CHAPTER 5

DETERMINATION OF THIOCYANATE
IN BIOLOGICAL FLUIDS

A major metabolite of cyanide in human body is thiocyanate, which is usually present in low concentrations in biological fluids. The determination of thiocyanate in serum and urine has consequently been used for monitoring exposure to hydrogen cyanide from tobacco smoke and cyanogenic glucosides in certain vegetables. The anomalies from iodine deficiency and antithyroid properties of thiocyanate is dependent on thiocyanate concentration in the body and this needs to be monitored. So trace thiocyanate requires its determination by an instrumental method, since classical methods are not so sensitive. Different methods have been applied viz., spectrophotometry, amperometry, potentiometry, polarography, high performance liquid chromatography, voltametry, gas chromatography, ion-chromatography and atomic absorption spectrometry. The spectrophotometric method based on the formation of a red complex with iron(III) ion is not selective and is applicable to serum only. Several spectrophotometric methods are based on the synthesis of pyridine dyes in which cyanogen bromide or chloride produced by the reaction of thiocyanate or cyanide formed from thiocyanate with bromine.
or chlorine, reacts with pyridine and an aromatic amine to produce a coloured reaction product. These methods are laborious to perform, have limited applications and many involve the use of carcinogenic reagents. Michigami et al.\(^\text{26}\) have described a method for the determination of thiocyanate in human serum by ion-chromatography measuring the absorbance at 195 nm.

To attain higher sensitivity, indirect atomic absorption spectrometric methods (AAS) have been proposed. The first indirect method of SCN\(^{-}\) determination proposed by Danchik and Boltz\(^\text{11}\) employed the complex dithiocyanato dipyridine copper(II) extracted into chloroform; the method suffered serious interference from Ni\(^{2+}\), Hg\(^{2+}\), Ag\(^{+}\) and Fe\(^{2+}\). Takahiko\(^\text{27}\) employed the method of ion-pair extraction of Cu(I)-thiourea complex with SCN\(^{-}\) into IBMK. Stratis and Vasilikiotis\(^\text{28}\) extracted: 2,2'-dipyridyl-2-pyridyl hydrazine-Cu-SCN into IBMK and measured the copper signal in AAS in process of determining thiocyanate. Yonggen et al.\(^\text{29}\) estimated thiocyanate using copper(I) neocuproine complex extracted into chloroform; the method determined 0.06 - 1.30 µgml\(^{-1}\) of thiocyanate. Chakraborty and Das\(^\text{30}\) measured the molybdenum signal for thiocyanate determination after extraction of the Mo(V)-SCN complex; the sensitivity was low.

The present method\(^\text{31}\) is based on the formation of the complex \([\text{Cu(BPTC)(SCN)}]\), extraction of the complex into isopentyl acetate over a wide pH range, determining the copper
signal in the organic extract by air-C\textsubscript{2}H\textsubscript{2} flame of AAS, thereby indirectly determining thiocyanate. The present work involves determination of thiocyanate levels in human blood serum, urine and saliva in both male smokers and non-smokers and female non-smokers.

5.1. EXPERIMENTAL

Reagents

\([\text{Cu(BPTC)Cl}]\) solution - BPTC (2-benzoylpyridine thiosemicarbazone) and the complex \([\text{Cu(BPTC)Cl}]\) was prepared as described in Chapter 4. A stock solution of the complex (4000 \(\mu\)gml\(^{-1}\) of Cu\textsuperscript{II}) was prepared in the aqueous medium and standardised titrimetrically\(^{32}\).

Thiocyanate solution - The stock solution of 5250 \(\mu\)gml\(^{-1}\) of thiocyanate was prepared by dissolving potassium thiocyanate (Central Drug House, Bombay) in doubly distilled water and standardised gravimetrically\(^{32}\), and was diluted as required.

Buffer solution - The buffer solution at pH 7.0 was prepared by mixing 0.2 M \(\text{Na}_2\text{HPO}_4\) and 0.1 M citric acid in the ratio 4.67 : 1.

Diverse ion solution - Stock solutions of various cations were prepared by dissolving mainly their chlorides and sulphates in water or dilute acids. Anionic diverse solutions were prepared by dissolving sodium, potassium or ammonium...
salts of the anions. The concentration of these interfering ions were estimated either titrimetrically or gravimetrically.

