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Determination of Cyanide in Blood Using Differential Pulse Voltammetry

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Abstract

Cyanide is present in two major forms such as sodium cyanide (NaCN) and potassium cyanide (KCN) and is highly toxic. Cyanide is the inhibitor of cytochrome C oxidase. Most hazardous compound is hydrocyanic acid that can be inhaled as gas at ambient room temperature. Oral ingestion of cyanide in liquid and solid form i.e. 200 mg or inhalation of 270 ppm in air causes death within minutes. Cyanide in body fluid can be determined using spectrophotometric techniques which are very time consuming and requires lot of sample pre-treatment. An attempt has been made to develop the new method for determination cyanide in blood using Multi Mode electrode in

differential pulse voltammetry. Blood was processed using microwave assisted closed vessel digestion using 35% nitric acid and ultrapure water. The buffer of pH 10.2 was used with a sweep rate of 0.01V/s and pulse amplitude 0.05V by HMDE by standard addition method. The solution was stirred during pre-electrolysis at 0.00 V for purge time of 300 seconds from 0.00V to -0.500V.

Keywords: Cyanide, Voltammetry, Dropping Mercury Electrode, Trace Metal Analyser, differential pulse etc.

Introduction

Cyanide is a chemical compound having triple bonded carbon atom with nitrogen atom, a monovalent combining group CN[1]. Cyanide is the inhibitor of cytochrome C oxidase. Most hazardous compound is hydrocyanic acid that can be inhaled as gas at ambient room temperature. Oral ingestion of cyanide in liquid and solid form i.e. 200mg or inhalation of 270 ppm in air causes death within minutes[2,3]. Cyanide is used in mining of gold and silver as it helps to dissolve the metals. It is also used in certain kinds of photography, as a insecticide for fumigation in ships, killing of ants, rat poison and a food additive[4,5,6,7]. Its medical use include its use as sodium nitroprusside to measure ketone bodies as a follow up to diabetic patients. It is used to produce a rapid decrease in blood pressure in humans, as a vasodilator in vascular research. Cobalt in artificial vitamin B12 contains a cyanide ligand as an artefact in purification process. Copper cyanide earlier was also used for treatment of tuberculosis and leprosy during world war II[8].

Symptoms of cyanide poisoning include general weakness, malaise & collapse, headache, vertigo, dizziness, giddiness, inebriation, confusion generalised seizures, coma, abdominal pain, nausea, vomiting, shortness of breath associated with chest pain and apnea[9]. It can be used as suicidal, homicidal and terrorist activities. Some environmental exposure and accidental consumption was also reported. Antidote for cyanide is hydroxycobalamin which reacts with it to form cyanocobalamin which can be safely eliminated by kidneys. Earlier antidote used was amyl nitrite, sodium nitrite and sodium thiosulphate[10,11]. Cyanide is rapidly distributed in the body through blood. Since it forms volatile hydrocyanic acid gas, so its preservation and storage needs more precautions. Cyanide in body fluid can be determined using spectrophotometric techniques which are

very time consuming and requires lot of sample pre-treatment[9]. An attempt has been made to determine cyanide in blood using dropping mercury electrode in voltammetry. The major advantage of the techniques is that it is quicker, sensitive and simpler method with less interferences.

Materials and methods

Apparatus and accessories

1. Trace Metal analyser model 797 VA Computrace from Metrohm AG Ltd, switerzerland(Fig 1) was used, which contains following electrodes:
   Working electrode - Multimode Mercury Electrode (MME)
   Auxillaryelectrode - Platinum Electrode (PE)
   Reference Electrode - Ag/AgCl electrode filled with 3M KCl solution
2. Nitrogen gas of purity 99.99% from laser gas, India was used.
3. Micropipette of Eppendorf make of volume 10-100 ml and 100-1000 ml was used.
4. Microwave digestion system of Aurora instruments Ltd., Canada was used.

Chemicals and Reagents

Suprapure potassium hydroxide, boric acid and potassium cyanide from Merck, Germany,
Ultrapure water from Rions, India were used.

Glasswares

Volumetric flask of 100 ml capacity from borosil India was used. Glasswares used were thoroughly washed and rinsed 2-3 times with ultrapure water and dried in digital oven.

