl sulphate, gives fairly high yields. It is interesting that the derivatization yield of S^{2-} by trimethyl phosphate is much higher than that by dimethyl sulphate or methyl *p*-toluenesulphonate.

TABLE III

OPTIMUM REACTION CONDITIONS AND DERIVATIZATION YIELDS

Anion (1.0 ml)	*Methylating reagent (0.1 ml)*	*Concentration of KOH (0.5 ml)*	*Reaction time (h)*	*Derivatization yield* (%)*
SCN^-	$(CH_3)_2SO_4$	–	0.5	102.9 ± 1.7
SCN^-	PTS-Me	–	4	90.9 ± 0.7
I^-	$(CH_3)_2SO_4$	–	0.5	72.8 ± 1.7
I^-	PTS-Me	–	3	79.0 ± 1.0
Br^-	$(CH_3)_2SO_4$	–	0.5	*ca.* 65
S^{2-}	$(CH_3)_2SO_4$	6 *N*	0.5	42.3 ± 0.8
S^{2-}	PTS-Me	5 *N*	0.5	51.8 ± 0.9
S^{2-}	$(CH_3)_3PO_4$	1.5 *N*	1	62.5 ± 0.8
CN^-	$(CH_3)_2SO_4$	0.5 *N*	0.5	53.5 ± 1.5 101.1 ± 3.1**
CN^-	PTS-Me	0.5 *N*	1	48.5 ± 1.0 90.0 ± 1.0**
CN^-	$(CH_3)_3PO_4$	0.5 *N*	2	44.4 ± 2.1 84.0 ± 1.8**

* The concentration of inorganic anion in the sample is 0.05 *M*. Number of data: 5.
** Value corrected for extraction loss.

Optimum reaction conditions

In order to ascertain the optimum reaction conditions, the effects of pH and reaction time on the derivatization yield were examined.

The pH effect was studied as follows. To 1.0 ml of each aqueous solution of inorganic anion were added 0.5 ml of H_2SO_4 or KOH aqueous solution at different concentrations. Methylation was carried out for 1 h, and the derivatization yield was obtained as above. Fig. 1 shows the effects of the concentrations of H_2SO_4 and KOH on the derivatization yields of SCN^- and Br^- by each methylating reagent. In the cases of SCN^- and Br^-, the yields decreased with increasing KOH concentration, but did not vary with H_2SO_4 concentration. The derivatization yields for I^- do not vary with H_2SO_4 or KOH concentration in the range of 0–1.0 *N*. In subsequent studies of methylation of these three anions, neither H_2SO_4 nor KOH solution was added.

Figs. 2 and 3 show the effects of KOH concentration on the derivatization yields of CN^- and S^{2-}, respectively. The yields decreased to zero on addition of H_2SO_4 in the concentration range 0.1–1.0 *N*. The yields for CN^- became constant at a KOH concentration of *ca.* 0.2 *N*. Thus, in subsequent studies of methylation of CN^-, 0.5 *N* KOH solution was used. All curves in Fig. 3 show maximum derivatization yields; the KOH concentrations which give the maximum yields are shown in

