CHAPTER - VI

SPECTROPHOTOMETRIC DETERMINATION OF ACRYLONITRILE:
A FUMIGANT IN AIR*

SUMMARY

A simple spectrophotometric method is described for the determination of acrylonitrile in air. The acrylonitrile present in air is absorbed in dilute alkaline potassium permanganate and subsequently oxidized to cyanide. The cyanide thus formed is converted to cyanogen bromide with bromine and reacted with pyridine to form glutarconic aldehyde by breaking of the heterocyclic linkage which is subsequently coupled with sulphanilic acid to give yellow orange polymethine dye. The dye has maximum absorbance at 460 nm and obeys Beer's law in the range of 0.5 - 5 ppm of acrylonitrile. The optimum reaction conditions and important analytical parameters have been studied and successfully applied for the determination of acrylonitrile in air and biological samples.

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Acrylonitrile is widely used as a fumigant for individual machines in flour mills and packaged cereal products under the trade names Acrylon, Carbacryl and Ventox. Alternative names of this compound are cyano-ethylene, propene nitrile and vinyl cyanide. In a mixture with carbon tetrachloride, it is used for fumigating stored tobacco, nuts and dates to control insects. It is an important industrial monomer produced in large quantities to use in the manufacture of acrylic and modacrylic fibers, resins, adiponitrile, pipe fittings, auto instrument panels, drinking tumblers and house-ware items (1-7).

It is toxic to man and animals in both acute and chronic experiments and, also, a proved animal carcinogen and suspected human carcinogen, when taken into the body via inhalation (8-10). It has been detected in effluent streams of an acrylic fiber manufacturing plant, in the smoke of U.S. cigarettes, in interstellar space, in commercial acrylamide, in polyether polymer polyols and as impurity in organic soil consolidating agents. It has been detected in workplace air in thermo-setting plastic plants, rubber foot wear plants and in sewing factories
using synthetic and natural fibers. It has also been found in shelled walnuts 38 days after fumigation with it (12).

Acrylonitrile is reported to be mutagenic, embryotoxic and teratogenic (12,13), and is very active as a primary irritant on the skin or eye. It is readily absorbed by mouth, through intact skin, or by inhalation. When inhaled, a part of it is hydrolyzed with release of cyanide. This rapid process determines the relative toxicity of acrylonitrile. The remaining portion is reportedly converted to an organic acid (14). Acrylonitrile depletes GSH in the liver and other tissues in a reaction catalyzed by GSH. S-transferases and yielding S-cyano ethyl glutathione which is then metabolized to acrylonitrile mercapturic acids (14-20). Acrylonitrile has also been reported to exert hepatotoxic and testicular effects (8,21). The threshold limit value reported by ACGIH for acrylonitrile has now been reduced from 20 ppm to 2 ppm (14,22). The acute oral LD$_{50}$ in mouse is 40-46 mg/kg (4).

Due to the high toxicity of acrylonitrile, a suitable method for its determination in industrial raw materials, air, waste water, crops, food stuffs and in various physiological systems is needed. Established methods for the determination of acrylonitrile are titrimetric (23), potentiometric (24), polarographic(25,26),
gas chromatographic (27-29), gas liquid chromatographic (30, 31), high performance liquid chromatographic (32), absorptiometric (33), ultra violet spectrophotometric (34), infra red spectrophotometric (35), mass spectrophotometric (36) and visual spectrophotometric (37-42), which have their own merits and demerits.

A survey of the literature reveals only a few visual spectrophotometric methods for its determination. Most of them are either less sensitive, less selective or use toxic reagents. In the present work a new spectrophotometric method is proposed for the determination of acrylonitrile in air. The method is a modification of the method reported for the determination of cyanide from this laboratory (43). Acrylonitrile present in air is absorbed in dilute alkaline potassium permanganate, where it is oxidized to cyanide (40). The cyanide thus formed is converted into cyanogen bromide with bromine, which later reacts with pyridine to form glutaric aldehyde by breaking of the heterocyclic linkage and then couples with sulphanilic acid to give a polymethine dye having absorbance maxima at 460 nm.

Beer's law is obeyed in the range of 0.5 - 5 ppm of acrylonitrile. Various reaction conditions have been optimized. The method has been found to be highly specific, fast and sensitive in comparison to other reported methods and is successfully applied for the determination of
acrylonitrile in air and other complex biological materials, i.e., cystein, blood, urine and human saliva.

