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Simultaneous Determination of Zinc (Zn), Cadmium (Cd), Lead (Pb) and Copper (Cu) in Blood Using Differential- Pulse Anodic-Stripping Voltammetry

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Abstract: ***The salts of Zinc (Zn), Cadmium (Cd), Lead (Pb), Copper (Cu), are of great toxicological importance and can causes poisoning. Therefore quantitative determination of traces of zinc, cadmium, lead, copper, in blood is very essential. Routinely, inductive coupled plasma, atomic absorption spectrometry, graphite furnace atomic absorption spectrometry were used for analysis. An attempt has been made to develop new method for simultaneous determination of traces of zinc, cadmium, lead, copper, in blood done by differential-pulse anodic-stripping voltammetry. Blood was processed by wet digestion method using concentrated nitric acid and sulphuric acid. Determination of zinc, cadmium, lead, copper, was made in acetate buffer (pH 4.6) with a sweep rate (scan rate) of 60.0 mV/s and pulse amplitude 50 mV by Hanging Mercury Dropping Electrode (HMDE) by standard addition method. The solution was stirred during pre-electrolysis at -1150 mV (vs. Ag/ AgCl) for 90 s and the potential was scanned from -1150m V to +100m V (vs. Ag/ AgCl). Under these conditions the limit of detection of zinc, cadmium, lead, and copper were 1.0 µg/L, 1.0 µg/L, 0.1 µg/L, 1.0 µg/L and respectively.***

Keywords-Blood, Voltammetry, Anodic Stripping, Zinc, Cadmium, Lead, Copper.

I. INTRODUCTION

Zinc is an essential trace mineral. The functions of zinc are enzymatic. There are over 70 metalloenzymes known to require zinc for their functions. The main biochemical in which zinc has been found to be necessary includes enzymes and enzymatic function, protein synthesis and carbohydrate metabolism. Zinc is a constituent of insulin and male reproductive fluid. Zinc is necessary for the proper metabolism of alcohol, to get rid of the lactic acid that builds up in working muscles and to transfer it to the lungs. Zinc is involved in the health of the immune system, assists vitamin A utilization and is involved in the formation of bone and teeth. Zinc sources include nuts, bolts, and zinc oxide based skin creams. The clinical signs of zinc toxicosis include vomiting, diarrhea, red urine, yellow mucous membranes, liver failure, kidney failure, anemia etc [1-2].

Cadmium and solutions of its compounds are toxic, particularly in soluble and respirable forms, being more easily absorbed through inhaled dusts and fumes. Chronic dust or fume exposure can irreversibly damage the lungs, producing shortness of breath and emphysema. Breathing high levels of cadmium may severely damage the lungs and can cause death. Eating food or drinking water with very high levels severely irritates the stomach, causing vomiting and diarrhea. A balanced diet can reduce the amount of cadmium taken into the body from food and drink. Animal studies suggest that more cadmium is absorbed into the body if the diet is low in calcium, protein, or iron, or is high in fat. The major route for cadmium intake for non smokers is ingestion of trace cadmium in foodstuffs of natural origin or from the use of phosphate fertilizers and sludge on agricultural soils. Smokers have elevated blood and tissue concentrations of cadmium from cigarette smoke. Whole blood and urine levels are useful indicators of cadmium exposure [3-4].

Lead poisoning is caused by increased levels of the heavy metal lead in the body. Lead interferes with a variety of body processes and is toxic to many organs and tissues including the heart, bones, intestines, kidneys, and reproductive and nervous systems. It interferes with the development of the nervous system and is therefore particularly toxic to children, causing potentially permanent learning and behavior disorders. Symptoms include abdominal pain, headache, anemia, irritability, and in severe cases seizures, coma, and death. Lead can enter our bodies through ingestion (eating and swallowing) of lead contaminated food, water, soil, dust or paint chips and through inhalation of lead dust particles. A common way of absorbing lead, particularly for young children, is through contaminated hand to mouth movements Occupational exposure is a common cause of lead poisoning in adults. One of the largest threats to children is lead paint that exists in many homes, especially older ones; thus children in older housing with chipping paint are at greater risk. Prevention of lead exposure can range from individual efforts [5-7].