All other solutions were prepared with doubly distilled water using analytical reagent or guaranteed reagent grade chemicals.

**Instrumentation** - The copper signal in the organic phase was made with a Shimadzu Atomic Absorption Spectrometer (Model AA 646), the operating conditions for copper signal measurement has been presented in Table 5-1.

**Table 5-1. Operating conditions of AAS**

<table>
<thead>
<tr>
<th>Operating condition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper hollow cathode lamp current</td>
<td>7 mA</td>
</tr>
<tr>
<td>Slit width</td>
<td>0.38 nm</td>
</tr>
<tr>
<td>Wavelength</td>
<td>324.7 nm</td>
</tr>
<tr>
<td>Burner Height</td>
<td>4 mm</td>
</tr>
<tr>
<td>Air flow rate</td>
<td>10 L min⁻¹</td>
</tr>
<tr>
<td>Acetylene flow rate</td>
<td>2 L min⁻¹</td>
</tr>
</tbody>
</table>

The measurements of pH were determined with a Systronics Digital pH meter Model TMS-30.

**Standard procedure** - The following solutions were quantitatively transferred into a 100 ml separatory funnel in the following sequence: 1.0 ml of [Cu(BPTC)Cl] solution (≈ 200 μgml⁻¹ with respect to Cu(II)), diluted to 10.0 ml, the pH
was adjusted to 7.0 using Na$_2$HPO$_4$ - citric acid buffer, 1.0 ml of sample solution and 6.0 ml of isopentyl acetate. For the blank, 1.0 ml of process blank was used. The mixture was shaken for 45 s and allowed to stand for 4 min to establish equilibrium. The procedure was repeated using 3.0 ml of the solvent and the combined organic phase was washed using 3.0 ml of buffer solution. Finally the organic phase was diluted to 10.0 ml in a volumetric flask and the copper signal was measured in the organic phase using air-C$_2$H$_2$ flame AAS against blank.

**Collection and treatment of samples** - Human blood serum samples were collected from different persons by heparnised syringe and stored at -20°C in contamination free tubes (cf. Chapter 3). To 0.5 - 1.5 ml serum samples, 4.0 ml of 10% KNO$_3$ was added and centrifuged; the supernatant was again treated with 2.0 ml of 10% KNO$_3$ and centrifuged. The final supernatant was diluted to 10.0 ml.

Human saliva samples (non-stimulated) was collected from several subjects. To 1.0 - 5.0 ml of these samples, 2.0 ml of 2% KNO$_3$ was added and centrifuged; it was finally diluted to 10.0 ml.

Urine samples collected were centrifuged prior to determination to remove suspended solid matter.
All the above samples were collected from male active smokers and non-smokers, and from female non-smokers. Male smokers included both cigarette smokers and country tobacco ('bidi') smokers.

Process blank - Process blanks for the above samples were prepared using doubly distilled water instead of samples and subjecting to the above sample treatment procedure.

5.2. OPTIMIZATION OF DIFFERENT PARAMETERS

Effect of pH - There was not much variation in the percentage of extraction of the copper complex over a wide pH range. However, the extraction was found to be maximum for the pH range 5.2 - 9.0 (Fig.5.1). Further methodical parameters were optimized by setting the pH at 7.0 using Na₂HPO₄-citric acid buffer.

Choice of the extracting solvent - Several pure solvents were investigated as possible extractants of the complex. Table 5-2 enumerates the extraction efficiency of different solvents for the copper complex at pH 7.0.

From the Table 5-2, it is evident that isopentyl acetate is the best extractant of the complex. It may be recalled that in case of the cyanide complex using the complex system \([\text{Cu(BPTC)}]^+\) (Chapter 4) the pH (8.2) and solvent (IBMK-isopentyl-alcohol) used are quite different from the present work using the same complex system.
Fig. 5.1. Extraction of the complex at different pH
Tabel 5-2. Extraction of 3.0 μgml⁻¹ of SCN⁻ into various pure solvents