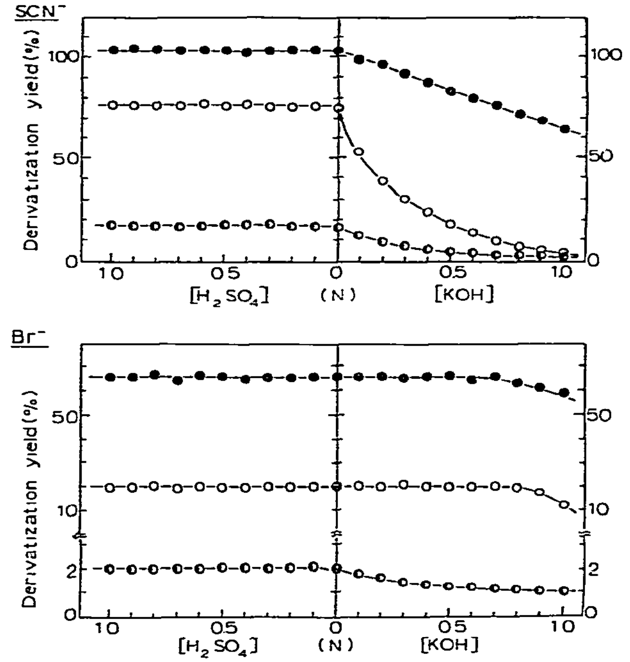


Fig. 1. Effects of H_2SO_4 and KOH concentrations on the derivatization yields of SCN^- and Br^-. Methylating reagent: ●, $(CH_3)_2SO_4$; ○, methyl *p*-toluenesulphonate; ◐, $(CH_3)_3PO_4$.

Table III. KOH solutions with concentrations of 6, 5 or 1.5 *N* were used for subsequent studies of derivatization of S^{2-}.

Methylation of other anions shown in Table II (*i.e.*, NO_2^-, CH_3COO^- and $HCOO^-$) gave relatively low yields (<50%) under a variety of conditions. Each reaction of SCN^-, I^- and Br^- with trimethyl phosphate and that of Br^- with methyl *p*-toluenesulphonate also gave fairly low yields, although the concentration of H_2SO_4 or KOH solution was varied. It is not expected that derivatization reactions with low

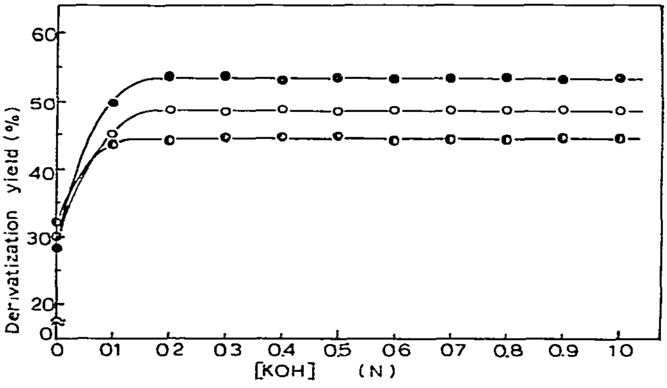


Fig. 2. Effect of KOH concentration on the derivatization yields of CN^-. Methylating reagents as in Fig. 1.

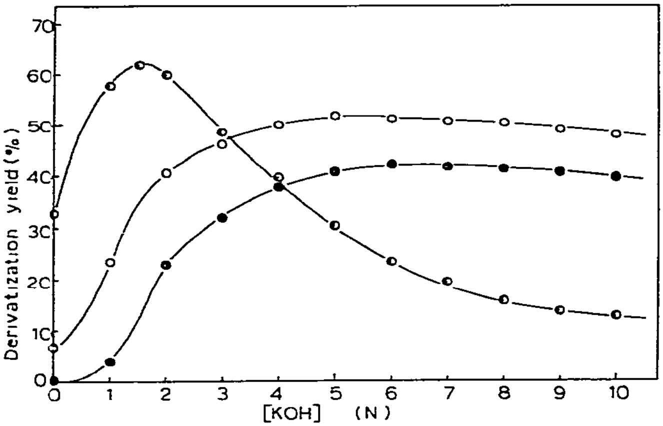


Fig. 3. Effect of KOH concentration on the derivatization yields of S^{2-}. Methylating reagents as in Fig. 1.

yields will be of practical use for the determination of inorganic anions. Therefore, these methylation reactions were not investigated further.

The effect of reaction time on the derivatization yield of each anion (SCN^-, CN^-, I^-, Br^- and S^{2-}) was examined, at the optimum concentration of KOH solution. The results obtained are shown in Figs. 4 and 5. In this study, the 'reaction time' means the period for which the reaction solution is shaken in an incubator at 70°C. It seems that methylation of each anion by dimethyl sulphate proceeds faster than by methyl *p*-toluenesulphonate or trimethyl phosphate. The methylation yield of S^{2-} by dimethyl sulphate reaches its maximum value in a reaction time of 0 min. This shows that this reaction proceeds during the extraction step of shaking at room temperature.