Copper toxicity refers to the consequences of an excess of copper in the body. Copper toxicity can occur from eating acid food that has been cooked in un-coated copper cookware, or from exposure to excess copper in drinking water or other

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environmental sources. Excessive copper absorption can occur through the skin, by inhalation or by ingestion. Copper is regularly used in agricultural chemicals for mildew prevention, and as algaecides in water treatment of industrial waters. It is also used as a preservative for wood, leather, and fabrics. Copper is a component of many body proteins, almost all of the body's copper is bound to copper proteins. Unbound (free) copper ions are toxic. Genetic mechanisms control the incorporation of copper into apoproteins and the processes that prevent toxic accumulation of copper in the body. Its toxicity is usually due to excessive supplementation, the increasingly common problem of low levels of zinc in the diet, contaminated food and drinking water due to contact with metallic copper [8].

Keeping in view of the above quantitative determination of zinc, cadmium, lead, copper is very essential [9-14]. Commonly used method for determination of traces of zinc, cadmium, lead, copper are atomic absorption spectrometry (AAS) [15], neutron activation , inductively coupled plasma atomic emission spectroscopy (ICP-AES) [16-20] x-ray fluorescence [21] and Cathode Ray Polarography [22]. These techniques are expensive and sophisticated. Keeping in view of the above an attempt has been made to develop new method for simultaneous determination of zinc, cadmium, lead, copper in blood sample using differential pulse stripping voltammetry which is relatively inexpensive, reproducible and is one of the most selective and sensitive technique in the determination of traces amount of the metals.

In this technique blood sample was processed by wet digestion and determination of zinc, cadmium, lead, copper was made in acetate buffer (pH 4.6) with a sweep rate (scan rate= 60.0 mV/s) which is the ratio of voltage step to voltage step time and pulse amplitude (pulse height =50 mV) by Hanging Mercury Dropping Electrode (HMDE). The solution was stirred during pre-electrolysis (Deposition potential) at -1150 mV (vs. Ag/ AgCl) for 90 s and the potential was scanned from -1150m V to +100mV.

II. EXPERIMENTAL

Apparatus and Accessories

1. Trace metal analyser model 797 VA Computrace from Metrohm AG Ltd, Switzerland (Fig 1) was used, which contains following electrodes:
   Working Electrode - Hanging Mercury Dropping Electrode (HMDE)
   Auxillary Electrode - Platinum (Pt)
   Reference Electrode - Ag/ AgCl (KCl 3mol/L)
2. Nitrogen gas of purity 99.99% from laser gases, India was used.
3. Micropipette of Eppendorf make of volume 10 - 100µl and 100- 1000 µl were used.
4. pH measurements were made with model Inolab WTW series pH meter at ambient temperature.
5. Whatman filter papers 41 Ashless Circles of 125mm from Whatman International England were used.

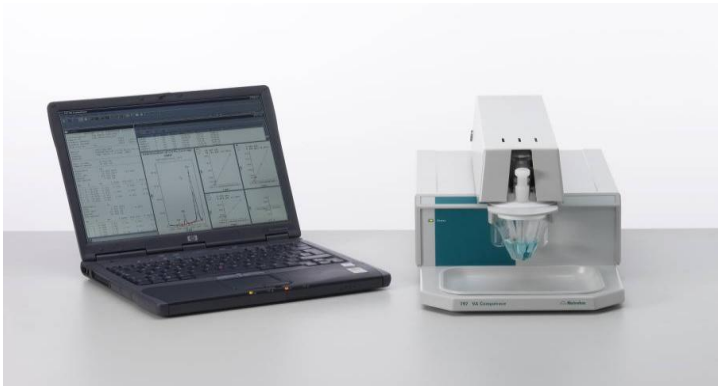

Figure 1: 797 VA Computrace (Trace Metal Analyser)

Chemicals

1. Suprapure acetic acid [CH_3COOH] from Merck, Germany.
2. Nitric acid [HNO_3], Zinc nitrate [$Zn(NO_3)_2$], Cadmium nitrate [$Cd(NO_3)_2$], Lead nitrate [$Pb(NO_3)_2$], Cuprous nitrate [$Cu(NO_3)_2$], from Merck, Mumbai.
3. Liquor ammonia (NH_3), Ammonium oxalate ($C_2H_8N_2O_4$), Sulphuric acid (H_2SO_4) from Qualigens Fine Chemicals, Mumbai.
4. Ultrapure water from Merck Millipore, Germany.