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Extraction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isopentyl acetate</td>
<td>96.0</td>
</tr>
<tr>
<td>Isobutyl methyl ketone</td>
<td>81.0</td>
</tr>
<tr>
<td>n-Butanol</td>
<td>43.0</td>
</tr>
<tr>
<td>n-Butyl acetate</td>
<td>43.0</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>40.0</td>
</tr>
<tr>
<td>Chloroform</td>
<td>35.0</td>
</tr>
<tr>
<td>Carbon tetrachloride</td>
<td>30.0</td>
</tr>
<tr>
<td>1,2-Dichloroethane</td>
<td>10.0</td>
</tr>
</tbody>
</table>

**Shaking time and equilibration time** - The shaking time required for maximum extraction of the complex into isopentyl acetate at pH 7.0 was found to be 45 s, after which the extraction was almost constant for different periods of time (Table 5-3).

The time required to achieve the extraction equilibrium was studied with the solvent mixture at pH 7.0; the equilibrium was reached in about 4 mins.

**Stability of the complex** - The stability of the complex was found to be almost similar to that of the cyanide complex of [Cu(BPTC)]⁺; the complex was appreciably stable in the organic phase.
Table 5-3. Optimization of shaking time

<table>
<thead>
<tr>
<th>Time (s)</th>
<th>Extraction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>64.5</td>
</tr>
<tr>
<td>20</td>
<td>81.2</td>
</tr>
<tr>
<td>30</td>
<td>92.3</td>
</tr>
<tr>
<td>45</td>
<td>96.0</td>
</tr>
<tr>
<td>60</td>
<td>96.1</td>
</tr>
<tr>
<td>120</td>
<td>96.2</td>
</tr>
<tr>
<td>180</td>
<td>95.8</td>
</tr>
</tbody>
</table>

Choice of the complex system - The stability of the 2-benzoyl pyridine Schiff bases with metal is quite high as established by Dutta and De. Generally, the ligating property of thiocyanate is used for its determination and in few cases, the formation of ion-pair has been utilized. The present method, considering the above points, utilizes the ligating property of thiocyanate in conjunction with $[\text{Cu}^{+}]$ . This was found to be suitable after studying with several other systems, viz., $[\text{Co}^{+}]_2$ , $[\text{Ni}^{+}]_2$ , $[\text{Co}^{+}][\text{BPINH}]$2+ (BPINH = 2-benzoyl pyridine isonicotinoyl hydrazone), $[\text{Ni}^{+}][\text{en}^+]$2+ (en = ethylenediamine) and $[\text{Cu}^{+}][\text{BigH}]_2$2+ (BigH = biguanide).

Effect of foreign ions - Modern analytical methods are aimed towards specific applications for determination of the analyte in complex matrices, and in view of this, validity
of the method in presence of several foreign ions and compounds is to be tested. The spectral interference in the determination of 30 µg thiocyanate at pH 7.0 from several cations, anions and organics is being presented in Table 5-4. The tolerance limits with the error in such determination of thiocyanate are also presented in this Table. No serious interference except for iodide, was observed in this determination. The complexing ability of thiocyanate by iron is well known and the interference was avoided in two ways: firstly, by using citrate as a masking agent and secondly working at a higher pH e.g. 8.5 - 9.0.

Table 5-4. Determination of 30 µg thiocyanate from binary mixtures of various foreign ions

<table>
<thead>
<tr>
<th>Ions</th>
<th>Tolerance limit (µg)</th>
<th>Error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SO₄²⁻</td>
<td>3500</td>
<td>- 0.9</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>2000</td>
<td>+ 1.1</td>
</tr>
<tr>
<td>VO₃⁻</td>
<td>1800</td>
<td>+ 1.4</td>
</tr>
<tr>
<td>CH₃COO⁻</td>
<td>1800</td>
<td>- 0.8</td>
</tr>
<tr>
<td>Citrate</td>
<td>1500</td>
<td>- 1.2</td>
</tr>
<tr>
<td>Br⁻</td>
<td>1400</td>
<td>+ 1.1</td>
</tr>
<tr>
<td>S₂O₃²⁻</td>
<td>750</td>
<td>+ 1.5</td>
</tr>
<tr>
<td>WO₄²⁻</td>
<td>450</td>
<td>+ 0.8</td>
</tr>
<tr>
<td>PO₄³⁻</td>
<td>400</td>
<td>- 1.0</td>
</tr>
<tr>
<td>I⁻</td>
<td>250</td>
<td>+ 1.4</td>
</tr>
<tr>
<td>K⁺</td>
<td>4500</td>
<td>- 0.3</td>
</tr>
<tr>
<td>Na⁺</td>
<td>4500</td>
<td>- 0.3</td>
</tr>
</tbody>
</table>

Contd.
Table 5-4 (Contd.)

