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Research Article

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Spectrophotometric determination of acrylonitrile in biological samples

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ABSTRACT

A new specrophotometric method is described for the trace determination of acrylonitrile in wastewater and biological sample. First the traces of acrylonitrile, is oxidized by dilute potassium permanganate into cyanide. The cyanide further reacts with bromine to form cyanogen bromide. The cyanogen bromide reacted with pyridine gives glutaconic aldehyde through the breakage of heterocyclic linkage, which then coupled with 4-amino salicylic acid. In alkaline medium a yellow orange dye formed which is extractable in n- butanol in acid medium. The system obeys Beer's law from 0.015-0.15 ppm .The extract shows maximum absorbance at 520 nm. Molar Absorpitivity and Sandell's Sensitivity were found to be 7.4 x 10^4 l/mol/cm and 0.0007 µg/ cm respectively for extractive system. The Standard Deviation and Relative Standard Deviation were found to be ±0.0073 and + 1.47 % respectively for 10 µg of acrylonitrile in 100 ml. The method has been successfully applied for the determination of acrylonitrile in wastewater, urine, blood, cystein, saliva etc. and compared with other methods.

Keywords: Nitrobenzene, Indophenol dye, Spectrophotometry, Waters, Vegetables and Grains.

INTRODUCTION

Acrylonitrile is a man-made chemical with a sharp, onion-or garlic-like odour. The acrylonitrile is a colorless to pale yellow volatile liquid that is soluble in water and most common organic solvents such as acetone, benzene, carbon tetra chloride, ethyl acetate, and toluene. Acrylonitrile is a reactive chemical that polymerizes spontaneously and can explode when exposed to flame. Polymers are known as insulators. The water soluble polymers have wide application in industries due to its unique properties. Since they are biodegradable, their utilization is restricted [1-8].

Polymers are often used in pharmaceutical work i.e., to control the release rate of active substances from formulations and used as stabilizers in emulsions and suspensions etc., several factors may influence the behaviour of the polymers in the formulation [9].

Because acrylonitrile evaporates quickly, it is most likely to be found in the air around chemical plants where it is made. Acrylonitrile breaks down quickly in the air. It has been found in small amounts in the water and soil near manufacturing plants and hazardous waste sites. The primary stationary sources are synthetics, paints, and furniture and fixtures. Acrylonitrile is present in cigarette smoke [10].

Acrylonitrile is reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity in experimental animals. According to ACGIH TLV and OSHA PEL the maximum limit of acrylonitrile in environment

is 4.3 mg/m^3 [11-13]. As a chemical intermediates, it is used in the synthesis of antioxidants, pharmaceuticals, surface-active agents, dyes, etc. It has been found in shelled walnuts 38 days after fumigation with the mixture [14-20].

Acrylonitrile has been determined by various methods like –Gas chromatography [21-26], Titrimetry [27,28], Polarography [29], IR spectrophotometry [30], Iodometry [31] and Paper chromatography [32]. Different reagents proposed for its spectophotometric determination are sulphanilic acid [33], pyridine [34], lauryl mecaptans [35], aniline [36], phloroglucinol [37] etc.

EXPERIMENTAL SECTION

Apparatus: A systronics spectrophotometer 166 with matched 1-cm cells and systronics digital spectrophotometer model BSM-13 were used for all spectral measurements. All glassware's were calibrated

Reagents: All chemicals used were of analytical reagent grade and solution was prepared in distilled water.

Standard Acrylonitrile Solution: The stock solution of 1mg/ml of acrylonitrile was prepared in 5% ethanol and kept in an amber colour bottle. A working standard solution of 10 µg /ml was prepared fresh daily by appropriate dilution to the stock.

Pyridine Reagent: 3 ml conc. hydrochloric acid (BDH) was mixed with 18ml of freshly distilled pyridine then it was diluted with 12ml of distilled water. The solution was kept in an amber colour bottle.

n-Butanol: Anal R grade n –butanol (BDH) was redistilled before use.

Sodium Arsenite: A 0.5% (weight/volume) solution of sodium arsenite was prepared by dissolving 500 mg of sodium arsenite in 100 ml distilled water.

