CHAPTER II

SPECTROPHOTOMETRIC DETERMINATION OF HYDROGEN CYANIDE IN AIR AND ITS APPLICATION IN BIOLOGICAL FLUIDS

SUMMARY

A spectrophotometric method is described for the determination of hydrogen cyanide in air. Hydrogen cyanide present in air is absorbed in 0.002 M sodium hydroxide solution, which is then treated with bromine. The cyanogen bromide so formed reacts with pyridine to form glutaric aldehyde. The latter is condensed with anthranilic acid to form a yellowish orange polymethine dye which shows a maximum absorbance at 400 nm. Beer's law is obeyed in the range of 0.44 to 4.4 mg m$^{-3}$ of hydrogen cyanide. Other analytical parameters such as collection efficiency of absorbing solution have been investigated. The method has been successfully applied to the determination of hydrogen cyanide in biological samples such as cysteine, blood and urine.

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SPECTROPHOTOMETRIC DETERMINATION OF HYDROGEN CYANIDE IN AIR AND ITS APPLICATION IN BIOLOGICAL FLUIDS

Hydrogen cyanide is highly toxic and is a fairly widespread pollutant in the air. It is emitted into the atmosphere by the coal tar and plastics industries during the manufacture of acrylonitrile and various resins. Gas works, coke ovens and the purification of blast furnaces are also important sources of hydrogen cyanide. Hydrogen cyanide or hydrocyanic acid often called 'prussic acid', is a colourless gas with definite bitter almond like odour (1). It is manufactured chiefly by the reaction of ammonia, air and methane in the presence of a platinum catalyst (2). Hydrogen cyanide has a broad spectrum of usage involving fumigation, in electroplating and in mining, in production of various resin monomers such as acrylates, methacrylates and hexamethylenediamine, in the production of other nitriles. Cyanides are widely used in precious metal refining, manufacturing of organic chemicals, fertilizers, rhodanticides, explosives, hardening of steel, metal polishing, photography, etc. and in various organic reactions as a chemical intermediate (3,4). Hydrogen cyanide is extensively employed in fumigation of warehouses, flour mills, holds of ships, green houses and citrus trees. As gaseous fumigant hydrogen cyanide is generated on the spot from calcium
cyanide pellets by dropping it into sulphuric acid solution. Calcium cyanide and cyanogens also liberate hydrogen cyanide gas on exposure to moisture in the air even with 25% humidities. Hydrogen cyanide can also be used in the form of liquified gas absorbed in Kieselguhr or fibre disc and in discoids, containing definite concentration of gas. Liquid hydrocyanic acid is used in fumigation for the control of pests on citrus trees (5-7).

Derivation of acrylic plastics now in wide use in homes and offices and polymethane foams used as insulating materials, upon pyrolysis generate hydrogen cyanide and thus potentiate hazards to persons breathing air laden with their poisonous gas (g). Cyanides occurs in seeds of fruits such as apples, apricots, cherries, peaches and plums. Cyanide in plants is bonded to glucoside (sugar), called amygdalin, which on enzymatic or acidic hydrolysis gives hydrogen cyanide in the stomach (g). HCN is soluble in water. The toxic effect of low concentrations of cyanide on aquatic life and on the biota of waste-water treatment is well studied (10). Doudoroff et al. (11,12) studied the toxicity of complex cyanides to fish. They found that the complex cyanide is toxic due to formation of molecular hydrogen cyanide caused by the dissociation of the metal cyanide complexes. It has been reported to be present as HCN (150-300 µg) in the mainstream of cigarette smoke (13,14).
Hydrogen cyanide and its salts are amongst the most rapid acting of all known poisons. The physiological response of animal and man to various concentrations of HCN in air has been described (15). HCN is toxic by virtue of the cyanide ions. Cyanide and their simple soluble salts are very perilous as its concentrated doses suddenly resorb through skin. For instance a drop of liquid hydrogen cyanide leads to death in few seconds. While inhalation of small concentrations over longer periods cause less acute danger of its toxic effects. Nevertheless inhalation of 100–200 mg HCN m\(^{-3}\) for half to one hour is lethal (16).