<table>
<thead>
<tr>
<th>Ions</th>
<th>Tolerance limit (µg)</th>
<th>Error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca$^{2+}$</td>
<td>2000</td>
<td>- 0.4</td>
</tr>
<tr>
<td>Mn$^{2+}$</td>
<td>2000</td>
<td>- 0.3</td>
</tr>
<tr>
<td>Hg$^{2+}$</td>
<td>2000</td>
<td>- 0.9</td>
</tr>
<tr>
<td>Bi$^{3+}$</td>
<td>1700</td>
<td>- 0.3</td>
</tr>
<tr>
<td>Mg$^{2+}$</td>
<td>1600</td>
<td>+ 0.4</td>
</tr>
<tr>
<td>Pb$^{2+}$</td>
<td>1600</td>
<td>- 0.5</td>
</tr>
<tr>
<td>Zr$^{4+}$</td>
<td>1500</td>
<td>+ 0.6</td>
</tr>
<tr>
<td>Cr$^{3+}$</td>
<td>1400</td>
<td>+ 0.4</td>
</tr>
<tr>
<td>Fe$^{2+}$</td>
<td>1200</td>
<td>- 1.4</td>
</tr>
<tr>
<td>Fe$^{3+}$</td>
<td>800</td>
<td>- 1.2</td>
</tr>
<tr>
<td>UO$_2^{2+}$</td>
<td>700</td>
<td>- 1.4</td>
</tr>
<tr>
<td>Cd$^{2+}$</td>
<td>700</td>
<td>- 0.8</td>
</tr>
<tr>
<td>Zn$^{2+}$</td>
<td>550</td>
<td>- 1.2</td>
</tr>
<tr>
<td>Co$^{2+}$</td>
<td>400</td>
<td>- 1.4</td>
</tr>
<tr>
<td>Ni$^{2+}$</td>
<td>400</td>
<td>- 1.2</td>
</tr>
</tbody>
</table>

Analysis of synthetic mixtures - Synthetic mixtures of cations, anions and organics with 30 µg of thiocyanate were prepared and estimation of the latter by the general procedure was followed. The results are presented in Table 5-5.
Table 5-5. Determination of 30 μg SCN\(^-\) in synthetic mixtures

<table>
<thead>
<tr>
<th>Composition (μg)</th>
<th>Error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. ( \text{Mg}^{2+}(1000) + \text{P}O_4^{3-}(300) + \text{Cl}^- (800) )</td>
<td>+1.2</td>
</tr>
<tr>
<td>2. ( \text{Mg}^{2+}(1000) + \text{Ca}^{2+}(1500) + \text{P}O_4^{3-}(300) )</td>
<td>-0.8</td>
</tr>
<tr>
<td>3. ( \text{Fe}^{3+}(500) + \text{Mg}^{2+}(800) + \text{Na}^+ (1000) )</td>
<td>+0.9</td>
</tr>
<tr>
<td>4. ( \text{WO}_4^{2-}(400) + \text{V}O_3^- (200) + \text{S}_2\text{O}_3^{2-}(200) )</td>
<td>+1.9</td>
</tr>
<tr>
<td>5. ( \text{Co}^{2+}(200) + \text{Mg}^{2+}(700) + \text{Fe}^{3+}(500) ) + ( \text{SO}_4^{2-}(1000) )</td>
<td>-1.8</td>
</tr>
<tr>
<td>6. ( \text{CH}_3\text{COO}^- (1000) + \text{Urea} (800) + \text{Fe}^{3+}(400) )</td>
<td>-0.8</td>
</tr>
</tbody>
</table>

Choice of serum deproteiniser - As cited previously by Chakraborty et al\(^{30}\), potassium nitrate was found to be the best serum deproteiniser and thiocyanate extractant. The concentration of \( \text{KNO}_3 \) was optimised to be 6.0 ml of 10% \( \text{KNO}_3 \) solution.

