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Zinc (Zn) Analysis in Milk by Microwave Oven Digestion and Differential Pulse Anodic Stripping Voltametry (DPASV) Technique

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Abstract. Milk is very important component of human diet. The presence of over limit of heavy metal in milk may create significant health problems. In the present study, the direct determination of Zinc (Zn) heavy metal in milk samples of different brands was carried out by differential pulse anodic stripping Voltammetric technique at Hanging Mercury Drop Electrode (HMDE). Milk samples were processed by microwave oven digestion using HP/VHP Vessels and TFM Liners and nitric acid (HNO_3).Determination of Zn was made in acetate buffer (pH 4.6) with a sweep rate (scan rate) of 59.5 mV/s and pulse amplitude 50mV by HMDE by standard addition method. The solution was stirred during pre-electrolysis at -1150mV (vs. Ag/AgCl) for 90 seconds and the potential was scanned from -1150V to +100V (vs. Ag/AgCl). The zinc ions were deposited by reduction at -1150 mV on HMDE. The stripping current arising from the oxidation of metal was correlated with the concentration the metal in the sample. .As a result the minimum level of Zn observed in the milk sample of different brands was determined as 2.28 mgL^{-1}.

Keywords: Anodic stripping Voltametry, Hanging mercury dropping electrode, Heavy metals, Zinc, milk.

Introduction

Milk is an important source of minerals including trace elements for suckling as well as for human nutrition. Knowing the content of trace elements in the milk as a function of their intake prevents either insufficient or excessive quantity of trace elements from being supplemented. The content of micro-elements in the milk can be an important indicator whether the animals are being saturated with these elements and a parameters of the milk`s biological quality as a nutrient. [Bonzene et al, 2005] Increase in industrial and agricultural processes have resulted in increased concentration of metals in the air, water and soil. These metals are taken in by plants and consequently accumulate in their tissues. Animals that graze on such contaminated plants and drink from polluted waters also accumulate such metals in their tissues and milk if lactating [Yahaya M.I et al 2010]. A large amount of these metals taken in by plants and animals subsequently find their way into the food chain. This ever increasing pollution has given rise to concern on the intake of harmful metals in humans. Metals enter the human body through inhalation, ingestion or absorption through the skin [Ogabiela et al, 2010 and Ahmed WMS 2002]. The intake through ingestion depends on food habit. Cow milk which is a very important food stuff consumed by man is one of the major sources [Farid et al, 2004]. In recent times, the amount of metals in cow milk is widely studied, particularly in industrialized and polluted areas of the developed and the developing countries of the world since animals grazed freely on open fields are considered as bio-indicators of environmental pollution [Korenekong et al, 2002 and Li Quang et al 2009].Differential pulse anodic stripping Voltammetry (DPASV) is a powerful and established method for the analysis of trace metals in milk samples available in Delhi Market, India. It is possible to analyze very low level of Zn using ASV. It can detect levels in the range of part-per-million (ppm) or even part-per-billion (ppb) (i.e., ~10-10 M). Several other methods are also used routinely in addition to DPASV, such as Atomic Absorption Spectrometry (AAS) and X-ray fluorescence spectrometry, Inductive coupled plasma, but advantages of using DAPSAV are that it is a successful, new, rapid, simple, selective and inexpensive for qualitative and quantitative determinations of heavy metals. [6] The other reasons for using DPASAV technique are: It has extremely low detection limits, and it works in the presence high salt concentrations. It allows metal speciation and can differentiate between free and complexed metal ions. It can also analyze non-metals such as anions or organics. In this study, DPASV technique was used to determine Zinc metal in various milk samples of Delhi (India) using Hanging mercury

dropping electrode (HMDE) as a working electrode. Consequently, the most appropriate conditions were fixed to determine Zinc amount as an analytical application of DPASV on a HMD electrode.