Spiking of Sample

Blood sample was spiked with 10 ppm of standard cyanide solution

Preparation of standard

1000 ppm standard solution of cyanide was prepared using potassium cyanide (KCN) of known purity. Different concentrations were prepared by dilution method.

Preparation of buffer solution

1.12g of KOH was dissolved in 50 ml of ultrapure water and 1.14g of boric acid was added to it. The pH was adjusted to 10.2 and the volume was made upto 100 ml.

Sample Preparation using microwave digester

Vessel of microwave digester was cleaned up by Nitric acid (HNO_3) and water (H_2O) mixture (1:1) and dried. 1 ml blood sample was transferred into the linear microwave vessels, 15 ml of 35 % HNO_3 was added into the vessel and the mixture was left for few minutes for auto gas. In the reference vessel, 1 ml of water was added along with 15 ml of 35% HNO_3 for sample blank. Vessel carrousel was loaded in the microwave digestion oven and the digestion machine was run according to program given in Table 1.

Table 1: Programming conditions for the microwave digester

Step	Time (s)	Starting Temp (^oC)	Ending Temp (^oC)
1	210	28	100
2	600	100	160
3	600	160	170

After completion of run, microwave digestion was kept for cooling. After cooling, the vessels were opened and digestion material was completely transferred in 50 ml volumetric flask and final volume was made up to 50 ml with ultrapure water.

Fig. 1: Microwave digestion system

Anodic Stripping Voltammetric measurements

10 ml ultrapure water and 1ml of buffer solution (pH 10.2) was taken in polarographic vessel and then the measurement was started under the given parameters table 2. After this voltamogramme of the blank was recorded. 0.5 ml of prepared sample solution was added to polarographic vessel and then voltamogramme of the sample solution was recorded under the same conditions. After the sample voltamogramme was recorded, 0.5 ml of 5 ppm standard of cyanide was added twice and then voltamogramme of the standard was recorded. Finally the concentration of the metal was calculated by linear regression method (standard addition) using following formula

$$\text{Final Result} = \text{Concentration} \times \frac{\text{Cell Volume}}{\text{Sample amount}} \times \frac{\text{Multiplier}}{\text{Divisor}}$$

Where, Multiplier = Dilution

Divisor = Sample amount taken for preparation

Fig. 2: Trace Metal Analyser

Table 2 : Operating parameters of Trace Metal Analyzer for the determination of cyanide using Differential Pulse Voltammetry

Method parameters	
Method	Determination of Cyanide
Title	Determination of Cyanide
Blank solution in polarographic vessel	10 ml + 1 ml Buffer
Calibration Technique	Standard Addition
Addition	Manual
Sample amount(mL)	0.500
Cell Volume (mL)	11.500
Voltammetric parameters	
Working electrode	Hanging Mercury Dropping Electrode
Calibration	Standard addition method
Number of replications	2
Drop size	4
Stirrer speed	1200 rpm
Mode	DP-Differential pulse
Purge time	300 s
Equilibration time	5 s
Pulse amplitude	0.05 V
Start potential	0 V
End potential	-5 V
Voltage step	0.08 V
Voltage step time	0.8 s
Sweep rate	0.01 V/s
Peak potential CN^-	-0.24 V
Peak evaluation parameters	
Regression technique	Linear Regression
Peak evaluation	Height
Minimum peak width (V. Steps)	5
Minimum peak height (A)	1.000e-010
Reverse Peaks	No
Smooth Factor	4
Eliminate Substances	Yes

Results and discussion

Voltammetry/Polarography are names of analytical methods based on current potential measurements in electrochemical cells. Voltammetry includes all method in which the current potential measurements are made at stationary and fixed working electrodes. It consists of a three electrode system containing one working electrode i.e. dropping mercury electrode, reference electrode i.e. Ag/AgCl filled with 3M KCl solution and auxiliary electrode i.e. Pt electrode. In the first step the metals or ions are deposited on the electrode and in the second step metal or ions are stripped out of the electrode. The metals or ions stripped out are directly proportional to the current, greater the current greater will be the concentration of metals and lesser the current lesser will be the concentration of metals. All measurements were done by standard addition technique in which voltamogramme of blank was measured followed by sample and known standard. Result of blank was automatically subtracted and the extrapolation curve obtained shows the value of cyanide present in it.