The toxic action of hydrogen cyanide on warm-blooded animals is very fast (17). Hydrogen cyanide stops the oxidation of protoplasm in the tissue cells. Cyanide inhibit oxidative enzymes from mediating the process by which oxygen is utilized to complete the production of ATP in mitochondria and cells get energy. Hydrogen cyanide acts directly on the nervous system. Toxic action of cyanide on respiration together with asphyxia resulting from inactivation of cytochrome oxidase and other oxidative enzymes causes respiratory effort in the early stage of cyanide toxication (18,19). Inhalation of higher concentrations of hydrogen cyanide vapours causes symptoms of giddiness, headache, unconsciousness, collapse and convulsion with cessation of respiration as a result of paralysis of the respiratory centre in brain. Lower concentration of cyanide causes
symptoms such as weakness, headache, nausea, muscle cramps, loss of appetite, psychoses and vomiting. Hydrogen cyanide vapour is absorbed extremely rapidly through the respiratory tract, the liquid and possibly the concentrated vapour is absorbed directly through intact skin. Venous blood may appear a brighter red colour than normal. It causes anaemia by forming stable compound with haemoglobin. Halogenated cyanides, at low concentrations have more like the highly irritating vesicant gases and cause severe lachrymatory effects as well as both acute and delayed pulmonary irritant and pulmonary edema (1). The acute systemic toxicity of cyanide by instillation into the conjunctival sac has been studied (20). Contamination of the eye with cyanide could be hazardous route of exposure. As cyanides may be absorbed across conjunctival blood vessels to produce systemic toxicity.

The General Toxicology of Hydrogen Cyanide (21)

<table>
<thead>
<tr>
<th>Hydrogen cyanide concentration (ppm)</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2 - 5.0</td>
<td>Threshold of odour.</td>
</tr>
<tr>
<td>10</td>
<td>Threshold limit value.</td>
</tr>
<tr>
<td>18 - 36</td>
<td>Slight symptoms (headache) after several hours.</td>
</tr>
<tr>
<td>45 - 54</td>
<td>Tolerated for 1/2 to 1 hour without difficulty.</td>
</tr>
<tr>
<td>100</td>
<td>Death within 1 hour.</td>
</tr>
<tr>
<td>110 - 135</td>
<td>Fatal in 1/2 to 1 hour.</td>
</tr>
<tr>
<td>181</td>
<td>Fatal after 10 minutes.</td>
</tr>
<tr>
<td>280</td>
<td>Immediately fatal.</td>
</tr>
</tbody>
</table>
The burning example of toxicity of hydrogen cyanide has been seen in the industrial accident at Union Carbide Plant, Bhopal on the night of 2nd Dec., 1984. Thousands of people died due to the chronic effect of cyanide in the body after exposure to methylisocyanide (MIC) (22). According to the report of American Chemical Society this tragedy appears to have two aspects in the cyanide issue. One is the direct exposure and the other is the indirect exposure owing to unusual generation of toxic amounts of hydrogen cyanide in the body after exposure to methylisocyanide (MIC). The proposed mechanism for the generation of cyanide in the blood after exposure to MIC is given in Scheme 1.

The reported threshold limit value for hydrogen cyanide in air is 0.01 mg m\(^{-3}\) (23). Minute amounts of cyanide produces adverse toxicological effects in man and invasion of this substance into body by almost any route results in life threatening conditions (8). Sensitive methods are, therefore, required for its determination in trace amounts.

A large number of methods based on various principles have been reported in literature for the determination of cyanide. The methods based on titration involving visual end point indicator or instrumental method for determining end point. The best known Liebig
PROPOSED MECHANISM FOR GENERATION OF CYANIDE IN THE BODY AFTER EXPOSURE TO MIC (22)

Hb = Hemoglobin; AMP = Adenosine monophosphate; ATP = Adenosine triphosphate

In this scheme, the N-terminal ends of hemoglobin would react with methylisocyanate to produce the N-methylcarbamoyl hemoglobin. That product would react with the adenylyl group of adenosine triphosphate (ATP) to generate activated adenylyl hemoglobin. Following elimination of adenosine monophosphate (AMP), a series of tautomeric shift would result in the splitting off of hydrogen cyanide, which ionizes at physiologic pH. The liberated cyanide ion would bind to hemoglobin, cytochrome oxidase, and other physiologically critical enzymes, thus favouring equilibrium toward hydrogen cyanide production.
titrimetric method (24) is based on the formation of turbidity due to silver cyanide. This method is satisfactory for higher concentrations only as well as in alkaline and ammoniacal medium, is subjected to error (25,26). Various other titrimetric methods based on turbidity formation or using different indicators have also been reported (27,28). There are various instrumental methods reported for the determination of cyanide such as amperometric (29), coulometric (30), potentiometric (31), polarographic (32) and ion-selective electrode methods (33), etc. in addition to fluorimetric (34,35), flow injection analysis (36), atomic absorption spectrometry (37) and gas chromatography (38). Gas chromatography is used widely for the determination of HCN. The separation of HCN, water and permanent gases has been accomplished by various workers. Separated hydrogen cyanide is determined by different techniques (39-43).