4- Amino salicylic acid: 1 % (weight/volume) solution of 4-aminosalycylic acid (Loba Chemicals) was prepared by dissolving 1 g of 4-amino salicylic acid in 100 ml distilled water. The reagent is stable ~45 days at room temperature.

Bromine Water: Saturated solution of bromine in distilled water was used.

Hydrochloric Acid Solution: 6 M hydrochloric acid solution was prepared by appropriate dilution of Conc. HCl with distilled water.

Sodium Hydroxide: 3 M sodium hydroxide solution was prepared fresh daily by dissolving 12 gm NaOH in 100 ml distilled water and standardized by the standard method [38].

Potassium permanganate solution: Alkaline potassium permanganate solution (25ml of 0.1N) was mixed with 75ml of 0.1 N NaOH solutions.

Solutions of interfering ions: Solution of interfering ions was prepared according to west [39].

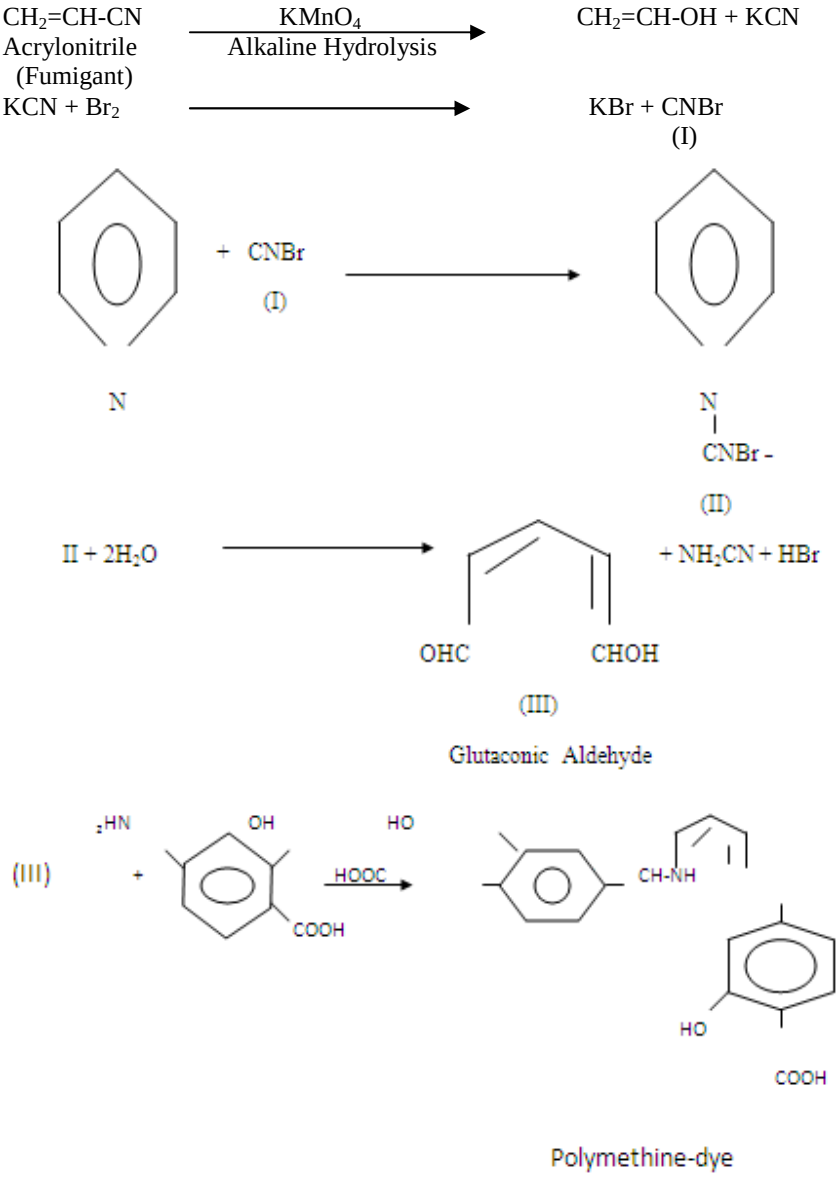
Procedure

An aliquot containing 4.5 to 45 µg (i.e. 0.45 to 4.5) of acrylonitrile was taken in a10 ml volumetric flask. 1 ml of alkaline potassium permanganate solution was added to it and the reaction mixture was kept for 2 minutes. Then the excess of potassium permanganate was decolourised with a few drops of sodium arsenite solution followed by 0.2 ml of 2 N hydrochloric acid. To it 0.5 ml of saturated bromine solution was added. After two minutes the excess of bromine was destroyed by the drop wise addition of sodium arsenite. To it 0.3 ml of pyridine reagent followed by 0.4 ml of 4-amino salicylic acid solution was added. The reaction mixture was kept for 5 minutes. The solution was made alkaline by adding 0.4 ml of 3M sodium hydroxide. The volume was made up to the mark with distilled water (Final pH~8.0). The absorbance of the yellow dye was measured at 400 nm against distilled water **[Fig-1]**.

4) Solvent Extraction

An aliquot of water sample (~ 100 ml) containing (1.5 to 15 µg) of acrylonitrile was taken in a 250 ml separating funnel. The yellow ploymethine dye added (formed as reported for aqueous procedure). Then 4 ml of 3M sodium hydroxide was added for complete color reaction. The final PH~8 was adjusted with 10 ml of 6M hydrochloric acid prior to extraction. The dye was then extracted with two 5 ml portions of n-butanol and dried over anhydrous sodium sulphate. The absorbance of red-purple dye was measured at 520 nm against similarly treated reagent blank. The calibration graph was prepared by treating standards in a similar fashion.