From these results, the optimum reaction conditions were chosen for each methylation reaction (Table III). Table III also shows the derivatization yield of each inorganic anion methylated under the optimum reaction conditions. In the case of CN^-, the resulting product, acetonitrile, was not completely extracted into the organic layer. The derivatization yields corrected for extraction loss by using the distri-

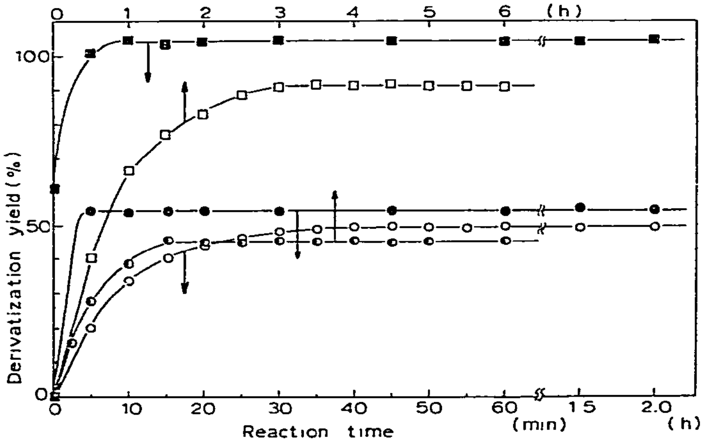
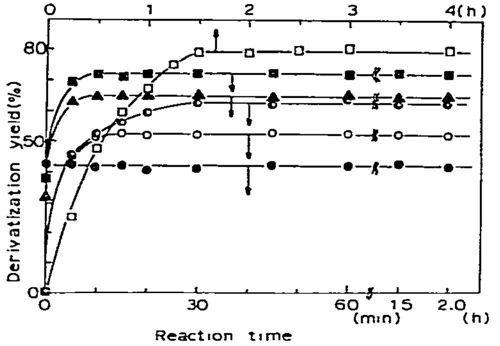


Fig. 4. Effect of reaction time on the derivatization yields of CN^- and SCN^-. Anion (methylating reagent): ●, CN^- ($(CH_3)_2SO_4$); O, CN^- (methyl *p*-toluenesulphonate); ◐, CN^- ($(CH_3)_3PO_4$); ■, SCN^- ($(CH_3)_2SO_4$); □, SCN^- (methyl *p*-toluenesulphonate).



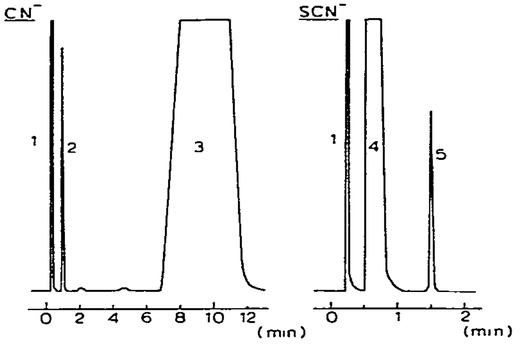


Fig. 5. Effect of reaction time on the derivatization yields of S^{2-}, I^- and Br^-. Anion (methylating reagent): ●, S^{2-} ($(CH_3)_2SO_4$); ○, S^{2-} (methyl *p*-toluenesulphonate); ◐, S^{2-} ($(CH_3)_3PO_4$); ■, I^- ($(CH_3)_2SO_4$); □, I^- (methyl *p*-toluenesulphonate); ▲, Br^- ($(CH_3)_2SO_4$).