Most of the methods used for detecting and determining cyanide involve the formation of hydrogen cyanide. Williams (44) proposed a method for decomposing complex cyanides to give hydrogen cyanide, by distilling after the addition of an acidic solution of cuprous chloride. This method is also recommended by British authorities (45). Kruse and Mellon (46) distilled complex cyanide with phosphoric acid in the presence of citric acid and ethylenediamine tetra acetic acid, under partial vacuum.
They also proposed solvent extraction to improve the sensitivity of determination (47). Serfass et al (48) decompose the complex cyanide by action of magnesium chloride, mercuric chloride and sulphuric acid. Recovered hydrogen cyanide was collected in absorption tubes by reflux method.

Colorimetric methods are quite sensitive and widely used. Various methods for detection and determination of hydrogen cyanide are summarised by Feigl (49) and Ruch (50,51). Several reagents are used for the detection of hydrogen cyanide such as phthalophenone (52), silver nitrate (53), benzidine-copper acetate (54), methyl orange-mercuric chloride (55), ferrous sulphate (prussian blue test) (56,57), o-tolidine (50), etc.

Formation of metal complexes form the basis of some colorimetric methods (58-71). Vadav et al (72) absorbed hydrogen cyanide in Ni²⁺ solution containing colour indicator muraxide. The formation of thiocyanate (73) from cyanide by reaction with ammonium polysulphide and then ferrithiocyanate, of blood red colour by ferric chloride is an excellent test for cyanide. Indicators like bromothymol blue, bromocresol green, etc. are used in indicator tube method for the determination of cyanide (74). Reilly determined hydrogen cyanide by formation of red complex of cyanide with alkaline sodium picrate (75). Recently Kratochvil (77) prepared detector
tubes using silica gel treated with sodium carbonate and
impregnated with solution containing p-nitrobenzaldehyde
benzyl pyridine, triethylene glycol and butyl ether in
cyclohexane for the determination of hydrogen cyanide in
air. Determination of cyanide by some indirect spectro-
photometric methods have also been proposed (77-87)
using different reagents such as chrome Azurol S-cetyl-
pyridinium chloride (77), cadion 2B-triton X-100 (80),
gold or silver sol (85,86), etc.

Hydrogen cyanide concentration is most frequently
determined in air by relatively simple spectrophotometric
methods. The best known spectrophotometric methods are
based on Konig reaction (88), which has a reasonably high
sensitivity and reproducibility. Konig reaction consists
of prior conversion of cyanide into a halogen cyanide
(cyanogen chloride or cyanogen bromide) which then on
reacting with pyridine forms glutaric aldehyde, which
then readily reacts with amines and compounds containing
an active methylene group to form polymethine dyes.
Aldridge method (89) is one of the example of Konig
reaction. The method has been further modified by the
other workers using different reagents (90-94). Colori-
metric method developed by Aldridge (89) and Epstein (95)
are standard methods for determination of cyanide in
water (96). Bark and Higson used benzidine (97), and
p-phenylenediamine (98) for the development of the colour
but these are carcinogenic in nature. Epstein (95) used
pyrazolane and Asimus (99) used barbituric acid in place of toxic amines. Several other workers used dicarboxy­
dine (100), p-toluidine (101), o-dinitrobenzene (102),
isonicotinic barbituric acid (103), ethyl acetoneor
benzoyl acetate (104), amsonic acid (105), anthranilic
acid (106), sulphanilic acid (107), phloroglucinol (108),
etc. to form polymethine dye from glutaronic aldehyde.

