Determination of thiocyanate by carbonyl sulphide (OCS) generation and gas-phase molecular absorption spectrometry

S. Cabredo Pinillos [a], I. Sanz Vicente [a], J. Galbán Bernal [b], J. Sanz Asensio [a, *]

[a] *Chemistry Department, Analytical Chemistry Section, University of La Rioja, Logroño 26001, Spain*
[b] *Analytical Chemistry Department, Faculty of Sciences, University of Zaragoza, Zaragoza 50009, Spain*

Abstract

A method for determination of thiocyanate by gas-phase molecular absorption spectrometry is described. The proposed method is based on the continuous generation of carbonyl sulphide and preconcentration in a liquid nitrogen trap. Later, the product is volatilised and the absorbance at 224 nm is measured. Under the optimised conditions, the calibration graph was linear for 2–80 μg ml^{-1}, with a detection limit of 1 μg ml^{-1}. No metal ions interfered. The method was tested with saliva samples from non-smokers and smokers; the results show that it is possible to distinguish between these two categories of individuals.

1. Introduction

Thiocyanate is present in humans as a result of the digestion of certain types of vegetables and as a metabolic degradation product of compounds in tobacco smoke that contain cyanide. The concentration of thiocyanate is considered to be a good indicator for distinguishing between smokers and non-smokers. Thiocyanate is also introduced into humans as a drug in the treatment of thyroid conditions and arterial hypertension, so the determination of thiocyanate is of considerable interest. In humans, thiocyanate can be found in saliva, urine and blood and, the values are reported to be 1 mmol l^{-1} [1,2], 0.3 mmol l^{-1} [1] and 0.05 mmol l^{-1} [3] for non-smokers and 2 or 4 times higher for smokers.

Chromatography is usually the technique chosen for thiocyanate determination in complex samples and the simultaneous determination of thiocyanate and other inorganic anions, including gas chromatography [4] (previous derivatisation to methane thiocyanate reversed-phase liquid chromatography [5] (previous formation of an absorbing derivative) or ion-pair [6] chromatography. Ion chromatography with a conductivity detector is the method most often employed. To this effect, most of the papers are directed towards finding mobile phases with high eluent capacity (thiocyanate ions tend to be strongly retained on the column) and low background signal. Using eluents such as mixtures of carbonate or hy-

drogencarbonate and different polar organic compounds [7,8] it is possible to obtain good detection limits (< 0.1 mg l^{-1}), although substantial time investment per sample is required (retention time of thiocyanate is 15–20 min); new possibilities are being tested [9].

Direct methods for thiocyanate determination are not frequent; for example, some types of titration (redox titration) [10] or the laborious method proposed by Iwata et al. [11] in which the absorbance of hydrated electrons produced by an Ar-F laser is measured at 720 nm, although other inorganic anions with a similar reaction interfere.

Many indirect methods have been reported. Some of them are electrochemical techniques, such as indirect voltammetry, based on the previous conversion of thiocyanate to cyanide [4]. However, the most frequently employed methods are spectrophotometric: (a) UV-visible molecular absorption spectrophotometry, which contains some of the most popular alternatives, such as formation of colored complexes with cationic species (particularly Fe(III)), and more recently others based on ion-pair formation [12], oxidation (reaction with 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol) or reduction reactions [13]; (b) molecular fluorescence by the modified König

method [14]; (c) indirect atomic absorption spectrometry, based on binary or ternary complex formation with some metal ions (mainly Cu and Mo) [15].

As can be seen, most of the methods employed are indirect and need a previous separation step (extraction and chromatography). Generation of volatile species is another separation alternative. Thiocyanate ion, in acid media, can generate volatile carbonyl sulphide (OCS). No references concerning OCS generation from thiocyanate solution have been found, but Radford-Dnoery and Cutter have determined the OCS species as such [16]. In this work, an analytical method for the determination of SCN$^-$ after OCS generation, and measurement of the volatile species by gas-phase molecular absorption spectrometry is proposed. The method has been applied to the determination of thiocyanate in saliva.