Glasswares: Beaker of 100, 500 ml capacity, Pipettes 5, 10, 25 ml capacity, Volumetric flask of 50 ml, 100 ml capacity and glass funnel from Borosil India were used. The glasswares were thoroughly washed and rinsed 2-3 times with ultrapure water and dried in digital oven.

Preparation of Ammonium oxalate solution: Saturated solution of Ammonium oxalate was prepared in ultrapure water.

Preparation of Ammonium acetate buffer: Ammonium acetate buffer was prepared by dissolving 5.55 ml of suprapure acetic acid in 10 ml ultrapure was added. Then 3.7 ml suprapure ammonia was added slowly and pH was adjusted to 4.6 by adding few drops of suprapure ammonia. Finally the volume was made up to 50 ml with ultrapure water.

Preparation of Standard Solution: 1 ppm mixed standard of zinc, cadmium, lead, copper was prepared by diluting 1000 ppm stock solution of zinc, cadmium, lead, copper using ultrapure water.

Sample Preparation: One ml of blood sample was taken in a beaker and kept over a wire gauge on the hot plate at 60˚C till it was completely charred. Then 1.5 ml concentrated sulphuric acid and 2.5 ml concentrated nitric acid was added to it and again heated on the hot plate near boiling to complete dissolution of the residue and then cooled. After cooling the contents of the beaker was heated till just carbonized. Concentrated nitric acid was added to the beaker dropwise with the help of a dropping funnel at the rate of about 20 drop/minute and heating is continued. Addition of nitric acid and heating was continued till the contents of the beaker becoming nearby colorless (When organic material is a not completely oxidized red fume will appear in the beaker and charring will occur on further heating). When the contents of the beaker become colorless, dropwise addition of the nitric acid and heating is stopped and kept for cooling. After cooling 25 ml of the saturated ammonium oxalate solution was added to the beaker and the boiling was continued till sulphuric acid begin to reflux. The sample was cooled,

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diluted with 20 ml ultrapure water and filtered. The sample was then quantitatively transferred to a 50 ml volumetric flask and make up to mark by ultrapure water.

Voltammetric determination: 1ml of ammonium acetate buffer along with 10 ml of ultrapure water was taken into the polarographic vessel and then the measurement was started under voltammetric condition given in Table 1. After the voltamogramme of the blank was recorded, 0.1 ml of prepared sample solution was added to the polarography vessel and then voltamogramme of the sample solution was recorded under the same conditions. The measurement was done twice to check the repeatability. After the sample voltamogramme was recorded, 100µl of 1ppm mixed standard of zinc, cadmium, lead copper was added twice and then voltamogramme of the standard was recorded. Finally the concentrations of the metals were calculated by linear regression method. All the measurement was done by standard addition technique to avoid the sample matrixes effect. Voltamogramme obtained for blank, sample and standard was given in Figure 2.

Table 1: Voltammetric Conditions for simultaneous determination of Zinc, Cadmium, Lead and Copper in blood using Differential- Pulse Anodic-Stripping Voltammetry

Parameters	Description
Working electrode	Hanging mercury drop electrode (HMDE)
Drop size	4
Stirrer speed	1200 rpm
Mode	Differential pulse (DP)
Initial purge time	300s
Addition purge time	10s
Deposition potential	-1150mV
Deposition time	90s
Equilibration time	10s
Pulse amplitude	50mV
Start potential	-1150mV
End potential	100mV
Voltage step	6mV
Voltage step time	0.1sec
Sweep rate	60.0mV/s
Peak potential Zn^{2+}	-980mV
Peak potential Cd^{2+}	-560mV
Peak potential Pb^{2+}	-380mV
Peak potential Cu^{2+}	-50mV