**EXPERIMENTAL**

**Apparatus:**

A Carl Zeiss spekol with 1 cm matched silica cells was used for all spectral measurements. Midget impingers of 35 ml capacity and a calibrated rotameter (PIMCO make) were used to check air flow for the absorption of acrylonitrile.

**Reagents:**

Standard acrylonitrile solution: A stock solution of 1 mg/ml of acrylonitrile was prepared in 5% ethanol. A working standard of 20 μg/ml was prepared daily by appropriate dilutions.

Alkaline potassium permanganate solution: A potassium permanganate solution (25 ml of 0.1 N) was mixed with 75 ml of 0.1 N sodium hydroxide solution.

Sodium arsenite solution: A 1.5% (w/v) solution of sodium arsenite in demineralized water was prepared.

Bromine water: A saturated solution of bromine in demineralized water was prepared.

Hydrochloric acid solution: A 0.2 M hydrochloric acid solution was prepared.
Pyridine reagent: This was prepared by mixing 3 ml of concentrated hydrochloric acid and 18 ml of freshly distilled pyridine. The solution was further diluted with 12 ml of demineralized water.

Sulphanilic acid solution: A 1% (w/v) solution in demineralized water was prepared.

Tris-HCl buffer (44): A 0.2 mol/lit., pH 7.6, solution was prepared by dissolving 0.60 g of tris (hydroxymethyl) aminomethane and 2.36 g of tris (hydroxymethyl) aminomethane hydrochloride in demineralized water to make 100 ml solution.

Cystein solution (44): This was prepared by placing 50 mg of L cystein HCl-H_2O in a test tube and adding one drop of bromocresol green indicator solution. Sodium hydroxide 0.5 M was added dropwise until all cystein dissolved and the solution remained blue after solubilization and mixing. 10 ml of tris-HCl buffer was added to the neutralized cystein, which was shaken and stored.

Solution of diverse ions: Solutions of foreign ions were prepared by the reported method of West (45).

All reagents unless mentioned otherwise used were of AnalaR grade.
Procedure:

Collection of sample:

Known amounts of acrylonitrile vapours were generated by dropwise addition of acrylonitrile solution with the help of a microburette into a flask kept on a hot plate using the method of Wilson (46) as modified by Gupta et al (47,48). The vapour was drawn into two impingers containing dilute alkaline potassium permanganate solution as absorber, connected in series to an air sampling train (Fig. 1). After sampling for 5 minutes, purified air was passed through the apparatus for additional 5 minutes to sweep out the acrylonitrile vapours.

Analysis:

An aliquot of collected acrylonitrile solution (containing 5 - 50 μg) was transferred quantitatively into 10 ml volumetric flask. The excess potassium permanganate was decolourized by adding a few drops of sodium arsenite and 0.5 ml of 2 M hydrochloric acid. The solution was allowed to stand until it became colourless. To it 0.3 ml of saturated bromine solution was added and kept for 1 minute for complete bromination. The excess bromine was destroyed by dropwise addition of sodium arsenite solution. Finally 0.3 ml pyridine reagent and 2 ml of sulphanilic acid solution were added. The solution was kept aside for 3-5 minutes for full colour development and then made up to the mark with demineralized water.
FIG. 1. SAMPLING TRAIN FOR THE DETERMINATION OF COLLECTION EFFICIENCY OF ABSORBING SOLUTION.
The absorbance was measured at 460 nm against demineralized water as reference.

RESULTS AND DISCUSSION

Spectral characteristics:

The absorption spectra of the dye is shown in Fig. 2. The spectra shows a maximum absorbance at 460 nm. The reagent blank has negligible absorbance in this range.

The colour reaction:

Four steps are involved in the colour reaction. In the first step acrylonitrile is oxidized to cyanide (I) with alkaline potassium permanganate reagent. In the second step cyanide reacts with bromine to form cyanogen bromide (II), which then reacts with pyridine to yield glutaconic aldehyde (III) by the hydrolytic opening of the pyridine ring in the third step. Finally glutaconic aldehyde couples with sulphanilic acid to form a yellow orange polymethine dye (IV) in the fourth step.