2. Experimental

2.1. Apparatus

All measurements were performed using a Hewlett-Packard model HP 8451A diode-array spectrophotometer furnished with a quartz flow cell of 1
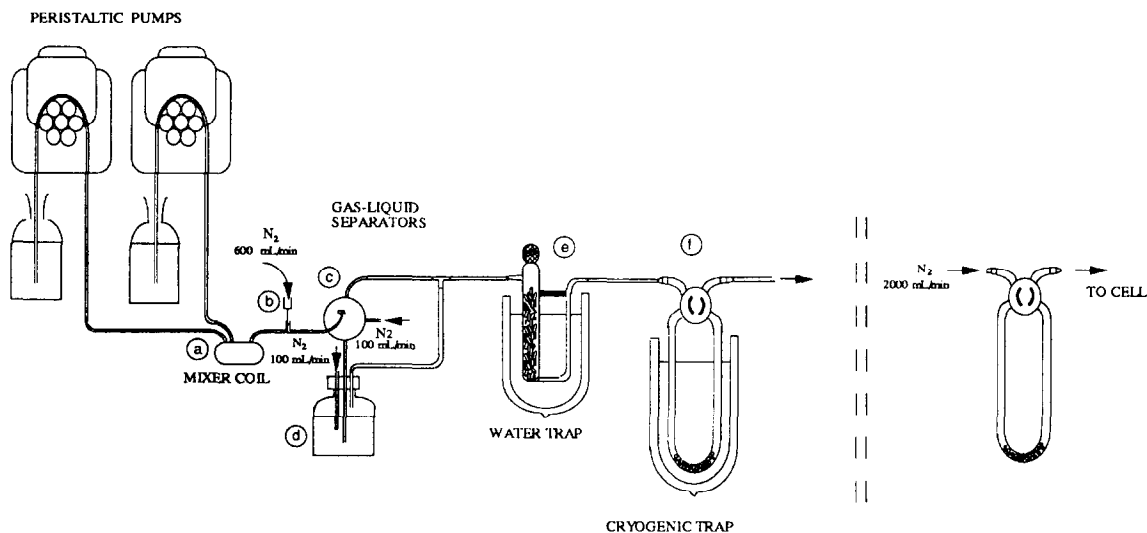

Fig. 1. Schematic diagram of the system (see explanation in the text).

cm path length (Hellma 174 QS) and equipped with a HP98155A keyboard (Hewlett-Packard), a floppy disk drive for bulk data storage (Hewlett-Packard HP9121) and a graphics plotter (HP7475A).

Masterflex models 7518-10 and 7016-20 peristaltic pumps (Cole Parmer, Chicago, IL), were employed throughout.

2.2. Reagents

All reagents used were of analytical grade quality. Doubly distilled water was used. Standard thiocyanate solution was prepared by dissolving KSCN in 100 ml of water. This and working standard solutions (prepared by serial dilution of the stock solution), were prepared daily.

2.3. Procedure

Fig. 1 shows a schematic representation of the continuous volatile generation and cold trap system used. Two peristaltic pumps are used to move the analyte solution (12 ml min^{-1}) and concentrated sulphuric acid (12 ml min^{-1}). These solutions (analyte and sulphuric acid) are mixed in mixer coil (a) in which there is an additional 600 ml min^{-1} flow of nitrogen gas auxiliary carrier (b). The OCS generated is separated first in a gas–liquid separator (c) where there is a nitrogen flow (transport flow, 100 ml min^{-1}). The remaining liquid phase reaches a second gas–liquid separator (d), where an additional 100 ml min^{-1} nitrogen flow is bubbled to improve the gas separation. The volatile is swept through a water trap (e) (a flask full of Raschig rings and immersed in an ice and salt bath at −10°C) and is finally condensed in the U-tube, immersed in liquid nitrogen, which contains a small quantity of glass spheres (2 mm diameter). The Raschig rings and the small glass spheres are both silanised.

After generation and trapping of the volatile compound for 4 min, the U-tube is closed, removed from the liquid nitrogen and left at room temperature. Ten min later, the vapour is transported to the continuous flow cell placed in the spectrophotometer, with a nitrogen flow (measuring flow) of 2000 ml min^{-1}. The transient signal peak height at 224 nm and the OCS absorption spectrum can be obtained with an adequate BASIC program.

3. Results and discussion

3.1. Molecular absorption spectrum

Carbonyl sulphide generation from thiocyanate is produced by direct hydrolysis in concentrated acid media. The information available about this reaction is only qualitative and indicates that the products of the reaction depend on the acidity, so in (1:1) acid/water media the reaction is:

$$SCN^- + H_2O + 2H^+ \Leftrightarrow OCS + NH_4^+$$

while in concentrated acid media, a mixture of volatiles is obtained, mainly SO_2 and OCS.

