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Differential Pulses Adsorptive Stripping Voltammetry (DPSAV) Determination of Mercury (HG) in Blood Using Gold Rotating Disc Electrode (RDE)

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Abstract

The salts of mercury are of great toxicological importance and can cause poisoning, therefore quantitative determination of traces of mercury in blood is very essential. Routinely, inductive coupled plasma, graphite furnace atomic absorption spectrometry are used for analysis. An attempt has been made to develop new method for determination of traces of mercury in blood using differential- pulse adsorptive stripping voltametry (DPASV). The analysis utilizes three electrode systems, Rotating Disc Electrode (RDE) as a working electrode, Ag/AgCl (filled with ammonium buffer) as a reference electrode and glassy carbon electrode as an auxiliary electrode. Blood was processed by closed digestion method using 34.5% Nitric acid (HNO3). Determination of mercury was made by primary solution with a sweep rate of 20 mv/s and pulse amplitude 50 mv by standard addition method. The solution was purged with nitrogen gas and cleaning was done at 1300 mv for 30 sec. and the potential was scanned from 500 mv to 850 mv on RDE with stirrer speed 2000 rpm. The mercury ions were deposited by adsorption at 370 mv for 60 sec. The deposited metal was stripped by scanning the potential from 500 mv to 850 mv using adsorptive stripping mode. The stripping current arising was correlated with the concentration of the metal in the sample. The peak potential for mercury is 640 mv .The detection limit of mercury by this method was 1.0 ug/l.

Keywords: Adsorptive stripping voltametry, RDE, Mercury, Blood, Heavy metal, etc

Introduction

Heavy metal is considered as a causal agent for causing morbidity and mortality. Mercury is one of the well known toxic heavy metal which causes poisoning. Mercury is only metal that is liquid under standard conditions. Due to this property it is used in thermometer, barometer, and blood pressure gauges. It exist in three basic forms i.e. organic (methyl mercury) inorganic (Hg^{2+}divalent mercury) elemental mercury vapour state[1-2]. It is found naturally in environment, so its major exposure sources are mercury contaminated soil, air and water. In water, mercury exists in cationic form and maximum contamination level for mercury in drinking water 1-2 ppb level. Level of Mercury in different biological material is given in table 1.

Table-1
Levels of mercury in biological material

Matrix	Normal Level	Toxic Level
Blood	< 2µg/L	> 3µg/L
Urine	< 10µg/L	>20µg/L
Nails	< 1µg/g	>2µg/g

It can enter in the body by eating food that acquired mercury residues during processing and also from breathing mercury vapours which is present in the air. Major pathway of mercury entrance is through skin and mucous membrane and second major pathway is through inhalation[3]. Inhalation of mercury vapours causes mercury poisoning. Mercury behaves likes neurotoxin. In human, it can damage the brain, lungs damage, central nervous system, immune system, reproductive system and nervous system[4-6].

In mercury toxicity cases, immediate chelation therapy is used for its treatment. Common chelator which is used for the treatment is N-acetyl D, L penicillamine (NAP), British anti leisite (BAL), 2, 3 dimercaptopropanesulfonic acid (DMPS) and dimercaptosuccinic acid. Experimentally it was proved that DMSA chelating agent help in increasing excretion of mercury to a greater extent than NAP. Other Experimental findings demonstrate that selenium also help to protect against organic mercury foam that is methyl mercury[7-10]. The analysis of blood mercury levels in the laboratory can be carried out in many ways, such as Inductive coupled plasma, Graphite furnace atomic absorption spectrometry, an attempt has been made to develop method for the determination of traces of mercury in blood by using a technique differential pulse adsorptive stripping voltametry. The analysis utilizes three electrode

systems, Rotating Disc Electrode (RDE) as a working electrode, Ag/AgCl (filled with ammonium buffer) as a reference electrode and glassy carbon electrode as an auxiliary electrode. Determination of mercury was made by primary solution with a sweep rate of 20 mv/s and pulse amplitude 50 mv by standard addition method. The solution was purged with nitrogen gas and cleaning was done at 1300 mv for 30 sec and potential was scanned from 500 mv to 850 mv on RDE for 60 second.