The concentration of 4 ppm soln of cyanide was 4.28 ppm respectively. 10 ppm spiked solution in blood after digestion was run as sample solution. The concentration of 10 ppm solution was 10.52 ppm. The advantage of this method over the earlier used methods is that it is quicker, more sensitive, simpler, cost effective and is not effected by the other interfering agents present in it.

Conclusion

The research paper describes the determination of cyanide using MME (Multimode electrode) on voltammetry. The Trace Metal Analyzer is advantageous in terms of the range of concentration to which it can measure. It becomes a useful technique as it can be used for the analysis of cyanide in biological samples such as blood, liver, vitreous humor, tissue etc.

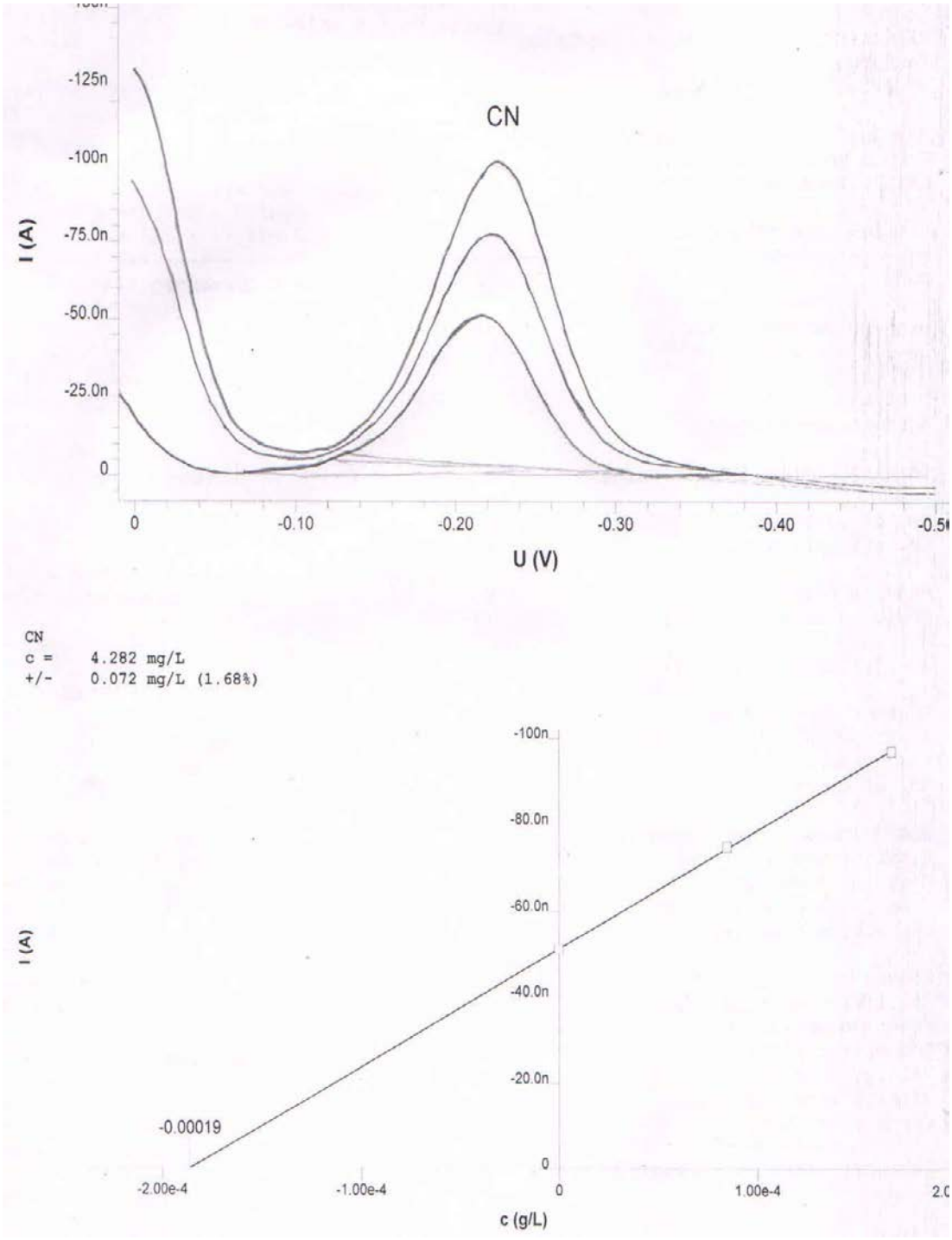

Fig. 3. Voltamogramme and Extrapolation curve of 4 ppm cyanide solution

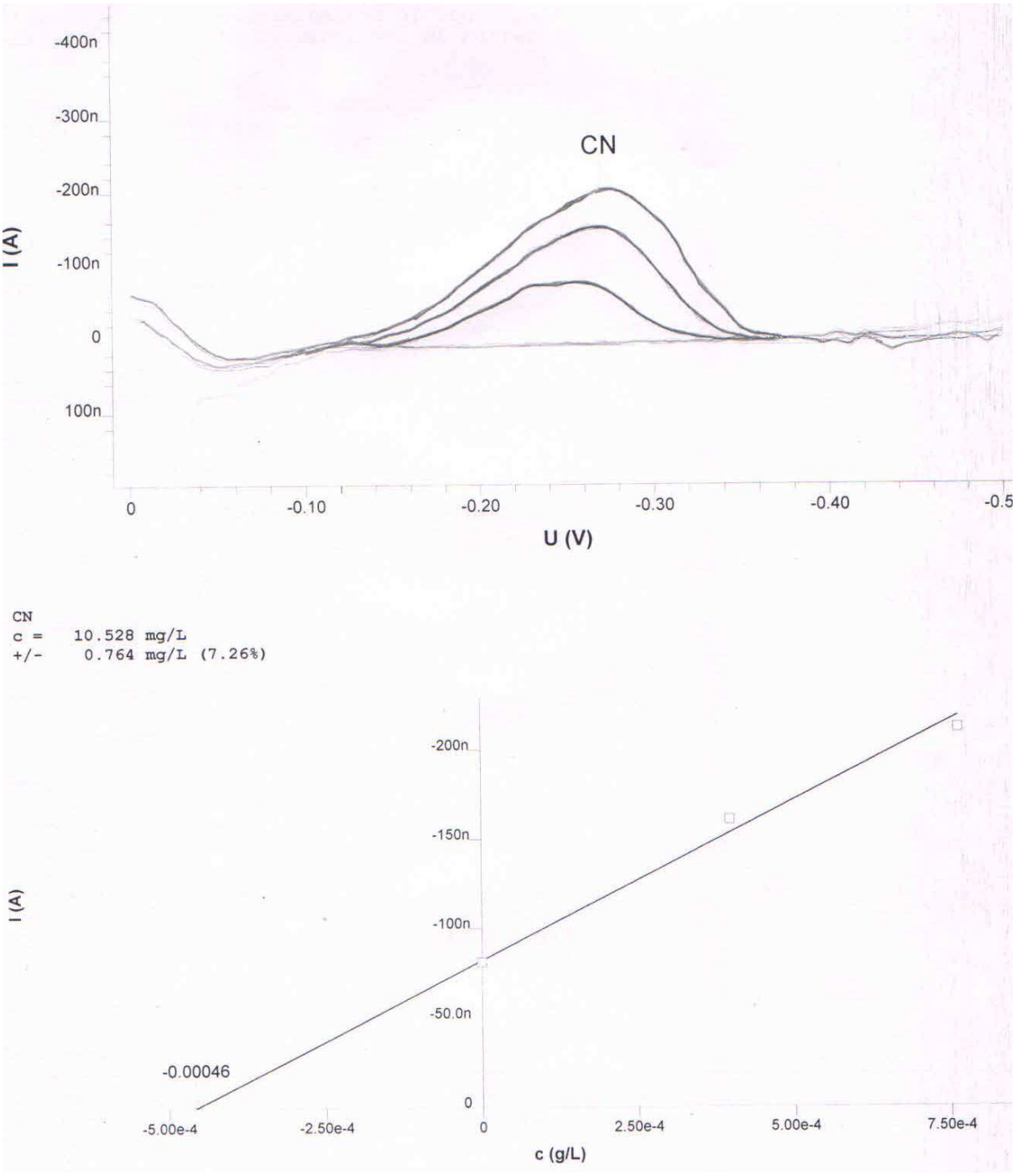

Fig. 4. Voltamogramme and Extrapolation curve of 10 ppm spiked cyanide solution

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