5) Colour reactions of acrylonitrile



2) Effect of temperature

Effect of temperature on the colour reaction was studied. The study reveals that the absorbance values remained constant in the temperature range between 15-40 ^{0}c. Below and above this temperature range the absorbance values decreased [Fig-2].

RESULTS AND DISCUSSION

Spectral Characteristics

The red-purple dye shows maximum absorption at 520 nm. The absorbance spectra of the dye are shown in **Fig. 1**. The reagent blank is colourless and shows negligible absorption at this range.

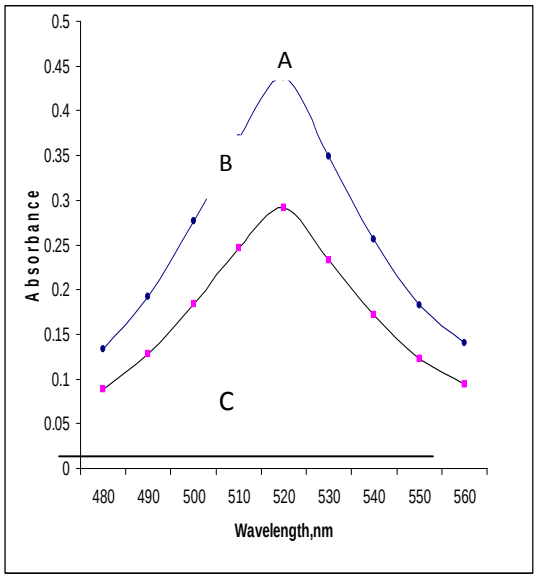


Fig. 1 Absorption Spectra of the Dye and Reagent Blank

A. Concentration of Acrylonitrile = 45 µg/10ml.
B. Concentration of Acrylonitrile = 23 µg/10ml.
C. Reagent Blank

Effect of Varying Reaction Conditions

1) Effect of temperature

Effect of temperature on the colour reaction was studied. The study reveals that the absorbance values remained constant in the temperature range between 15-40 ^{0}c. Below and above this temperature range the absorbance values decreased **[Fig-2].**

2) Effect of time

The time required for complete coupling reaction and color development was also studied. It was found that a minimum 5 minutes were required for full color development and up to 7 minutes constant absorbance values were obtained **[Fig-3]**. The dye formed was stable for 45 minutes.

3) Effect of Oxidizing Reagent

Effect of oxidizing reagent for complete oxidation of acrylonitrile into cyanide was studied. It was found that maximum absorption for colour reaction was obtained when 0.1 N potassium permanganate and 0.1 N sodium hydroxide were mixed in the ratio of 1:3. A minimum of 1 ml of oxidizing solution was found to be sufficient for complete oxidation of acrylonitrile. Excess of potassium permanganate caused no effect on the absorbance as the excess was destroyed by sodium arsenite solution followed by hydrochloric acid.