1. \[ \text{alkaline KMnO}_4 \quad \text{CN}^- \]
   \[ \text{(Acrylonitrile)} \quad \text{(I)} \]

2. \[ \text{CN}^- + \text{Br}_2 \quad \text{CNBr}^- \]
   \[ \text{(II)} \]
Effect of varying reaction conditions:

Studies on the vaporization of acrylonitrile and its absorption in dilute alkaline permanganate to optimize the analytical parameters, i.e. the effect of various
2. ABSORPTION SPECTRA OF THE POLYMETHINE DYE.
A. CONCENTRATION OF ACRYLONITRILE = 20 µg/10 ml.
B. CONCENTRATION OF ACRYLONITRILE = 30 µg/10 ml.
C. REAGENT BLANK.
concentrations of absorption/oxidizing solutions, flow rate, sampling time, effect of reagent on colour and temperature, were carried out.

Absorption efficiency:

Various concentrations of alkaline potassium permanganate were used to check the maximum absorption/oxidizing efficiency. It was found that a mixture of 25 ml of 0.1 N potassium permanganate and 75 ml of 0.1 N sodium hydroxide solution was best for the complete absorption as well as oxidation of the absorbed acrylonitrile for air throughputs of 0.1 - 0.5 lit/min, 5 - 50 μg of acrylonitrile in samples, and sampling time of 5 - 15 minutes. The results on the absorption efficiency are tabulated in Table I. It was found that the flow rate of 0.1 - 0.5 lit/min had no effect on the absorption efficiency. Lower flow rate caused incomplete absorption/oxidation of acrylonitrile vapours. Higher concentrations of potassium permanganate caused turbidity in the solution and lower concentrations resulted in incomplete oxidation of acrylonitrile (Fig. 5).

Effect of reagents on colour development:

It was found that at least 0.2 ml of bromine water was required for full colour development. Excess bromine caused no effect as it was removed with sodium arsenite solution. It was found that a minimum of 0.3 ml of freshly distilled pyridine reagent was required for
### TABLE - I

**EFFECT OF TIME ON ABSORPTION EFFICIENCY**

Air through put - 0.2 lit/min.

<table>
<thead>
<tr>
<th>Sampling time (min.)</th>
<th>Amount of acrylonitrile added (µg)</th>
<th>Amount of acrylonitrile found (µg)</th>
<th>% Absorbed</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>19.6</td>
<td>19.6</td>
<td>98.0</td>
</tr>
<tr>
<td>30</td>
<td>29.5</td>
<td>29.5</td>
<td>98.5</td>
</tr>
<tr>
<td>40</td>
<td>39.2</td>
<td>39.2</td>
<td>98.0</td>
</tr>
<tr>
<td>10</td>
<td>20.0</td>
<td>20.0</td>
<td>100.0</td>
</tr>
<tr>
<td>20</td>
<td>20.9</td>
<td>20.9</td>
<td>99.8</td>
</tr>
<tr>
<td>30</td>
<td>40.0</td>
<td>40.0</td>
<td>100.0</td>
</tr>
<tr>
<td>40</td>
<td>39.8</td>
<td>39.8</td>
<td>99.6</td>
</tr>
<tr>
<td>15</td>
<td>20.0</td>
<td>20.0</td>
<td>100.0</td>
</tr>
<tr>
<td>20</td>
<td>19.9</td>
<td>19.9</td>
<td>99.8</td>
</tr>
<tr>
<td>30</td>
<td>30.0</td>
<td>30.0</td>
<td>100.0</td>
</tr>
<tr>
<td>40</td>
<td>39.8</td>
<td>39.8</td>
<td>99.6</td>
</tr>
<tr>
<td>20</td>
<td>18.5</td>
<td>18.5</td>
<td>92.5</td>
</tr>
<tr>
<td>25</td>
<td>27.6</td>
<td>27.6</td>
<td>92.3</td>
</tr>
<tr>
<td>30</td>
<td>36.4</td>
<td>36.4</td>
<td>91.0</td>
</tr>
<tr>
<td>30</td>
<td>18.0</td>
<td>18.0</td>
<td>90.0</td>
</tr>
<tr>
<td>30</td>
<td>26.3</td>
<td>26.3</td>
<td>87.7</td>
</tr>
<tr>
<td>40</td>
<td>34.8</td>
<td>34.8</td>
<td>87.0</td>
</tr>
</tbody>
</table>
complete conversion into glutaconic aldehyde by cyanogen bromide (Fig. 3).

Constant absorbance values were obtained with the addition of 2-5 ml of 1% sulphanilic acid (Fig. 4) and 3-5 minutes were needed for full colour development. Varying temperature between 15-35°C had no effect on the final absorbance (Fig. 6). In this temperature range the coloured dye was stable for ~25 minutes. Above 35°C, there was decrease in the final absorbance value.