Materials and Methods

Instrumentation / Accessories and Operating Conditions

Voltammetric determination of Zn was performed with Trace Metal Analyzer (797 VA Computrace ,Metrohm AG Ltd, Switzerland) with three-electrode system consisting Hanging mercury drop electrode(HMDE) as working electrode, Platinum (Pt) as auxiliary electrode and Ag/AgCl/ KCl (3mol/L) as reference electrode. The operating parameters are given in Table 1.

Reagents/Chemicals

Suprapure acetic acid (Merck Darmstadt, Germany), Nitric acid, Liquor ammonia, ammonium oxalate, and sulphuric acid (Qualigens Fine Chemicals, A Division of GlaxoSmithKline Pharmaceuticals Limited, Mumbai, India), Ultra pure-deionize water was obtained from Milli-Q (Biocel System, Millipore)

Preparation of Supporting Electrolyte

*(*Ammonium acetate buffer) 55.5 ml of Suprapure acetic acid was taken in a 500 ml volumetric flask. To this about 100 ml of water was added. 37 ml of Suprapure ammonia was added slowly to the volumetric flask. Ammonia had to be added slowly, because heat will be generated while addition. After the addition the solution was diluted to 500 ml with ultra pure water. The ph of the buffer should be 4.6.

Preparation of Standard Solution

1mg L^{-1} standard of zinc was prepared for the Voltammetric analysis from stock solution of 1000 mg L^{-1}.

Sample collection and Preparation/Digestion

Milk samples were collected in 15 ml graduated centrifuge tubes (Tarson: Cat. No.500030) from the various milk sample of different brands of Delhi, India. For the determination of Zinc, Milk sample digested and analyzed in same day.

Anodic Stripping Voltammetric measurements

10 ml ultra pure water and 1ml of acetate buffer (pH 4.6) was taken in polarographic vessel and then the measurement was started under the given parameters Table 1, after this voltamogramme of the blank was recorded. 1 ml of digested milk sample was added to polarographic vessel and then voltamogramme of the sample solution was recorded under the same conditions. After the sample voltamogramme was recorded, 0.1 ml of 1 mg L^{-1} Zinc standard was added twice and then voltamogramme of the standard was recorded (Fig. 1). ASV is the two step measurement .In the first step the zinc metal ions present in the test solution are get deposited on the mercury 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 -1150 to + 100mV.

Tab.1 Operating parameters for the determination of Zinc in milk sample by DPASV

Parameters	**Description**
Working electrode	Hanging Mercury Dropping Electrode
Calibration	Standard addition method
Number of replications	3
Drop size	4
Stirrer speed	2000 rpm
Mode	Differential pulse
Initial purge time	300 s
Addition purge time	10 s
Deposition potential	-1.15 V
Deposition time	90 s
Equilibration time	10 s
Pulse amplitude	0.05 V
Start potential	-1.3 V
End potential	0.05 V
Voltage step	0.006 V
Voltage step time	0.1 s
Sweep rate	0.06 V/s
Peak potential (Zn)	-9.80 V
Peak potential (Cd)	-0.56 V
Peak potential (Pb)	-0.38 V
Peak potential (Cu)	-0.10V

All the measurements are done by standard addition technique in which first the sample was taken into the polarographic vessel and the current was measured. After the addition of 100 µl of standard soln. the procedure was repeated three times and the current was

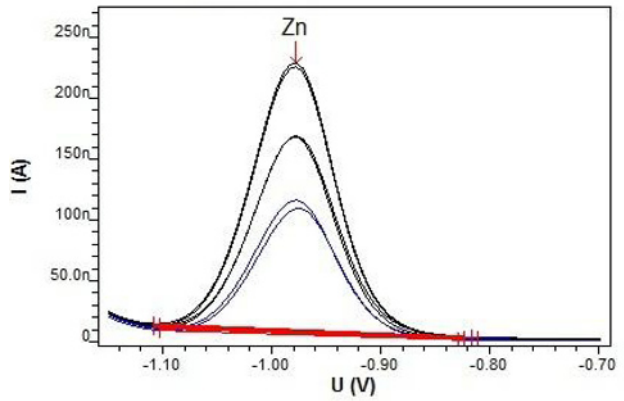

Fig.1 Voltommogramme obtanined for samples as well as for standards.Condition: scan rate, 59.5 mv/s;Pulse amplitude, 50 mV;Deposition potential, -1150 mV vs Ag/AgCl; deposition time , 90s, equilibration time,10s;Sacnning range, -1150 to 100 mV