Fig. 6. Gas chromatograms of the products of methylation of CN^- and SCN^- by $(CH_3)_2SO_4$ under the optimum reaction conditions. Peaks: 1 = CH_3OH (hydrolysis product of $(CH_3)_2SO_4$); 2 = CH_3CN; 3 = CH_2ClCH_2Cl (solvent); 4 = CH_2Cl_2 (solvent); 5 = CH_3SCN.

bution ratio are also shown in Table III. Methylation of each anion shown in Table III gives a satisfactorily high yield. In particular, CN^- and SCN^- are quantitatively methylated. These high derivatization yields demonstrate the possibility of using these methylation reactions for the determination of inorganic anions by GC with derivatization.

Gas chromatograms and calibration curves

Fig. 6 shows typical gas chromatograms of the products of methylation of CN^- and SCN^- by dimethyl sulphate under the optimum reaction conditions. Fig. 7 shows the calibration curve for CN^-, plotting the peak area of acetonitrile *vs.* the concentration of CN^- aqueous solution, together with the similar calibration curve for SCN^-. The peak area of the methyl derivative produced from 1.0 mg/ml of CN^- or SCN^- solution was arbitrarily assigned a value of 100. Both calibration curves pass through the origin. For the other methylation systems shown in Table III, a good linear relationship between the peak area of the methyl derivative and the concentration of inorganic anion (0.1–1.0 mg/ml) is also obtained.

Comparison with trimethylsilylation

Trimethylsilyl derivatives have been prepared for many anions by reaction with trimethylsilylating reagents. During derivatization, it is necessary to maintain anhydrous conditions since trimethylsilylating reagents and trimethylsilyl derivatives are sensitive to water. The silylation of anions in water, therefore, requires a pretreatment step: extraction of the anions into an organic solvent from an aqueous sample solution, evaporation to dryness, etc. In contrast, the methylating reagents used in this work are much more stable in water and give methyl derivatives which are insensitive to water. Consequently, methylation can be performed directly by adding the methylating reagents to an aqueous sample solution, without pretreatment, although the present inorganic anions methylated were not the same as those trimethylsilylated.

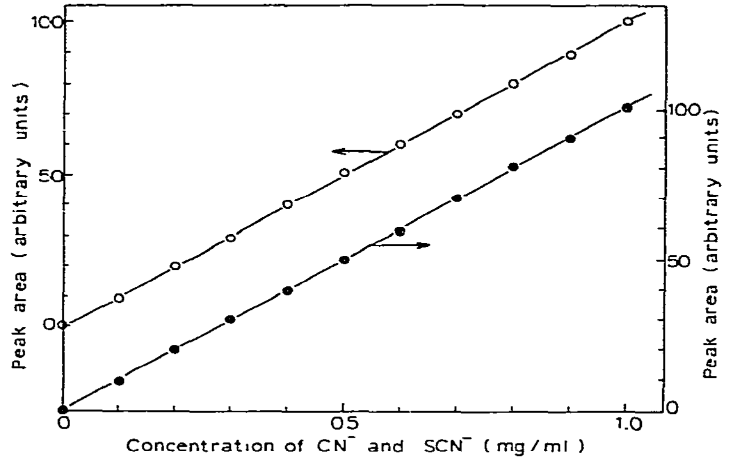


Fig. 7. Calibration curves for CN⁻ (○) and SCN⁻ (●). Methylating reagent: $(CH_3)_2SO_4$.

CONCLUSION

It is found that the inorganic anions CN⁻, SCN⁻, I⁻, Br⁻ and S^{2-} at concentrations of 0.1–1.0 mg/ml can be determined by methylation followed by flame ionization gas chromatography. This concentration range is much higher than the concentrations found in environmental or biological samples. Moreover, the methyl derivatives do not give as high a FID response as compounds containing more carbon atoms. Consequently, in order to determine these anions at low concentrations, a detector must be used which exhibits a higher response to the methyl derivatives. Fortunately, a FTD is highly sensitive to nitrogen-containing compounds (for CN⁻ and SCN⁻), an ECD to halogen-containing compounds (for I⁻ and Br⁻) and a FPD to sulphur-containing compounds (for S^{2-} and SCN⁻).

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