4) Effect of Bromine Solution

Effect of bromine solution was studied by adding 0.2 to 0.8 ml of saturated bromine solution. It was found that a minimum of 0.5 ml of bromine was required for complete bromination. Up to 0.8 ml constant absorbance value was observed. Excess of bromine caused no change in the absorbance values since the excess of bromine was destroyed by sodium arsenite solution.

5) Effect of Pyridine

It was found that a minimum of 0.2 ml pyridine was needed for the complete colour reaction. Up to 1 ml pyridine caused no change in the absorbance values **[Fig- 4]**

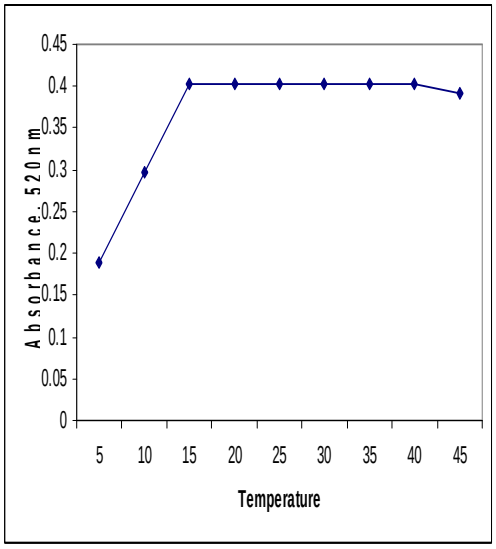


Fig. 2 Effect of Temperature on the Colour Reaction
Concentration of Acrylonitrile = 20 µg /10ml

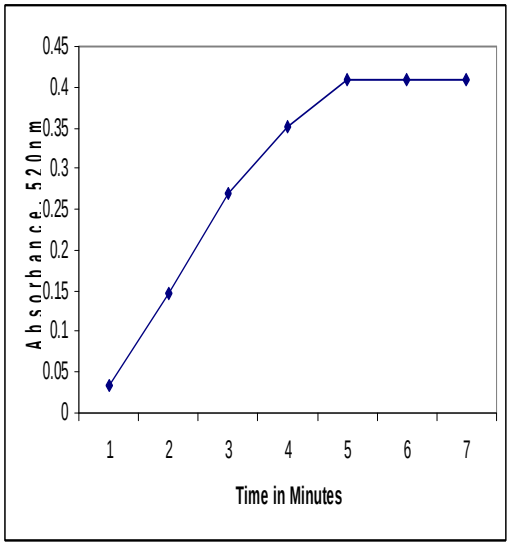


Fig. 3 Effect of Time on the Colour Reaction
Concentration of Acrylonitrile = 20 µg /10ml

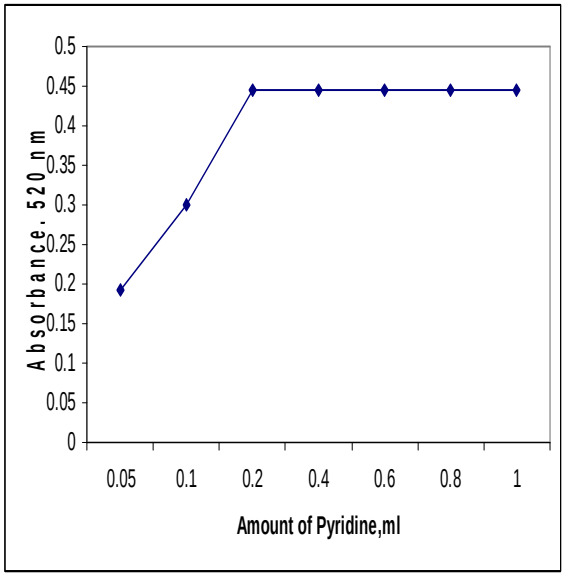


Fig. 4 Effect of Amount of Pyridine on the Colour Reaction
Concentration of Acrylonitrile = 20 µg /10ml