Material and Methods

Instrument/Accessories: Trace Metal Analyzer Model 797 VA Computrance from Metrohm AG (Switzerland Ltd) was used. It isthree electrode system which is consist of RDE as working electrode, Ag/AgCl (filled with ammonium buffer) as a reference electrode and glassy carbon electrode as auxiliary electrode. Nitrogen gas of high purity from Laser Gas India Pvt Ltd was used. Micropipettes of volume 10-100 µl and 100-1000 µl eppendroffs were used. Microwave digestion system model Mw 680 from Aurora Canada was used for digestion of blood sample.

Reagent/chemicals: Nitric acid, liquor ammonia, Mercury nitrate, Ethylenediaminetetraaceticacid (EDTA), percholric acid ($HClO_4$), from Merck India and ultra pure from ultra pure were used.

Glassware: Volumetric flasks, beakers, funnels from Borosil India Ltd were used.

Preparation of primary solution: 0.1755g of NaCl and 0.75g of EDTA was taken in a 500 ml standard flask and dissolved in 250 ml of water. To this 9.4ml of 70% $HClO_4$ was added and made the volume upto 500 ml by ultra pure water.

Pretreatment of Gold RDE: 10 ml water and 10 ml primary solution was taken in measuring vessel and pretreatment was done under the condition given in table 2.

Table-2
Condition for pre- treatment Gold RDE

Condition	Value
Working electrode stirrer speed	RDE 2000 rpm
Initial purge time	300 s
Conditioning cycle	-
Start potential	0 mV
End potential	0 mV
Number of cycles	0
Cleaning potential	1300 mV
Cleaning time	30 s
Sweep	-
Start potential	20 mV
End potential	1200 mV
Equilibration time	5 s

Preparation of standard solution of Mercury: 1000 ppm solution of mercury was prepared by using mercury nitrate. 1 ppm standard of mercury was prepared by diluting 0.1 ml of 1000 ppm stock solution to 100 ml ultra pure water.

Sample preparation: Vessels of microwave digester were cleaned up by nitric acid and water mixture (1:1) thoroughly and dried. 1 ml of blood sample was transferred into linear vessels and 15 ml of 34.5% HNO_3 was added into each vessel and the mixture was left for few minutes for autogas. In the reference vessel 1ml of water (instead of sample) was added along with 15 ml of 34.5% HNO_3 for sample blank. Vessel carrousel was kept in the microwave digestion oven and run the digestion machine according to program given in table 3. After digestion, the sample vessels were cooled down and then, each vessel were opened in the fume hoods. After opening the vessel liquid was transferred in 50 ml volumetric flask with the help of ultra pure water and final volume was made upto 50 ml with the help of ultra pure water.

Table-3
Microwave digestion program for blood

Step	Time (second)	Starting temp(^{0}C)	Ending temp(^{0}C)
1.	210	28	100
2.	600	100	160
3.	600	160	170

Adsorptive Stripping Voltametric Measurements: 10 ml of ultra pure and 1 ml of primary solution was taken in polarographic vessel and Voltamogramme of the blank was recorded under the condition given in (table-4**)** 0.1 ml of prepared sample solution was added to polorographic vessel and voltamogramme of the sample solution was recorded under the same conditions and finally 0.1 ml of 1 ppm standard of mercury was added twice and voltamogramme of the standard was recorded (figure 1).

Results and Discussion

In the present study, the concentration of the mercury metal in the blood was successfully determined by Differential Pulse Adsorptive Stripping Voltammetry Technique. DPASV voltamogramme of mercury obtained from standard addition technique in (figure 1 and figure 2). The instrument was calibrated by standard additions to the sample and the initial metal concentrations were calculated by extrapolation graph (figure 2). The automatic blank correction as feature of instrument was used to subtract the blank contribution due to chemicals, water etc. Finally the concentration of the metal was calculated by linear regression method using following formula

Final result = (Concentration x cell volume x multiplier) / (sample amount x divisor)

Where, Multiplier and divisor are dilution and sample amount respectively taken for preparation. The advantage of proposed techniques was sensitivity, rapidity, and less sophistication.

