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Trace level extraction and spectrophotometric determination of cyanide in waste water and biological fluid

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An extractive spectrophotometric method for the determination of cyanide in wastewater is developed. Cyanide is reacted with bromine to form cyanogen bromide, which subsequently reacts with pyridine. Glutaconic aldehyde is formed through the heterolytic cleavage of pyridine ring, which is then coupled with 4-amino salicylic acid. A yellow orange dye formed in alkaline medium is extractable in *n*-butanol in acidic medium. The extract shows absorbance maxima at 520 nm. The system obeys Beer's law in the range of 0.03-0.20 μg/mL. Important analytical parameters such as time, temperature, reagent concentration, acidity etc. have been optimized for complete colour reaction. Sandell's sensitivity and molar absorptivity for the system have been calculated. The method has been successfully applied for determination of cyanide in wastewater and biological fluid.

Keywords: Spectrophotometry, Cyanide, Pyridine, 4-Amino salicylic acid, *n*-Butanol, Sodium arsenite

Cyanides are extremely toxic and occur primarily in industrial effluents, metal cleaning and electroplating bath. Gas scrubbers, gas-work, coke oven and other chemical treatments are the main sources of cyanide found in industrial waste[1,2]. The maximum recommended amount of cyanide in water by WHO as CN^- is 0.01 mg/L for human beings and 0.03 mg/L for fishes. Cyanides act directly on the nervous system and readily absorbed through intact skin and by inhalation[3,4]. Repeated exposure to small concentrations of cyanides over a long period causes paralysis of the legs and arms, loss of appetite, muscle cramps, psychoses, nausea and weakness[2].

For the determination of cyanide, spectrophotometric methods[5-7] are more superior to the other methods such as polarographic[8], chromatographic[9], titrimetric[10] and fluorometric[11] techniques. Only few methods are sensitive and reliable. These methods have their own merits and demerits[6,12-14]. In the present communication an extractive method is modified for the determination of cyanide in wastewater[15] by its reaction with bromine solution and then subsequent heterocyclic cleavage of pyridine ring followed by coupling with 4-amino salicylic acid. The yellow-orange polymethine dye formed in alkaline medium (7.2-8.5) is extractable in acidic medium. The reddish-violet *n*-butanol extract shows absorbance maxima at 520 nm. Molar absorptivity and Sandell's sensitivity were found to be 1200 (±100) L/mol/cm and 0.0002 μg/cm respectively. Important analytical parameters have been studied and optimized for complete colour reaction. The method has been successfully applied for the determination of cyanide in biological samples also.

The solvent extraction is employed here to increase the sensitivity several times. The use of non-toxic and easily available coupling reagent (4-amino salicylic acid) also makes the method suitable for industrial hygienic work.

Experimental Procedure

Apparatus

A carl-zeiss spekol with 1 cm matched silica cells was used. All chemicals used were of analytical reagent grade.

Reagents

A stock solution (1 mg/mL) of cyanide was prepared by dissolving 250 mg of potassium cyanide (BDH) in 100 mL of distilled water. A working standard of 10 μg/mL was prepared fresh daily by the appropriate dilution of the stock. The pyridine solution was prepared by mixing 3 mL concentrated hydrochloric acid (BDH) with 18 mL of freshly distilled pyridine then it was diluted with 12 mL distilled water. A 0.5% (*w*/v) solution of sodium arsenite was prepared by dissolving the salt in distilled water. A 1% (*w*/v) solution of 4-aminosalycylic acid (Loba Chemicals) was prepared. The reagent is stable ~45 days at room temperature. AnalaR grade *n*-butanol (BDH) was redistilled before use. Saturated solution of bromine in distilled water was used. Solutions of sodium

hydroxide (3 M) and hydrochloric acid (6 M) (Loba chemicals) were used. Solutions of interfering ions were prepared according to West[16].

Method

An aliquot of water sample (containing 4-40 μg of cyanide) was taken in a 10 mL volumetric flask. To it 0.3 mL of saturated bromine solution was added. After two minutes the excess of bromine was destroyed by the dropwise addition of sodium arsenite. Then 0.4 mL of 4-amino salicylic acid solution was added. The reaction mixture was kept for 5 min. The solution was made alkaline by adding 0.4 mL of 3 M sodium hydroxide. The volume was made upto the mark with distilled water (Final *p*H ~8). The absorbance of the yellow dye was measured at 400 nm against distilled water.

Solvent extraction

An aliquot of water sample (~100 mL) containing (3 to 20 μg) of cyanide was taken in a 250 mL separatory funnel. The yellow ploymethine dye was formed as reported for aqueous procedure. Then 4 mL of 3 M sodium hydroxide was added for complete colour reaction. The final *p*H~8 was adjusted with 10 mL of 6 M 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.