**Beer's Law, Molar absorptivity and Sandell's sensitivity:**

The colour system was found to obey Beer's law in the range of 5-50 µg per 10 ml of acrylonitrile solution (Fig. 7). The molar absorptivity and Sandell's sensitivity were found to be 8.8x10⁻³ lit mol⁻¹ cm⁻¹ (+ 100) and 0.006 µg cm⁻², respectively.

**Reproducibility of the method:**

The reproducibility of the method was checked by taking replicate analysis of 20 µg of acrylonitrile per 10 ml (2 ppm) over a period of 7 days. The standard and relative standard deviations were found to be ± 0.0045 and ± 1.36% respectively (Table II).

**Effect of foreign species:**

To assess the validity of the proposed method, the effect of foreign ions normally found with acrylonitrile were studied by adding known amounts of foreign ions to the
FIG. 3. EFFECT OF AMOUNT OF PYRIDINE ON COLOUR DEVELOPMENT.

CONCENTRATION OF ACRYLONITRILE = 20µg/10ml.

FIG. 4. EFFECT OF AMOUNT OF SULPHANILIC ACID ON COLOUR DEVELOPMENT.

CONCENTRATION OF ACRYLONITRILE = 20µg/10ml.
FIG. 5. EFFECT OF OXIDIZING AGENT ON OXIDATION OF ACRYLONITRILE.
CONCENTRATION OF ACRYLONITRILE = 20 µg/10 ml.

FIG. 6. EFFECT OF TEMPERATURE ON FINAL ABSORBANCE.
CONCENTRATION OF ACRYLONITRILE = 20 µg/10 ml.
FIG. 7. CALIBRATION DATA FOR THE DETERMINATION OF ACRYLONITRILE.
standard solution of acrylonitrile. The results in
Table III show that the method is free from common interferents found in air. Cyanides and thiocyanates gave positive interference.

Application of the method:

Determination of acrylonitrile in biological materials, i.e., cystein, blood, urine and saliva:

The method has been applied for the determination of acrylonitrile in cystein, whole blood, urine and human saliva samples. The cystein present in the body reacts with the cyanide formed from acrylonitrile and helps in detoxification. Hence, the determination of acrylonitrile in cystein is important from the biological point of view. Several samples of cystein were tested and found to be free of acrylonitrile. A known amount of acrylonitrile was therefore added to 1 ml of cystein sample. The mixed solution was vapourized and then absorbed as well as oxidized in dilute alkaline potassium permanganate solution, which was then analysed by the proposed method. The results show a 100% recovery of acrylonitrile from cystein.

To determine acrylonitrile in several samples of urine and whole blood, the samples were tested and were found to be free of acrylonitrile. Known amounts of acrylonitrile were added to these samples and were analysed by the above method as well Aldridge's method, reported for cyanides after deproteinisation with trichloric
### TABLE - II

**REPRODUCIBILITY OF THE METHOD**

Concentration of acrylonitrile = 2 ppm

<table>
<thead>
<tr>
<th>No. of days</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.330</td>
</tr>
<tr>
<td>2</td>
<td>0.335</td>
</tr>
<tr>
<td>3</td>
<td>0.325</td>
</tr>
<tr>
<td>4</td>
<td>0.325</td>
</tr>
<tr>
<td>5</td>
<td>0.330</td>
</tr>
<tr>
<td>6</td>
<td>0.335</td>
</tr>
<tr>
<td>7</td>
<td>0.325</td>
</tr>
</tbody>
</table>

Mean = 0.329

Standard deviation = ± 0.0045

Relative standard deviation = ± 1.36%

### TABLE - III

**EFFECT OF FOREIGN SPECIES**

Concentration of acrylonitrile = 2 ppm

<table>
<thead>
<tr>
<th>Foreign ions (tolerance limit in ppm)</th>
<th>Amount of foreign species that cause ± 2% error.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene (2000), Ethanol (1500), Aniline (800),</td>
<td></td>
</tr>
<tr>
<td>Phenol (100), Acetonitrile (100), Formaldehyde (80),</td>
<td></td>
</tr>
<tr>
<td>Al³⁺, Fe³⁺, Fe²⁺, Cd²⁺, Cu⁺ (300), Ca²⁺, Mg²⁺ (600),</td>
<td></td>
</tr>
<tr>
<td>NO₃⁻, SO₄²⁻, PO₄³⁻ (150), SO₄²⁻ (50), NO₂⁻ (80), Ammonia (50),</td>
<td></td>
</tr>
<tr>
<td>CN⁻, CNS⁻ (+ve interference).</td>
<td></td>
</tr>
</tbody>
</table>