In the present investigation a method has been
developed for the determination of hydrogen cyanide in
air, making the use of Konig reaction. Hydrogen cyanide
is converted to cyanogen bromide which then reacts with
pyridine to form glutaronic aldehyde which is then reacted
with anthranilic acid to form polymethine dye (106).
Hydrogen cyanide from air was absorbed in dilute sodium
hydroxide and then analysed. Various analytical para­
meters such as concentration of absorbing solution, flow
rate etc. have been studied. The method is applied for
the determination of hydrogen cyanide in air and biological
fluids such as cysteine, blood and urine.

EXPERIMENTAL

Apparatus:

A Carl Zeiss Spekol spectrophotometer with matched
silica cells of 1 cm path length was used for all spectral
measurements. Midget impingers of 35 ml capacity were
used for the absorption of hydrogen cyanide from air. A
calibrated PINCO rotameter was used for air flow measurements.

Reagents

Standard cyanide solution - A stock solution of 1 mg/ml of cyanide was prepared by dissolving 0.250 g of potassium cyanide in 100 ml of distilled water. Working standard of 10 μg/ml was prepared by the appropriate dilution of the stock.

Pyridine reagent - 3 ml of concentrated hydrochloric acid was mixed with 18 ml of freshly distilled pyridine and 12 ml of distilled water were added to prepare pyridine reagent (98).

Sodium arsenite solution - 1.5% (m/v) solution of sodium arsenite was prepared in distilled water.

Bromine water - Saturated solution of bromine water was prepared.

Anthranilic acid - A 0.1% (m/v) solution of anthranilic acid was prepared in distilled water.

Absorbing solution - 0.002 M solution of sodium hydroxide was prepared for absorbing hydrogen cyanide.

Tris-HCl buffer - pH 7.6 - Prepared by dissolving 0.60 g of tris(hydroxymethyl)aminomethane and 2.36 g of tris(hydroxymethyl)aminomethane hydrochloride in 100 ml of water (8).
Cysteine solution (8) - Prepared by placing 50 mg of L-cysteine hydrochloride monohydrate in test tube, after which one drop of bromocresol green indicator solution was added. 0.5M sodium hydroxide was added dropwise until all of the cysteine dissolved. 10 ml of tris-HCl buffer was added to neutralise cysteine and thoroughly mixed. The pH of the solution should be ~7.4.

All chemicals used were of analytical reagents grade.

Procedure

Collection of Sample

Hydrogen cyanide was generated from acidified solution of cyanide and was absorbed in 0.002 M sodium hydroxide solution. For the generation of hydrogen cyanide a few millilitres of 5 M sulphuric acid were added to a flask containing 5 ml of standard cyanide solution and the flask was stoppered immediately. The flask was connected to two midget impingers containing 10 ml each of the absorbing solution connected in series to the source of suction. Just after the addition of sulphuric acid purified air was passed through the impinger for 30 min. (Fig. 1).

Analysis -

After sampling, aliquots of the absorbed solution were transferred into a 10 ml calibrated flask. To each flask was added 0.3 ml of bromine water and the mixture
FIG. 1 - SAMPLING TRAIN FOR THE DETERMINATION OF COLLECTION EFFICIENCY OF ABSORBING SOLUTION

P - PUMP
R - ROTAMETER
S - DRYING TOWER (SILICA GEL)
I 1 - IMPINGER WITH ABSORBING SOLUTION
I 2 - IMPINGER
C - CHAMBER
B - MICRO BURETTE
was allowed to stand for 1 min so that the bromination was complete. The excess of bromine was decolourised by the dropwise addition of sodium arsenite solution. Then 0.4 ml of pyridine reagent followed by 1 ml of anthranilic acid were added. The mixture was allowed to stand 10 minutes for full colour development. The volume was made up to the mark with dilute solution of sodium hydroxide to maintain a pH of ~ 7.2 and the absorbance was measured at 400 nm using distilled water as the reference. The same procedure was followed for the blank, which gave no colour under these conditions.

RESULTS AND DISCUSSION

Generation of Hydrogen Cyanide:

Hydrogen cyanide was generated by the reaction of sulphuric acid with potassium cyanide. The liberated hydrogen cyanide was efficiently trapped in a 0.002 M solution of sodium hydroxide by passing purified air through the solution at a rate of 0.5 lit min⁻¹. The absorbed hydrogen cyanide was then determined by the proposed procedure. It was found that the generation of hydrogen cyanide was quantitative.