Results and Discussion

The red-purple dye shows maximum absorption at 520 nm (Fig. 1). The dye is found to be stable for 45 min in the temperature range of 15-45°C. The time required for complete coupling reaction and colour development was also studied. Minimum 5-7 min (Table 1) were needed for full colour development in the *p*H range between 7.2-8.5.

A minimum of 0.2 mL of bromine solution was found to be sufficient for complete bromination of cyanide. Also 0.2 to 1 mL of sodium arsenite and pyridine reagent caused no change in the absorbance values. A minimum 2 mL of 4-amino salicylic acid was sufficient for complete colour reaction but its addition upto 4 mL had no effect on the absorbance values.

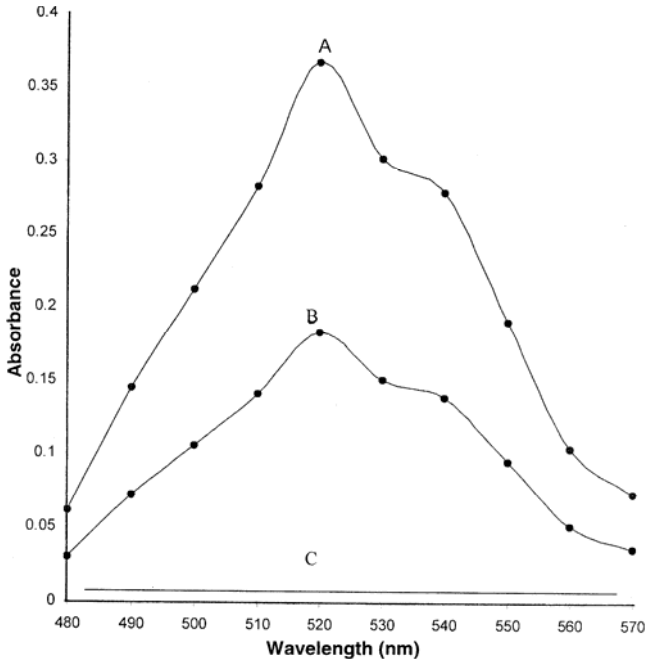

Fig. 1—Absorption spectra of the dye and reagent blank
A-Concentration of cyanide = 40 μg/25 mL
B-Concentration of cyanide = 20 μg/25 mL
C-Reagent blank

Table 1—Effect of time, amount of pyridine and 4-amino salicylic acid on the final absorbance
Concentration of cyanide = 40 μg /25 mL

S.No.	Time (min)	Absorbance (520 nm)	Pyridine (mL)	Absorbance (520 nm)	4-Amino salicylic acid (mL)	Absorbance (520 nm)
1	3	0.178	0.05	0.212	1.0	0.154
2	5	0.369	0.12	0.281	1.5	0.241
3	7	0.369	0.2	0.372	2.0	0.324
4	10	0.369	0.4	0.372	2.5	0.324
5	15	0.369	0.6	0.372	3.0	0.324
6	20	0.369	0.8	0.372	3.5	0.324
7	25	0.369	1.0	0.372	4.0	0.324
8	30	0.369	1.1	0.333		
9	35	0.369	1.2	0.301		
10	40	0.369	1.4	0.235		
11	45	0.369				

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

The proposed method is reproducible and obeys Beer's law in the range of 0.03 to 0.2 μg/mL, molar absorpitivity and Sandells sensitivity were found to be 1200 (± 100) L/mol/cm, 0.0002 μg/cm respectively for the extractive system. The standard deviation and relative standard deviation were found to be ±0.012 and ± 2.64% for 10 μg of cyanide in 100 mL.

Effect of interferring species

To asses the validity of the method, effect of co-existing species was studied with 10 μg of cyanide per 10 mL. The method was found to be free from most of the interferents. Thiocyanate shows positive interference since it also forms cyanogen bromide when treated with saturated bromine water. The tolerance limits shown in Table 2 are the concentration of interferring species that cause ± 2% error. Oxidizing and reducing agent if present were removed by sodium arsenite and bromine water.

Table 2—Effect of interfering species on determination of 1 μg/mL of cyanide.
(10 μg of cyanide in 10 mL aqueous solution)

Interfering species (Tolerance limit in μg)*
Benzene (2000), Benzaldehyde (800), Phenol, F^- (1000), Nitrobenzene, Aniline (200),
Zn^{2+}**, Cd^{2+}, Pb^{2+} (10,000), SO_4^{2-}, PO_4^{3-}, Sulphide(10,000), Ca^{2+}, Ba^{2+} (5,000),
Fe^{3+}***, Al^{3+}**, Cr^{3+}** (500), Se^{4+} (500), Se^{4+}, Mg^{2+}**, Mn^{2+}**(500), Cu^{2+}* (300).