6) Effect of Sodium Arsenite

It was found that a minimum of 0.2 ml pyridine was needed for the complete colour reaction. Up to 1 ml pyridine caused no change in the absorbance values **[Fig- 5]**

7) Effect of 4-amino Salicylic Acid

A minimum 2 ml of 4-amino salicylic acid was sufficient for complete color reaction but addition up to 4 ml of it had no effect on the absorbance values **[Fig-6].**

Beer's law, Molar absorptivity, Sandell's sensitivity and Reproducibility

The colour system was found to obey Beer's law in the range of 0.015 to 0.15 µg/ml. Molar absorpitivity and Sandell's sensitivity were found to be 7.4 x 10^4 l/mol/cm, 0.0007 µg/ cm respectively for extractive system.

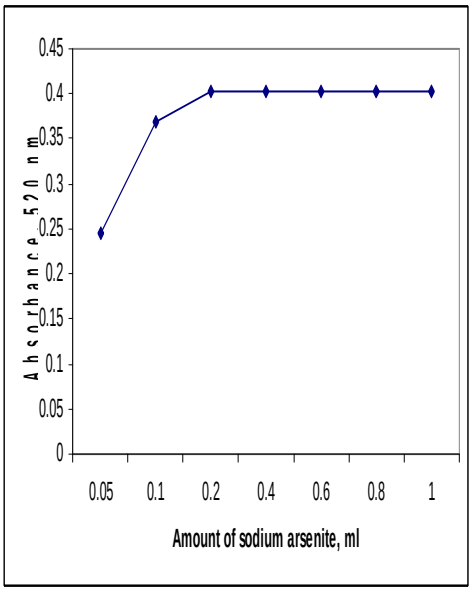


Fig. 5 Effect of Amount of Sodium Arsenite on the Colour

Reaction Concentration of Acrylonitrile = 20 µg /10ml

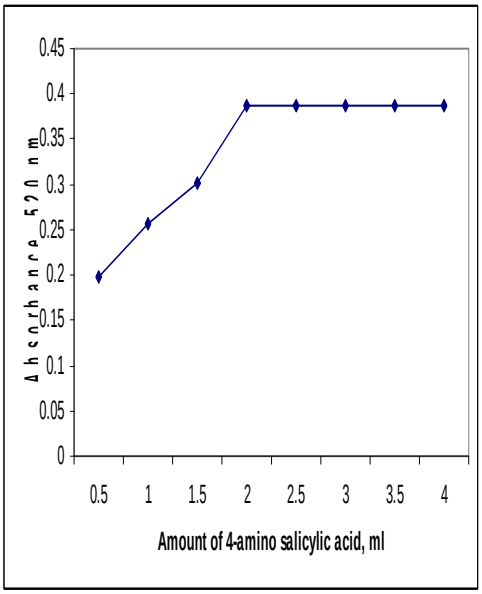


Fig. 6 Effect of Amount of 4-Amino Salicylic Acid on the Colour

Reaction Concentration of Acrylonitrile = 20 µg /10ml

The reproducibility of the colour reaction was checked by 7 replicate analysis over a period of 7 days [**Table I**]. The standard deviation and relative standard deviation was found to be ±0.0073 and + 1.47 % for 10 µg of acrylonitrile in 100 ml.

Table-I: Reproducibility of the Method

Concentration of Acrylonitrile = 45 µg/ 10ml

S.No.	No. of Days	Absorbance at 520 nm*
1	1	0.505
2	2	0.495
3	3	0.510
4	4	0.501
5	5	0.490
6	6	0.505
7	7	0.501

Mean = 0.501Standard Deviation = ±0.0073, Relative Standard Deviation = 1.47%,
** Mean of three repetitive analyses*

Effect of Foreign Species- The effect of foreign species commonly found with acrylonitrile was studied. The known amounts of foreign species were added to 3 µg of acrylonitrile in 10 ml of the final solution prior to analysis and then analyzed by the proposed method. The method is found to be free from the interference of most of the organic compounds viz. phenol, aniline, benzene, benzaldehyde, nitrobenzene etc. Masking of metal ions with EDTA and sodium potassium tartrate solution increases the tolerance limit to considerable extent. Cyanides and thiocyanates show positive interference with the determination of acrylonitrile. The tolerance limits shown in **Table II** are the concentration of interfering species that cause ± 2% error. Oxidizing and reducing agent if present were removed by sodium arsenite and bromine water.