Sulphuric acid was used in all cases because the high temperature produced when analyte and acid solutions are mixed favours the volatilization of OCS. Hydrochloric acid was also tested but no better results were obtained. In addition, a previous paper [18] for simultaneous determination of other sulphur-containing anions showed best results when H_2SO_4 is employed.

In Fig. 2 three OCS spectra, obtained in different situations, are shown: (a) batch method, (b) continuous method with (1:1) sulphuric acid and (c) continuous method with concentrated sulphuric acid. As can be observed, in (a) and (b), a maximum appears between 196 and 200 nm, probably due to SO_2, while in all three there is a maximum at 224 nm due to OCS. The SO_2 maximum does not depend only on the acidity and perhaps the most important difference between the three spectra is the contact time between SCN^- and H^+ during the generation. In the batch method, this time is very short (in the generation system [17] employed, the reaction is almost instantaneous), while in the continuous method it is higher and increases with the acidity (at high acidity, the probability of interaction between thiocyanate and H^+ is higher, for the same real contact time in the solution). The results obtained later confirm this hypothesis. Therefore:

(1) using concentrated sulphuric acid, the spectrum shape does not change if the reactor is longer, but the peak height is increased by 10% for a 30 cm reactor length compared with 3 cm. A slight decrease in absorbance is observed with a reactor longer than 30 cm. The signal increase observed using a 30 cm reactor has an inconvenience known as a memory
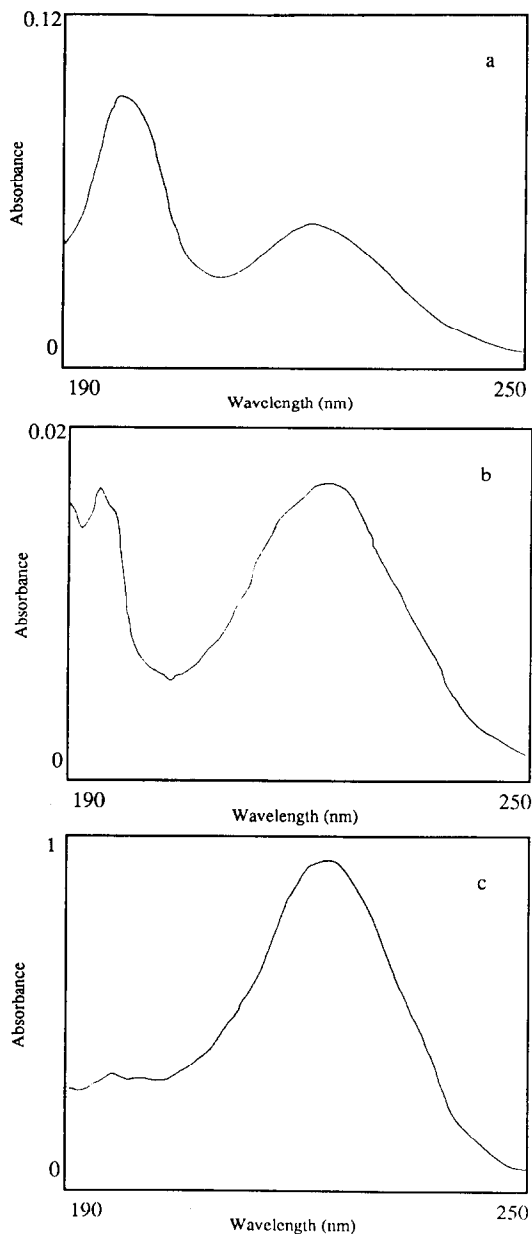
Fig. 2. Different molecular absorption spectra of carbonyl sulphide: (a) batch method and concentrated H_2SO_4; (b) continuous method and (1:1) H_2SO_4; (c) continuous method and concentrated H_2SO_4.

effect: the system has to be perfectly washed between two consecutive measurements. These extra efforts are not worthwhile given the limited sensitiv-

Table 1
Variation of OCS absorbance at 224 nm with analyte/acid flow ratio (flow $H_2SO_4 = 12$ ml min^{-1})

Analyte/acid flow ratio	Absorbance at 224 nm
4.0	0.010
2.0	0.039
1.5	0.285
1.0	0.475
0.5	0.512

ity increase obtained, thus a 3-cm reactor is recommended;

(2) using concentrated sulphuric acid, the signal at 224 nm is increased if the analyte flow/acid flow ratio is decreased (see Table 1); the increase at 224 nm means a slight decrease in the signal at 198 nm.