III. RESULTS AND DISCUSSIONS

Voltammetry is the two step measurement, In the first step the metal ions like Zinc, Cadmium, Lead ,Copper present in the test solution is deposited on the electrode surface (amalgamation) at deposition potential of -1150 mV. In the second step all the deposited ions are anodically stripped by scanning the potential range from -1150mV to + 100mV. All the measurements are done by standard addition technique in which first the sample is taken into the polarographic vessel and current is measured, then standard of known concentration is added twice to the sample solution and the current is measured. After all the measurement extrapolation curve is plotted between current vs. concentration. The extrapolation curve will show the amount of metals present in the sample solution. All the analysis was done with automatic blank subtraction, which is feature of the instrument.
Voltamogramme of blank, sample and standard was given in fig 2. Extrapolation curve of Zn, Cd, Pb and Cu were given in fig 3, 4, 5 and 6 respectively. Concentration of different metals in blood was given in table 2. Concentration of Cd, Pb and Cu was found zero, while the concentration of Zn was varied from 0.00 to 10.86 ppm. Recoveries of zinc, cadmium, lead and copper were checked by their standard solution.
Determination of zinc, cadmium, lead, copper using deferential-pulse anodic stripping voltammetry is discussed in a number of papers [23-31]. The advantages of proposed differential-pulse anodic stripping votammetric method over the other known techniques were matrix interferences, sensitivity, rapidity and cost factor. Therefore this method can be recommended for simultaneous determination of zinc, cadmium, lead and copper in blood samples.

IV. CONCLUSION

In the present work, the most appropriate conditions were fixed to simultaneous determine the amount of zinc, cadmium, lead and copper in blood sample by differential-pulse anodic stripping Voltammetry using acetate buffer (pH 4.5) has been successfully demonstrated.

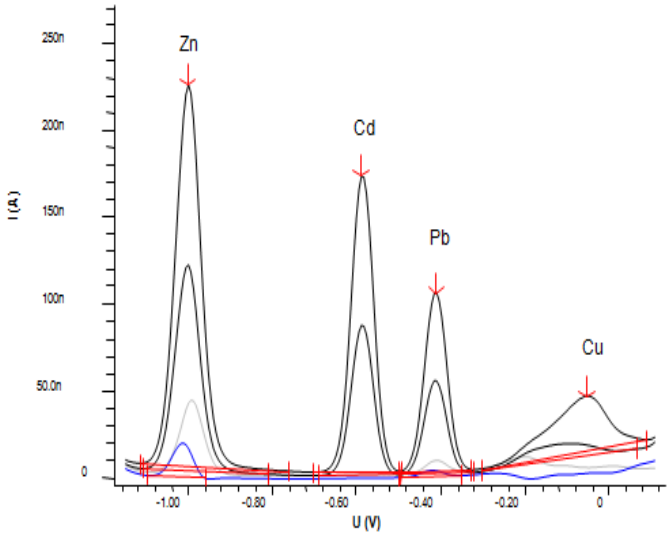

Figure 2 : Voltommogramme obtanined for samples as well as for standards.Condition: scan rate, 60.0 mv/s;Pulse amplitude, 50 mV;Deposition potential, -1150 mV vs Ag/AgCl; deposition time , 90s, equilibration time,10s;Sacnning range, -1150 to 100 mV.

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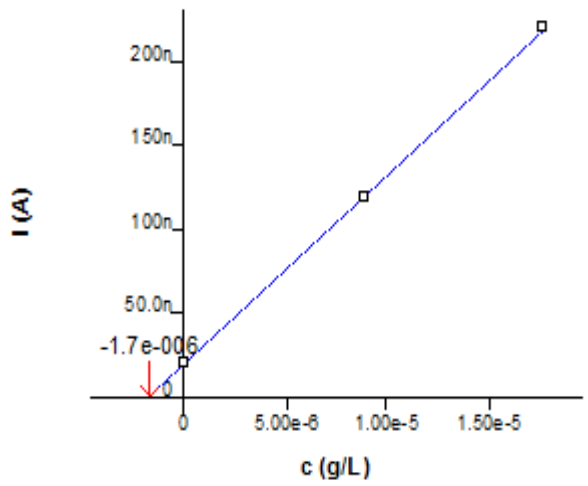