Absorption Efficiency

Two midget impingers (35 ml capacity) each containing 10 ml of 0.002 M sodium hydroxide solution, were connected in series. An air sample was passed
through them for various lengths of time at different flow rates. After sampling, hydrogen cyanide was determined by the proposed procedure. It was found that 99-100% of the hydrogen cyanide was absorbed in the first impinger. The second impinger gave a negative result for cyanide. Concentrations of sodium hydroxide solution from 0.001 to 0.01 M had no effect on the absorption efficiency (Table I).

Effect of flow rate and temperature variation on absorption efficiency were studied (Table II). It was found that a variation of the flow rate from 0.25 to 2 lit min\(^{-1}\) and of the temperature from 15° to 35° C during the collection of the sample had no effect on the absorption efficiency.

In the time period 10 - 60 minutes of sampling, no change in absorption efficiency was noted (Table III).

### Stability of the Collected Hydrogen Cyanide Samples

Stability of collected hydrogen cyanide sample was studied. It was found that the hydrogen cyanide absorbed in 0.002 M sodium hydroxide solution was stable for many days.

### Absorption spectra

The absorption spectra of the dye is shown in Fig. 2. The absorption spectra of the dye shows maximum absorption at 400 nm. The reagent blank has negligible absorption at this wavelength.
TABLE - I

EFFECT OF CONCENTRATION OF SODIUM HYDROXIDE
ON ABSORPTION EFFICIENCY

Flow rate = 0.5 lit min\(^{-1}\)

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Concentration of NaOH/M</th>
<th>Cyanide passed/ug</th>
<th>CN(^{-}) found* in 1st impinger/ug</th>
<th>Absorption %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 0.001</td>
<td>10</td>
<td>9.85</td>
<td>98.50</td>
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<td></td>
<td>40</td>
<td>39.95</td>
<td>99.87</td>
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<tr>
<td>2. 0.004</td>
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<td>100.00</td>
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<td>40</td>
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<td>9.85</td>
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<td>40</td>
<td>39.70</td>
<td>99.25</td>
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</tr>
</tbody>
</table>

* Mean of three repetitive analyses.
**TABLE II**

**EFFECT OF FLOW RATE ON ABSORPTION EFFICIENCY**

Concentration of NaOH solution = 0.002 M

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Flow rate/ ( \text{lit. min}^{-1} )</th>
<th>( \text{CN}^- ) passed in 1st impinger (ug)</th>
<th>( \text{CN}^- ) found* (ug)</th>
<th>Absorption %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0.25</td>
<td>10</td>
<td>9.95</td>
<td>99.50</td>
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<tr>
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<td>20</td>
<td>19.80</td>
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<td>40</td>
<td>39.50</td>
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<td>98.75</td>
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</tbody>
</table>

* Mean of three repetitive analysis.
**TABLE III**

**EFFECT OF TIME ON ABSORPTION EFFICIENCY**

Concentration of NaOH solution = 0.002 M  
Flow rate = 0.5 lit min$^{-1}$

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Sampling time (min)</th>
<th>CN$^-$ passed (µg)</th>
<th>CN$^-$ found* in 1st impinger (µg)</th>
<th>Absorption (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>10</td>
<td>10</td>
<td>9.85</td>
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<td>29.98</td>
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</table>

* Mean of three repititive analyses.
FIG. 2. ABSORPTION SPECTRA OF THE DYE.

A. CONCENTRATION OF CYANIDE = 20 μg/10 ml.

B. CONCENTRATION OF CYANIDE = 30 μg/10 ml.
**Effect of Varying Reaction Conditions**

The amount of bromine water needed for bromination was checked by adding various amounts of saturated bromine water. A minimum of 0.2 ml of bromine water was required for complete bromination of cyanide to cyanogen bromide (Fig. 3). Any excess of bromine was decolourised by the dropwise addition of sodium arsenite solution.

Effect of addition of sodium arsenite was studied. No change in the absorbance was observed for 0.2 to 1 ml of 1.5% sodium arsenite solution. Addition of more than 1 ml of sodium arsenite solution causes decrease in absorbance (Fig. 4).