Choice of electrolyte for saliva - The problem with thiocyanate determination in saliva arises from slow equilibration; this was avoided by addition of electrolyte to the saliva which set rapid equilibration. Use of 2.0 ml of 2.0% \( \text{KNO}_3 \) was sufficient to equilibrate the extracting system within 4 min and to avoid emulsion formation.
5.3. **COMPOSITION OF THE EXTRACTED SPECIES**

The molar-ratio method was used to ascertain the composition of the extracted species; the ratio was found to be \( \text{Cu}^{2+} : \text{SCN}^- = 1.12 : 1 \) (Fig. 5.2). Hence the species extracted presumably by the solvent is \([\text{Cu}(\text{BPTC})(\text{SCN})]\).

5.4. **APPLICATION**

The present method of thiocyanate determination was applied in human fluids, viz. blood serum, urine and saliva. The values obtained for male smoker, non-smoker and female non-smokers in different biological fluids have been presented in Fig. 5.3. As is evident from the figure, the concentration of thiocyanate in male saliva is higher than that in female saliva. Its concentration in smokers is greater than that in non-smokers, probably due to formation of cyanogen compounds primarily during smoking and then subsequent interaction with sulphur compounds in body fluids to form thiocyanate. The concentration of thiocyanate decreased from saliva to serum to urine; hence it may be concluded that thiocyanate may be accumulated in body fluids and it becomes diluted when excreted.

The results thus obtained have been compared with a comparative spectrophotometric method proposed by Danchik and Boltz\(^{11}\), and the results are given in Table 5-6. The latter method gave higher values of thiocyanate in the samples, probably due to positive interference present in the matrix, which have been removed in the present technique. In their
SCN⁻ = 1 µg ml⁻¹

Fig. 5.2. Molar-Ratio method
Fig. 5.3. Thiocyanate in human fluids: MS = male smoker; MNS = male non-smoker; and FNS = female non-smoker. 

\([\text{SCN}^-] \) = concentration of thiocyanate in \( \mu \text{g ml}^{-1} \)
method, the extraction equilibration was delayed up to ten minutes owing to haziness from colloidal suspension of biological materials.

Table 5-6. Comparison of results between present method and comparative technique

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sex</th>
<th>Status</th>
<th>Thiocyanate added (μg)</th>
<th>Thiocyanate found (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Present method</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saliva</td>
<td>M</td>
<td>Smoker</td>
<td>0.0</td>
<td>61.1</td>
</tr>
<tr>
<td>Saliva</td>
<td>F</td>
<td>Non-smoker</td>
<td>30.0</td>
<td>34.8</td>
</tr>
<tr>
<td>Serum</td>
<td>M</td>
<td>Smoker</td>
<td>0.0</td>
<td>5.3</td>
</tr>
<tr>
<td>Serum</td>
<td>F</td>
<td>Non-smoker</td>
<td>10.0</td>
<td>11.6</td>
</tr>
<tr>
<td>Urine</td>
<td>M</td>
<td>Smoker</td>
<td>20.0</td>
<td>21.5</td>
</tr>
<tr>
<td>Urine</td>
<td>F</td>
<td>Non-smoker</td>
<td>30.0</td>
<td>30.6</td>
</tr>
</tbody>
</table>

A good correlation between thiocyanate added to the samples and thiocyanate found was observed in the present method (Fig. 5.4). The slope values of these linear fits were found to be close to 1.0, proving the feasibility of the method.

5.5. **ANALYTICAL FIGURES OF MERIT**

The proposed method gave a linear calibration graph for 0.1 - 4.7 μg/ml⁻¹ of thiocyanate (Fig. 5.5). The limit of detection and sensitivity values were respectively 4 ng/ml⁻¹ and 0.133 ml μg⁻¹. The relative standard deviation (RSD) for 6 blank determinations
Fig. 5.4. Recovery of SCN added to human fluids

Plot A: Urine; $Y = 0.9934X + 1.6836$
Plot B: Serum; $Y = 0.9956X + 5.5140$
Plot C: Saliva; $Y = 0.9860X + 17.6700$
Fig. 5.5. Calibration plot for thiocyanate
was 1.8%. The RSD values for determination of 10 \( \mu g ml^{-1} \) thiocyanate in saliva, serum and urine were 2.3%, 5.8% and 3.5% respectively.