Table-II: Effect of Interfering Species
(20 μg of Acrylonitrile in 10 ml Aqueous Solution)

S. No.	Interfering Species	(Tolerance Limit in μg)*
1	Benzene	2000
2	Benzaldehyde	800
3	Phenol, F^-	1000
4	Nitrobenzene, Aniline	200
5	Zn^{2+}**, Cd^{2+}, Pb^{2+}	10,000
6	SO_4^{2-}, PO_4^{3-}, Sulphide	10,000
7	Ca^{2+}, Ba^{2+}	5000
8	Fe^{3+}***, Al^{3+} *, Cr^{3+}**	500
9	Se^{4+} Mg^{2+}**, Mn^{2+}**	500
10	Cu^{2+}*	300

**Tolerance limit causes ±2% error.*
***Masked with 1ml of 10% sodium potassium tartrate solution.*
**** Masked with 1ml of 10% EDTA solution.*

Solvent Extraction- The limit of detection/determination could be considerably improved by employing solvent extraction from 0.4 to 4.5 μg/ml of acrylonitrile in aqueous system to 0.015 to 0.15 ppm of acrylonitrile in extractive system of the various solvents tested, n-butanol was found to be the best. The molar absorpitivity was found to be lower when higher alcohols such as hexanol, iso-amyl alcohol, methyl propyl alcohol and octanol were used. Extraction was not possible with benzene, chloroform and carbon tetrachloride.

7) APPLICATION

The proposed method has been successfully applied for the determination of acrylonitrile in water, blood, urine, saliva and cystein.

(A) In Water: Since water sample was found to be free from acrylonitrile, synthetic samples were prepared by adding known amounts of acrylonitrile to each sample prior to analysis. The recovery ranges from 96-99% [**Table III**].

(B) In Biological Samples: Since biological samples were found to be free from acrylonitrile, synthetic samples were prepared by adding known amounts of acrylonitrile to each sample prior to analysis after deproteination with trichloroacetic acid as recommended. The recovery ranges from 94-99% [**Table III].**

Table-III Determination of Acrylonitrile in Wastewater and Biological Samples

Sample[a]	Set No.	Acrylonitrile, μg added	Acrylonitrile, μg found	Recovery %
Water	1	20	19.6	98.00
	2	30	29.5	98.83
	3	40	38.7	96.75
Blood	1	20	19.2	96.00
	2	30	29.0	96.70
	3	40	38.4	96.00
Urine	1	20	19.3	96.50
	2	30	29.0	96.70
	3	40	37.9	94.75
Cystein	1	20	19.5	97.50
	2	30	29.7	99.00
	3	40	39.3	98.25
Saliva	1	20	19.8	99.00
	2	30	29.5	98.30
	3	40	39.2	98.00

Note- Mean of three replicate analyses.
[a] Amount = 1 ml.

CONCLUSION

For acrylonitrile determination the proposed method is rapid, more sensitive as compared to other spectrophotometeric methods [**Table IV**]. Some advantages of this method are rapid color development, stability, easy availability of the reagent, reproducibility and freedom from a large group of interfering species. The extraction

method is advantageous because it lowers the detection limit by the concentration effect. The method has been successfully applied for the analysis of acrylonitrile in water and biological samples and can be applied for industrial hygienic work.

Table-IV Comparison of methods reported for the determination of acrylonitrile

Set. No.	Reagents (Ref)	Range of Determination (ppm)	Remarks
1.	Pyridine [34]	5-30	dye less stable
2.	Aniline-pyridine [36]	10-40	low sensitivity
3.	Laurylmercaptan [35]	5-50	reagent unstable
4.	Phloroglucinol [37]	1-7	less sensitive & selective
5.	Sulfanilic acid [33]	0.5-5.0	Interference of amines and NO_2
6.	4-amino salicylic acid	0.12-0.80	proposed method

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