* Amount of foreign species that cause ± 2% error.
acetic acid (49). The recoveries are shown in Table IV. An acrylonitrile free sample of human saliva was also mixed with a known amount of acrylonitrile and acrylonitrile was determined by the above process without any pretreatment. The recovery was found to be from 95% to 100%.

Determination of acrylonitrile in air sample:

Acrylonitrile was converted to vapour in a fume cupboard by a gentle heating on a water bath. The air from the fume cupboard was trapped in alkaline potassium permanganate with the help of a suction pump placed outside the cupboard. The air was sampled for 10 minutes and then the acrylonitrile in absorbed solution was analysed by the proposed method as well as reported method (41) (Table V).

The method has been compared with other reported methods for the determination of acrylonitrile and found to be more sensitive in comparison to the available methods (Table VI).

CONCLUSION

The proposed method is fast, sensitive and gives reproducible results for the determination of acrylonitrile in air and complex materials and can be easily applied for industrial hygienic work.
TABLE - IV

APPLICATION OF THE METHOD ON THE DETERMINATION OF ACRYLONITRILE IN BIOLOGICAL SAMPLES

<table>
<thead>
<tr>
<th>Sample*</th>
<th>Set No.</th>
<th>Acrylonitrile added in µg</th>
<th>Acrylonitrile found in µg**</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>1</td>
<td>20</td>
<td>19.4</td>
<td>97.0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>30</td>
<td>29.2</td>
<td>97.3</td>
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<tr>
<td></td>
<td>3</td>
<td>40</td>
<td>38.8</td>
<td>97.0</td>
</tr>
<tr>
<td>Urine</td>
<td>1</td>
<td>20</td>
<td>19.0</td>
<td>95.3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>30</td>
<td>28.5</td>
<td>95.0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>40</td>
<td>38.0</td>
<td>95.0</td>
</tr>
<tr>
<td>Cystein</td>
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<td>20</td>
<td>19.7</td>
<td>98.5</td>
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<td></td>
<td>2</td>
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<td>98.5</td>
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<tr>
<td></td>
<td>3</td>
<td>40</td>
<td>39.1</td>
<td>97.8</td>
</tr>
<tr>
<td>Saliva</td>
<td>1</td>
<td>20</td>
<td>20.0</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>30</td>
<td>30.0</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>40</td>
<td>39.8</td>
<td>99.5</td>
</tr>
</tbody>
</table>

* Amount = 1 ml

** Mean of three replicate analysis.
### TABLE - V
**DETERMINATION OF GENERATED ACRYLONITRILE IN AIR**

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Acrylonitrile found (Present method) µg</th>
<th>Acrylonitrile found (Laynard, Hall &amp; Steven's method) µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.5</td>
<td>8.6</td>
</tr>
<tr>
<td>2</td>
<td>5.6</td>
<td>5.7</td>
</tr>
<tr>
<td>3</td>
<td>11.2</td>
<td>11.7</td>
</tr>
</tbody>
</table>

### TABLE - VI
**COMPARISON OF THE METHOD REPORTED FOR THE DETERMINATION OF ACRYLONITRILE**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Reagent</th>
<th>Range of determination in ppm</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Pyridine - Aniline</td>
<td>10 - 40</td>
<td>(40)</td>
</tr>
<tr>
<td>2.</td>
<td>Pyridine - Lithium hydroxide + Sodium hypochlorite</td>
<td>5 - 30</td>
<td>(41)</td>
</tr>
<tr>
<td>3.</td>
<td>Laurylmercaptan</td>
<td>5 - 50</td>
<td>(38)</td>
</tr>
<tr>
<td>4.</td>
<td>Phloroglucinol</td>
<td>1 - 8</td>
<td>(42)</td>
</tr>
<tr>
<td>5.</td>
<td>Sulphanilic acid (Present method)</td>
<td>0.5 - 5</td>
<td>Present method</td>
</tr>
</tbody>
</table>
REFERENCES


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49. W.N. Aldridge, Analyst, 69, (1944), 262.