**Comparison with other methods** - The reliability and superiority of the present method was finally established by comparing the sensitivity and limit of detection (LOD) values of various methods used in the determination of thiocyanate in different samples. Table 5-7 shows the sensitivity and LOD of different methods along with the present method.

**Table 5-7. Comparison of different methods**

<table>
<thead>
<tr>
<th>Method</th>
<th>Sensitivity ((ml \mu g^{-1}))</th>
<th>LOD ((\mu g ml^{-1}))</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spectrophotometry</td>
<td>-</td>
<td>0.08</td>
<td>14</td>
</tr>
<tr>
<td>Cathodic Stripping Volta-(\text{metry})</td>
<td>-</td>
<td>(1.16 \times 10^{-3})</td>
<td>20</td>
</tr>
<tr>
<td>Ion-chromatography</td>
<td>-</td>
<td>0.004*</td>
<td>25</td>
</tr>
<tr>
<td>Indirect AAS</td>
<td>0.088</td>
<td>-</td>
<td>11</td>
</tr>
<tr>
<td>Indirect AAS</td>
<td>-</td>
<td>0.004</td>
<td>29</td>
</tr>
<tr>
<td>Indirect AAS</td>
<td>0.022</td>
<td>0.071</td>
<td>30</td>
</tr>
<tr>
<td>Present method</td>
<td>0.133</td>
<td>0.004</td>
<td>31</td>
</tr>
</tbody>
</table>

* For 2 \(\sigma\) value
5.6. ANALYTICAL CHARACTERISTICS

A summary of the analytical parameters for the extraction-indirect AAS determination of thiocyanate is presented in Table 5-8.

Table 5-8. Analytical characteristics

<table>
<thead>
<tr>
<th>Extracting complex</th>
<th>[Cu(BPTC)(SCN)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH range</td>
<td>5.2 - 9.0</td>
</tr>
<tr>
<td>Solvent</td>
<td>Isopentyl acetate</td>
</tr>
<tr>
<td>Shaking time</td>
<td>45 s</td>
</tr>
<tr>
<td>Equilibration time</td>
<td>4 min</td>
</tr>
<tr>
<td>Working range</td>
<td>0.1 - 4.7 (\mu g ml^{-1})</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>0.133 ml (\mu g^{-1})</td>
</tr>
<tr>
<td>Limit of detection</td>
<td>4 ng ml(^{-1})</td>
</tr>
<tr>
<td>Limit of quantitation</td>
<td>13.3 ng ml(^{-1})</td>
</tr>
<tr>
<td>R.S.D. (blank)</td>
<td>1.8%</td>
</tr>
<tr>
<td>R.S.D. (saliva)</td>
<td>2.3%</td>
</tr>
<tr>
<td>R.S.D. (serum)</td>
<td>5.8%</td>
</tr>
<tr>
<td>R.S.D. (urine)</td>
<td>3.5%</td>
</tr>
</tbody>
</table>

5.7. DISCUSSION

Takahiko\textsuperscript{27} proposed a method for salivary thiocyanate determination while the present method has proven versatile by application to other biological fluids also. As is evident from spectral interference study, the method is almost free
from most ion interference at the concentration level that are present in the biological fluids. The present method has a very good sensitivity and LOD values. The only method which has a better LOD value is that of Bilewicz et al.\textsuperscript{20} uses cathodic stripping voltametric technique but suffers from interferences due to Cl\textsuperscript{-}, Br\textsuperscript{-} and Fe\textsuperscript{3+}. The proposed method, which uses a stable complex [Cu(BPTC)(SCN)] employing the ligating property of thiocyanate, extracts the complex into isopentyl acetate, the copper signal is measured in the organic phase by AAS leading to enhanced sensitivity, and hence the thiocyanate concentration is indirectly determined. The method is well suited for measuring the thiocyanate level in human blood serum, urine and saliva. The total time including sample preparation, extraction and aspiration in AAS is about 20 min, proving the rapidity of the method.
REFERENCES