The pyridine reagent was added for conversion of cyanogen bromide into glutaric aldehyde. A minimum of 0.2 ml of pyridine reagent was needed for the reaction. The addition of up to 1 ml of pyridine reagent had no effect on the absorbance. Decrease in absorbance was observed when more than 1 ml of pyridine reagent was added (Fig. 5). Constant absorbance values were obtained with the addition of 1-5 ml of 0.1% anthranilic acid solution (Fig. 6).

**Effect of Time and Temperature**

The effects of time and temperature on the colour development were studied. It was observed that 2-3 minutes were sufficient for full colour development and the colour remained stable for 15 minutes in the range of 15° to 35°C.
FIG. 3. EFFECT OF BROMINATION ON REACTION
CONCENTRATION OF CYANIDE = 30 μg/10 ml

FIG. 4. EFFECT OF SODIUM ARSENITE ON COLOUR REACTION
CONCENTRATION OF CYANIDE = 30 μg/10 ml
FIG. 5. EFFECT OF PYRIDINE ON COLOUR REACTION.
CONCENTRATION OF CYANIDE = 30 µg/10 ml.

FIG. 6. EFFECT OF ANTHRANILIC ACID ON COLOUR REACTION
CONCENTRATION OF CYANIDE = 30 µg/10 ml.
At higher temperatures there was a decrease in the absorbance (Fig. 7 and 8).

**Beer's Law, Molar Absorptivity, Sandell's Sensitivity and Reproducibility:**

Beer's law is obeyed in the range of 4 to 40 \( \mu g \) per 10 ml (0.44 - 4.4 mg m\(^{-3}\)) of hydrogen cyanide. Molar absorptivity and Sandell's sensitivity of the colour reaction was found to be \( 6 \times 10^3 \) lit mol\(^{-1}\) cm\(^{-1}\) (± 100) and 0.005 \( \mu g \) cm\(^{-2}\) respectively.

The reproducibility of the method was checked by replicate determination of 20 \( \mu g \) cyanide over a period of seven days. The standard deviation and relative standard deviation were found to be ± 0.008 and 2.0% respectively (Table IV).

**Effect of Diverse Ions:**

To assess the applicability of the method effect of various diverse ions commonly present with cyanide have been studied. Organic pollutants such as benzene, phenol and aniline and metal ions such as zinc, cadmium, lead, mercury and iron did not interfere. Oxidising and reducing agents, if present in small amounts, are removed by the sodium arsenite and bromine water respectively and hence do not interfere. The tolerance limits are shown in Table V.
**FIG. 7. EFFECT OF TIME ON COLOUR DEVELOPMENT.**

CONCENTRATION OF CYANIDE = 30 μg / 10 ml.

**FIG. 8. EFFECT OF TEMPERATURE ON COLOUR DEVELOPMENT.**

CONCENTRATION OF CYANIDE = 30 μg / 10 ml.
FIG. 9. CALIBRATION CURVE FOR THE DETERMINATION OF HYDROGEN CYANIDE.
TABLE IV

REPRODUCIBILITY OF THE METHOD

Amount of cyanide = 20 \mu g

<table>
<thead>
<tr>
<th>No. of Days</th>
<th>Absorbance*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.40</td>
</tr>
<tr>
<td>2</td>
<td>0.41</td>
</tr>
<tr>
<td>3</td>
<td>0.39</td>
</tr>
<tr>
<td>4</td>
<td>0.40</td>
</tr>
<tr>
<td>5</td>
<td>0.40</td>
</tr>
<tr>
<td>6</td>
<td>0.41</td>
</tr>
<tr>
<td>7</td>
<td>0.39</td>
</tr>
</tbody>
</table>

Mean = 0.40

Standard deviation = ± 0.008

Relative standard deviation = 2.0%

* Mean of three repetitive analyses.
<table>
<thead>
<tr>
<th>Diverse ions (Tolerance limit in ppm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene (2000), Na⁺ (500)</td>
</tr>
<tr>
<td>Ethyl phenol (1200), K⁺ (450)</td>
</tr>
<tr>
<td>Phenol (1000), Mg²⁺ (350)</td>
</tr>
<tr>
<td>Benzaldehyde (800), Fe³⁺, Al³⁺ (300)</td>
</tr>
<tr>
<td>Formaldehyde (700), Fe²⁺, SO₄²⁻ (250)</td>
</tr>
<tr>
<td>Hydroxylamine (500), Cd²⁺, Cu²⁺ (200)</td>
</tr>
<tr>
<td>Aniline (500), Pb²⁺ (150)</td>
</tr>
<tr>
<td>Nitrobenzene (100), Zn²⁺, Hg²⁺, PO₄³⁻ (100)</td>
</tr>
<tr>
<td>Thiocyanate (interfere) NO₂⁻ (80)</td>
</tr>
</tbody>
</table>