3.2. Blank signal

Volatile compound generation gas-phase molecular absorption spectrometry has already been employed by us for the determination of several species. Although an acidic medium has been used in all cases, a blank signal had not been observed. In this study, however, a considerable blank signal is shown (Fig. 3a), probably due to SO_3 dissolved in sulphuric acid. This signal which appears near to 198 nm, does not interfere with absorbance measurements at 224 nm, but the precision is worse because 'chemical noise' is introduced. In order to avoid this problem,
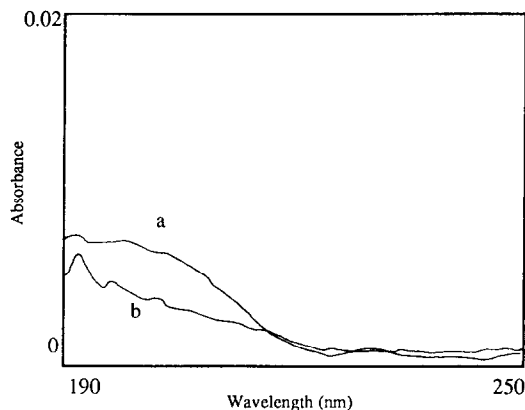

Fig. 3. Molecular absorption spectra of blank without (a) and with (b) 5 min previous bubbling of nitrogen in the concentrated sulphuric acid.

Table 2
Interference study in SCN^- determination

Ion	Concentration ($\mu g\ ml^{-1}$) [a]
Mn^{2+}, Zn^{2+}, K^+, Na^+	20000 [b]
Co^{2+}, Cr^{3+}, Ni^{2+}, Mg^{2+}	2000 [b]
Ca^{2+}, Ba^{2+}, As(III), Cl^-	
Fe^{3+}, Br^-	1000
Al^{3+}	800
NO_3^-	200
I^-	50
$S_2O_8^{2-}$	15
$S_2O_3^{2-}$	10
S^{2-}, SO_3^{2-}	5

[a] Maximum concentration that does not interfere.
[b] Maximum value tested.

some alternatives were tested. In particular, the blank signal is almost eliminated if a nitrogen flow is bubbled for 5 min in concentrated sulphuric acid before the volatile generation process (Fig. 3b).

3.3. Optimization of parameters

The generation system shown is similar to others described previously for hydride generation [19], but the nature of OCS is quite different to hydrides, so many parts of the system had to be redesigned (some of them have been cited above, length reactor for example), and an exhaustive optimization of the generation conditions was made (the effect of analyte and acid flows has been also discussed above). The most significant aspects are mentioned below.

(1) Auxiliary nitrogen flow. When low flows (ca. 40 ml min^{-1} were employed, retention of the nitrogen gas in the cryogenic trap was observed and later, when the trap warmed to room temperature, the nitrogen suddenly escaped from the valve and allowed the volatile product to escape. In hydride generation [19] this effect was not observed because the generated hydrogen in the reaction gives the same effect as the action of the nitrogen. Auxiliary and transport flows must be optimized simultaneously, and the optimum values are 600 and 100 ml min^{-1}, respectively.

(2) The second gas–liquid separator is essential because it produces an increase in absorbance of about 50%.