Figure 3: Extrapolation graph of Zn

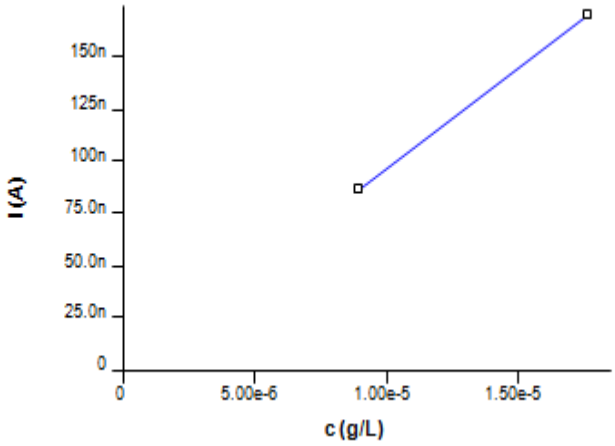

Figure 4: Extrapolation graph of Cd

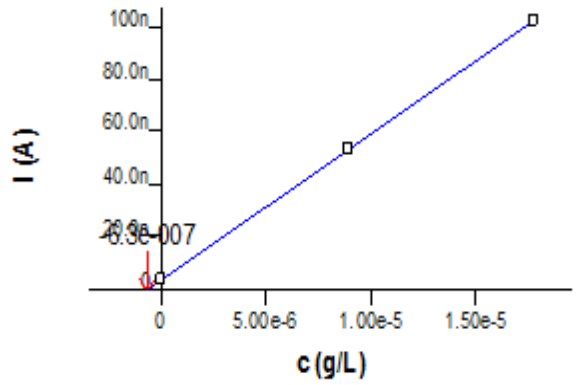

Figure 5: Extrapolation graph of Pb

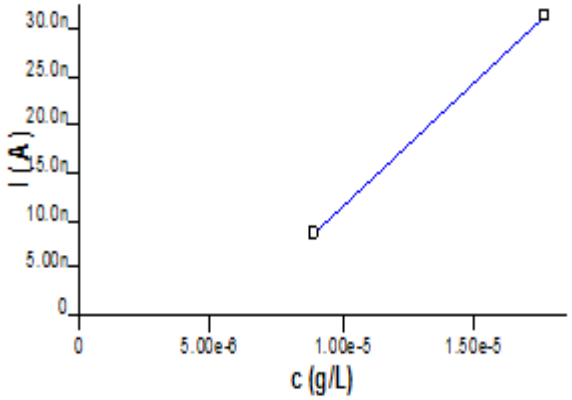

Figure 6: Extrapolation graph of Cu

Table 2: Amount of Zn, Cd, Pb and Cu in different blood samples

Sample No	Zn in ppm	Cd in ppm	Pb in ppm	Cu in ppm
1	04.236	00.000	00.000	00.000
2	00.208	00.000	00.000	00.000
3	01.733	00.000	00.000	00.000
4	01.741	00.000	00.000	00.000
5	06.186	00.000	00.000	00.000
6	10.800	00.000	00.000	00.000
7	05.862	00.000	00.000	00.000
8	05.324	00.000	00.000	00.000
9	03.274	00.000	00.000	00.000
10	17.252	00.000	00.000	00.000
11	13.570	00.000	00.000	00.000
12	03.036	00.000	00.000	00.000
13	11.075	00.000	00.000	00.000
14	07.045	00.000	00.000	00.000
15	10.394	00.000	00.000	00.000
16	00.000	00.000	00.000	00.000
17	10.011	00.000	00.000	00.000
18	04.383	00.000	00.000	00.000
19	15.340	00.000	00.000	00.000
20	02.766	00.000	00.000	00.000
21	02.931	00.000	00.000	00.000
22	00.000	00.000	00.000	00.000
23	00.000	00.000	00.000	00.000
24	05.569	00.000	00.000	00.000
25	00.000	00.000	00.000	00.000
26	00.596	00.000	00.000	00.000
27	09.283	00.000	00.000	00.000
28	00.000	00.000	00.000	00.000
29	00.000	00.000	00.000	00.000
30	00.000	00.000	00.000	00.000
31	00.000	00.000	00.000	00.000

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