* Amount of diverse ions which cause ± 2% error.
Application of the Method

Determination of Hydrogen cyanide in air

Hydrogen cyanide was generated in a fume cupboard by the gradual addition of sulphuric acid to a solution of potassium cyanide (3). The air from the fume cupboard was trapped in sodium hydroxide solution with the help of a suction pump placed outside the chamber. The air was sampled for 30 minute and then the solution was analysed by the recommended procedure and compared with the standard benzidine method proposed by Aldridge (89). The results obtained by both the methods were found to be identical as shown in Table VI.

Determination of hydrogen cyanide in biological samples

The method has also been applied to the determination of cyanide in cysteine, urine and whole blood samples. It has been reported that cysteine reacts with cyanide in the body and helps in the detoxification of cyanide. Hence the determination of cyanide in cysteine is important from biological point of view (1). To the 3 ml of cysteine solution were added known amounts of cyanide and purified air was passed through the solution. The liberated hydrogen cyanide was absorbed in 0.002 M sodium hydroxide solution and determined by the proposed procedure. The results in Table VI shows a 100% recovery of hydrogen cyanide from cysteine, which is in agreement with the results of the pyrazolone method (8).
### TABLE VI

**APPLICATION OF THE METHOD**

a. Analysis of generated hydrogen cyanide in air.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>HCN found</th>
<th>Aldridge method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Present method</td>
<td>( \mu g )</td>
</tr>
<tr>
<td>1</td>
<td>6.5</td>
<td>6.2</td>
</tr>
<tr>
<td>2</td>
<td>18.0</td>
<td>18.3</td>
</tr>
<tr>
<td>3</td>
<td>31.3</td>
<td>31.5</td>
</tr>
</tbody>
</table>

b. Determination and Recovery of hydrogen cyanide from cysteine, whole blood and urine samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>HCN added (3 ml)</th>
<th>HCN found</th>
<th>Recovery</th>
<th>HCN found</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \mu g )</td>
<td>( \mu g )</td>
<td>%</td>
<td>( \mu g )</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>Present method</td>
<td>Pyrazolone method</td>
<td></td>
<td>Present method</td>
<td>Pyrazolone method</td>
</tr>
<tr>
<td>Cysteine</td>
<td>1</td>
<td>8</td>
<td>7.9</td>
<td>98.75</td>
<td>7.6</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>15</td>
<td>14.8</td>
<td>98.66</td>
<td>14.9</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>25</td>
<td>24.5</td>
<td>98.00</td>
<td>25.0</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>30</td>
<td>29.7</td>
<td>99.00</td>
<td>29.5</td>
</tr>
<tr>
<td>Whole Blood</td>
<td>1</td>
<td>5</td>
<td>4.90</td>
<td>98.00</td>
<td>4.95</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>15</td>
<td>14.80</td>
<td>98.66</td>
<td>14.75</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>20</td>
<td>19.95</td>
<td>99.75</td>
<td>19.85</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>35</td>
<td>34.90</td>
<td>99.71</td>
<td>34.90</td>
</tr>
<tr>
<td>Urine</td>
<td>1</td>
<td>5</td>
<td>4.92</td>
<td>98.40</td>
<td>4.95</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10</td>
<td>9.70</td>
<td>98.00</td>
<td>9.84</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>15</td>
<td>14.71</td>
<td>98.06</td>
<td>14.90</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>20</td>
<td>19.20</td>
<td>96.00</td>
<td>19.70</td>
</tr>
</tbody>
</table>

*Mean of three repetitive analyses.*
To determine the recovery of cyanide from urine and whole blood, known amounts of cyanide were added to urine and blood, and cyanide was determined by the proposed procedure after deproteinisation of sample with trichloroacetic acid. The results are given in Table VI.

CONCLUSION

The proposed method for the determination of hydrogen cyanide is rapid and simple. No use of carcinogenic compounds is made and it can be applied to the determination of hydrogen cyanide in biological fluids. The sensitivity of the method is comparable to other reported methods.
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