(3) OCS revolatilization conditions from the liquid nitrogen trap. In hydride generation, a stabilization time at room temperature and a heating time of 1 min was necessary. In the present system, leaving
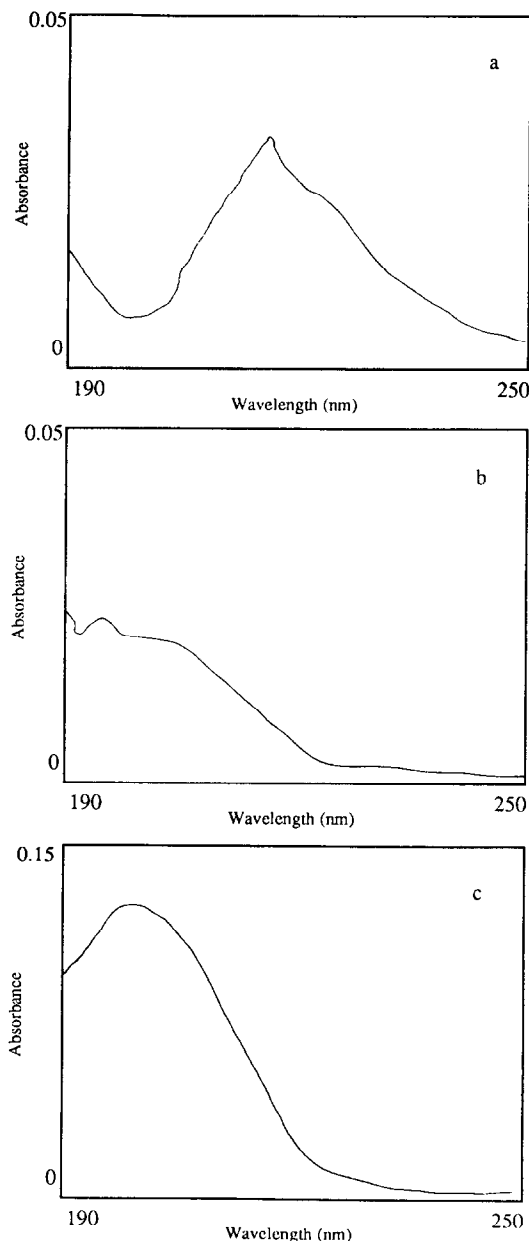

Fig. 4. Molecular absorption spectra of the volatile species generated from nitrate (a), bromide (b), iodide (c).

Table 3
Level of SCN^- ($\mu g\ ml^{-1}$)in saliva

Non-smokers			Smokers		
Sample number	Proposed method	Fe^{3-} method	Sample number	Proposed method	Fe^{3+} method
1	74	80	5	228	217
2	77	70	6	331	296
3	61	77	7	230	215
4	60	50	8	220	211

the U-tube at room temperature for 5 min is enough to give the greater analytical signal (the boiling point of OCS is $-78°C$).

3.4. Analytical characteristics

Under the optimum conditions (see Procedure), the calibration graph was linear from 2 $\mu g\ ml^{-1}$ to at least 80 $\mu g\ ml^{-1}$. The detection limit (calculated as a signal twice the height of the background of the blank measurement) was 1 $\mu g\ ml^{-1}$. It is important to note that the linear response and the detection limit are calculated for 50 ml of the sample. Because of the cryogenic preconcentration system, it is possible to get an improved detection limit if the sample volume is increased. Thus, if 200 ml of sample is used, a detection limit of 0.25 $\mu g\ ml^{-1}$ is obtained (although the generation time is 16 min). The relative standard deviation (RSD) was 2.8% for 8 determinations of 10 $\mu g\ ml^{-1}$ SCN^-.

3.5. Interferences

A study of the effect of different cations and anions on the analytical signal of SCN^- (20 $\mu g\ ml^{-1}$) was made. Table 2 shows the maximum concentration values that do not interfere.

It is appropriate to underline the small interference produced Fe^{3+}, Ni^{2+} or Co^{2+}, probably due to the high acidity medium which inhibits complex formation. With respect to sulphur-containing anions, sulphide, sulphite and thiosulphate produce an increase in absorbance. These species can generate volatile species in acidic media and although their spectra are displaced with respect to the OCS spectrum, the residual absorbance at 224 nm is important if the concentrations are high. It is also interesting to note the positive interference of nitrate, bromide and

iodide; this could be due to generation of absorbent volatile species. The spectra are shown in Fig. 4.

3.6. Application

As was indicated in the introduction, the most important SCN^- determination is in human biological fluids. In this paper, the determination of thiocyanate in saliva samples from non-smokers and smokers is described. The first assays indicated that spectral interferences were present, probably due to an organic fraction sample, so a preliminary sample preparation was necessary; 0.5 M $HClO_4$ was employed for deproteinizing the saliva samples.

Eight saliva samples of 5 ml (from four non-smokers and four smokers) were collected, treated with 0.5 M $HClO_4$ and centrifuged for 5 min. They were then diluted to 50 ml and analysed by the proposed method. In order to test this method, the samples were also analysed using the Fe^{3+} spectrophotometric method. The results obtained (Table 3) by the proposed method agreed fairly well with those of the reference method and with those given by other authors, and giving thiocyanate concentrations in smokers about 3 times higher than those of the non-smokers.

Acknowledgements

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