*Tolerance limit causes ±2% error.

**Masked with 1 mL of 10% sodium potassium tartrate solution.

***Masked with 1 mL of 10% EDTA solution.

Solvent extraction

The limit of detection/determination could be considerably improved by employing solvent extraction (for detection from 0.025 μg to 0.0025 μg and for determination from 0.4 to 4 mg/mL of cyanide in aqueous system and 0.03 to 0.2 ppm of cyanide 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.

A comparison of the sensitivity and determination range of the present method with the previously developed literature methods shows that the present method is comparable with most sensitive ones and has minimum interference (Table 3).

Applications

The method has been successfully applied to the determination of cyanide in wastewater, cystein and blood serum.

In wastewater

The samples were collected from the effluents of tube manufacturing factory where cyanides are used for galvanizing. 100 to 250 mg/mL of cyanides were found to be present in the samples of effluents before these were sent for final treatment. The results obtained by the proposed method are in agreement with those obtained by the Aldridges method and method using anthranilic acid[17] (Table 4).

Blood serum

Several samples of blood serum obtained from Bioscience Department of Ravishankar University

Table 3—Comparison of various spectrophotometric methods for cyanide

S.No.	Reagents	λmax (nm)	Range of determination	Remarks/References
1	Benzidine	520	0.1-20 mg/mL	Carcinogenic[12]
2	Sulphanilic acid	460	0.08-0.75 mg/mL	Stability is 25 min[18]
3	Chloroanilic acid	540	0.5-25 mg/mL	Less sensitive[19]
4	Benzidine	508	0.2-20 mg/mL	Carcinogenic[20]
5	Phloroglucinol	540	0.4-3.2 mg/mL	Simple, sensitive and rapid[21]
6	Anthranilic acid	400	----------	Simple and sensitive[17]
7	Picric acid	505	0.2-20 μg/g	Simple, sensitive and specific method[22]
8	Ninhydrine (NH)	590	0.04-0.24 μg/cm^3	Less sensitive, unstable reagent and interference of amino acids[23]
9	Pyridine	520	0.03-0.20 μg/mL	Rapid, stable reagent, more sensitive and free from most of the interference except thiocyanate (proposed method)

Table 4—Analysis of wastewater
Amount of sample = 1 mL diluted into 25 mL.

S.No.	Amount of diluted sample taken	Cyanide found μg* Proposed method	Aldridges method	Anthranilic acid method
1	15	12.00	11.96	12.20
2	25	24.50	24.20	24.48
3	40	36.50	36.20	37.00
4	50	48.50	48.20	48.75

*Mean of four repetitive analyses

Table 5—Recovery of cyanide from biological samples
Volume of blood serum and cystein = 1 mL.

Samples	Set No.	Cyanide added (μg)	Cyanide found by Proposed method	Recovery %
Blood serum	1	10	9.82	98.2
	2	20	19.60	98.0
	3	40	39.14	97.85
Cystein	1	10	9.48	94.8
	2	20	19.12	95.6
	3	40	38.00	95.0
			by Aldridges method	Recovery %
Blood serum	1	10	9.79	97.9
	2	20	19.57	97.85
	3	40	39.02	97.55
Cystein	1	10	9.50	95.0
	2	20	19.00	95.0
	3	40	37.92	94.8
			by Antharalic acid method	Recovery %
Blood serum	1	10	9.80	98.0
	2	20	19.57	97.85
	3	40	39.20	98.0
Cystein	1	10	9.50	95.0
	2	20	19.00	95.0
	3	40	37.84	94.6

were found to be free of cyanide. Hence synthetic samples were prepared by adding known amounts of cyanide to these samples and then analysed by the proposed method, Aldridges[12] and anthranilic acid method[17] after deproteination with trichloroacetic acid. The results show that the recoveries from the blood serum samples were ~98% (Table 5).

Cystein

The cystein present in body reacts with cyanide and helps in its detoxification, hence the determination of cyanide in cystein is important from biological point of view[1]. Known amounts of cyanides were added to 2 mL of cystein samples. After deproteination with trichloroacetic acid the mixed solution was treated with 5 M sulphuric acid. The generated hydrogen cyanide was absorbed in dilute sodium hydroxide. The results show ~95% recovery of cyanide from cystein analysed by proposed method (Table 5).

Conclusion

The proposed method is more sensitive as compared to other spectrophotometric methods for cyanide determination. The rapid colour development, reproducibility, stability and easy availability of the reagent and freedom from a large group of interfering species are some advantages of the method. The extraction method is advantageous because it lowers the detection limit by the concentration effect.

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