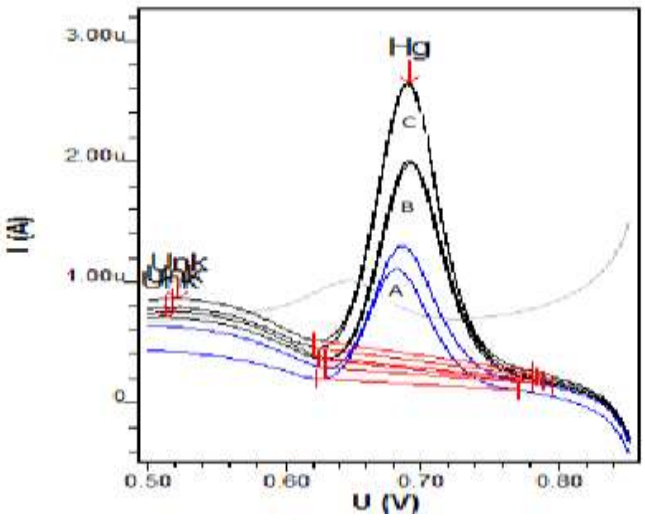

Figure-1

Voltammogram of Hg obtained from standard addition technique with number of replications being two A) 0.1 ml sample in 1ml primary solution + 10 ml ultra pure water, B) A + 0.1 ml standard solution of Hg (1 ppm), C) B + 0.1 ml standard solution of Hg (1 ppm)

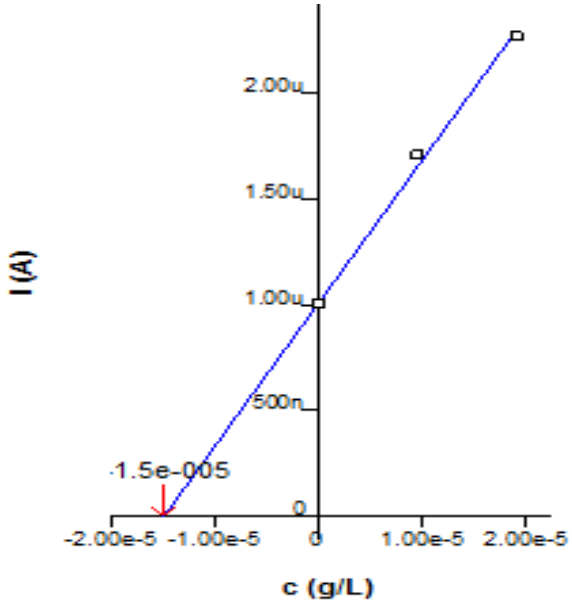

Figure-2

The extrapolation curves of Hg obtained from standard addition technique

Conclusion

In this work, mercury determination was carried out, under the most appropriate condition which was fixed. Direct determination of mercury (Hg) in blood sample is possible by DPASV technique. Under the working conditions given in table 1, amount of mercury in the blood sample has been successfully determined. The demand of this method for detection and quantitation of mercury refers mainly to trace analysis in blood. DPASV method satisfies most of needs, which is required for traces of mercury present in blood sample. DPASV technique is simple, selective and reproducible technique for qualitative and quantitative determination of mercury (Hg) in blood.

Table-4

Operating condition for the determination of Mercury by DPASV technique

Conditions	Value
General	-
Working electrode	RDE
Reference electrode	Ag/AgCl
Auxiliary electrode	Glassy electrode
Drop size	4
Stirrer Speed	2000 rpm
Initial purge	300 s
No. of addition	2
No. of replications	2
Measure blanks	Yes
No. of blanks	1
Addition purge time	20 s
Pretreatment	-
Cleaning potential	1300 mV
Cleaning time	30 s
Deposition potential	400 mV
Equilibrium time	10 s
Mode	Adsorptive stripping voltammetry
Sweep	-
Pulse amplitude	50 mV
Start potential	500 mV
End potential	850 mV
Voltage step	2 mV
Voltage step time	0.1 sec
Sweep rate	20 mV/s
Peak potential Hg^{2+}	640 mV

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