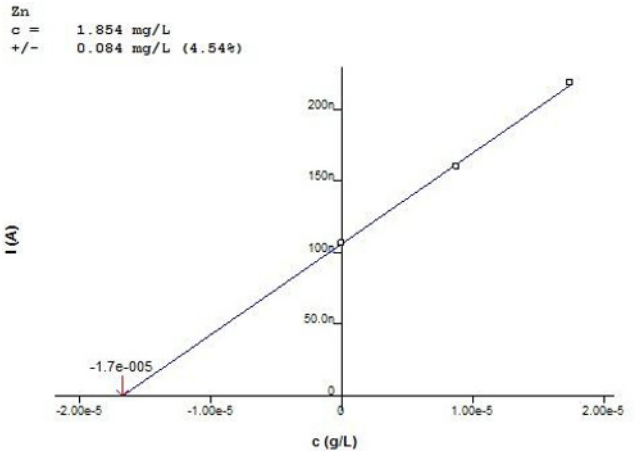

Fig.2 Extrapolation graph of Zn

measured. After all the measurement extrapolation curve was 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 feature of instrument. Voltamogramme of the standard and sample were shown in Fig. 1 and the exploration graph of Zn was shown in Fig. 2.

Results and Discussion

In this study, the concentration of the zinc metal in milk sample of different brands in Delhi, India was successfully determined by ASV technique .The mean concentration of Zn metal in milk sample of different brands of Delhi (India) was found to be 2.28 mgL^{-1}. Comparison of the elemental concentration of Zn in fresh milk in this study with published values is shown in Table No 2. Consequently, it is understood that the concentration of Zn in milk sample of different brands in Delhi (India) have no influence on the human health. Voltamogramme of the standard and sample were shown in figure 1. The sensitivity was calibrated by standard additions to the sample and the metal concentrations initially were calculated by exploration the extraploration graph of Zn was shown in Fig. 2.

Many analytical methods has been published for the determination of Zinc but using one method in DPASAV for the analysis of four metal combined respectively Zn is presented in this work. The advantages of the proposed Voltammetric method over the other known techniques(AAS, ICPOES,etc) are that it is a successful, new, rapid, simple, selective and inexpensive technique for quantitative and qualitative determination of heavy metals and it has extremely low detection limits and can also works in the presence of high salt concentrations.

Conclusion

In this work, the most appropriate conditions were fixed to determine the amount of Zn through DAPSAV using single method on Hanging mercury drop electrode (HMDE). Direct determination of Zn in the milk sample of different brands.

Delhi (India) is possible by DAPSAV. Under working conditions the amount of Zn in milk sample has been successfully determined. It can be very useful for human health concern. This method is rapid, sensitive and less costly as compared to other analytical methods.

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Tab.2 Comparison of the elemental concentration of Zn in fresh milk of different brands in this study with published values

Heavy Metal	In This study Value of Zn (mg L^{-1})	Bis Indian Standards (IS 105000: 1991) (mg L^{-1})		WHO (mg L^{-1}) Maximum allowable concentration	EPA (mg L^{-1})	Semaghiul et al (2008) (mg L^{-1})	Abdallah (2011) (mg /Kg)	Farid et al (2004) (µg /Kg)	Ostapezul et al (1987) (µg /Kg)	Bulinski et al (1992) (µg /Kg)	Cortes et al (1994) (µg /Kg)	Ogabiela et al (2011) (mg L^{-1})
		Desirable limit	Permissible limit									
Zn	2.28	5.0	15	5.0	5.00	0.98	3.661 ± 0.003	944.9 ± 2.4	3730.0	3770.0	3000.0	3.239-5.521

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