Research Article

Flame Atomic Absorption Spectrophotometric and Polarographic Methods for Zinc Determination in Infant's Milk Samples

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ABSTRACT

Zinc plays an important role in normal growth and development in the first 3 years of children's life; it also has a significant activity in the immune system. Therefore it is one of the most important essential trace elements in infant nutrition. It's necessary to supply infant formulas by trace elements like zinc in order to make their composition similar to that of human milk. Zinc contents were determined in infant milk formula samples from different manufactures commercially available in markets by polarographic method after extraction using trichloroacetic acid (TCA). The results were found to be in good agreement with those obtained by flame atomic absorption spectrophotometric method (FAAS), where both methods showed high precision, accuracy and high sensitivity.

Keywords: zinc, polarographic method, flame absorption spectrophotometric method, milk formula.

INTRODUCTION

Zinc is an essential component of a large number of enzymes, and plays a central role in cellular growth and differentiation in tissues that have a rapid differentiation and turnover, including those of the immune system and those in the gastrointestinal tract. The positive impact of zinc supplementation on the growth of some stunted children, and on the prevalence of selected childhood diseases such as diarrhea, suggests that zinc deficiency is likely to be a significant public health problem, especially in developing countries.[1-3] Zinc deficiency causes dermato-sclerosis, growth retardation, hypogonadism and hepatosplenomegaly.[4] All population age groups are at risk of zinc deficiency, but infants and young children are probably the most vulnerable.

Zinc supplementation is a powerful therapeutic tool in managing a long list of illnesses. Zinc is a constituent of numerous pharmaceutical preparations and food products including infant milk formulas. Various instrumental methods have been used to determine zinc compounds, e.g., inductively coupled plasma-mass spectrometry,[5] atomic absorption spectrometry (AAS) in biological materials,[6,7] foodstuffs,[8] and nutrients;[9] spectrofluorimetry,[10], and spectrometry[11] in pharmaceutical preparations; liquid chromatography[12] and ion-exchange chromatography;[13] and electrochemical methods including flow potentiometry in soil and waste water,[14] voltammetry at the mercury drop electrode in foodstuffs,[15,16] biological materials,[17,18] insulins[19] and plants.[20]

However, the best methods to determine metals in pharmaceutical, environmental and foodstuff materials, are the electrochemical methods and AAS which serves as a complementary method. Analysis by atomic absorption spectroscopy (AAS) has been shown to be quick and simple. Although there are several references to this method, there are also a number of factors generally overlooked, such as interference, matrix effects and losses through volatilization at high temperatures. The limit of detection is also poor compared to voltammetric methods and thus pre-concentration is usually needed. Electrochemical methods have the advantage that they require relatively inexpensive instrumentation, are capable of determining elements accurately at trace and ultra-trace levels[21,22] and have demonstrated an ability for multi-element determination.[23,24]

This paper deals with application of polarography and flame atomic absorption spectrometry (FAAS) techniques for determination of Zinc.

MATERIALS AND METHODS

Chemicals and Reagents: 24% trichloroacetic acid solution (C2HCl3O2) and 10 ppm zinc standard solution were purchased from MERCK, 5% lanthanum solution ($LaN_3O_9.6H_2O$) was obtained from FLUKA, blank solution (containing 500mg/l lanthanum and 1.2% TCA), Redistilled deionized water (d. water) was purchased from SHARLOU, acetic acid/sodium acetate buffer at pH 4.6 was prepared.

Instruments and apparatus: Flame atomic absorption spectrophotometer (SHIMADZU AA – 6300), Electrochemical Analyzer (Polarographe) (797VA Computrace Analyzer-Metrohm/Switzerland), hanging mercury drop electrode (HMDE), pH meter (model ion check) from Radio meter company.

Collection of Samples: For the present study, 10 infant milk samples for the first and second age produced by 8 different companies were collected from local supermarkets.

Extraction procedure: 5g of milk was dissolved in 20ml d.water and 50 ml of 24% TCA, followed by dilution with d.water to 100ml. The mixture was shaken for a period of

30 min and filtered 25ml of the filtrate containing milk metals was transferred to a 50ml volumetric flask, 1ml of 5% lanthanum solution was added, and diluted to the mark with d.water. The determination methods were applied to the final solution.[25,26] The extraction method's recovery was 97.1% and RSD=0.46%.

Zinc determination by FAAS method:

Preparation of sample: 5 g of sample was weighed for determination and 5 ml of standard solution was added, then the sample was extracted using TCA method.

A standard curve was prepared by transfer (0.5, 0.75, 1, 1.25, 1.5, 1.75) ml of zinc standard solution into 10ml volumetric flask, and diluted to the mark with the blank solution.[27-29] The absorbance of sample solution and standard solution series were determined under the following conditions:

Wavelength: 213.9 nm

Flame – gases: Air – acetylene

Slit width: 0.2 mm

Air flow: 6L/min

Acetylene flow: 1000 ml/min

Burner's high: 4 mm

Zinc determination by polarographic method:

Preparation of sample: 5 g of sample was weighed and extracted using TCA method. 0.2 ml of resulting solution was transferred into a 10 ml volumetric flask containing 0.5 ml of buffer, and diluted to the mark with d.water. The polarographic method's blank was prepared by transferring 0.2 ml of blank solution into a 10 ml volumetric flask containing 0.5 ml of buffer, and diluted to the mark with d.water. Samples were measured with three addition of standard (0.05 ml every time) to cancel interferences.

A standard curve was prepared by transferring (1,2,2.5,3,4,4.5,5) ml of zinc standard solution into 25ml volumetric flask. Then 0.5ml of buffer was added, and diluted to the mark with d.water. The buffer used for measurement also was diluted 20 times.[30]

The measure of peak current was done under the conditions which are given in **Table 1**.

Table 1: The optimum parameters established for determination of zinc using HMDE

Cell volume	10ml	Pulse time	0.02 sec
Sample volume	0.2 ml	Scan rate	0.0198V/s
No. of standard addition	3	Precipitation time	30 sec
Purge time	300 sec	Potential Precipitation	-1.2 V
Potential range	(-1.2) to (-0.8) V	working electrode	HMDE
Pulse amplitude	0.05 V	Auxiliary electrode	Calomel

RESULTS AND DISCUSSION

Analytical methods validation:

In the proposed validation study the following parameters were considered: linearity, precision, accuracy and sensitivity.

FAAS method:

Linearity: Linearity was evaluated through graphical representation of the measured absorbance at λ = 213.9 nm depending on total zinc concentration of standard solutions and depending on linear regression (least square technique) of the obtained calibration data.

The linear equation was y=0.3514x with a correlation coefficient of 0.9973.

Precision: The solution 10 ppm has been measured ten times. Standard deviation and relative standard deviation of the response have been calculated and the results are illustrated in **Table 2**. The RSD% for repeatability of sample preparation is1.9%.

Table 2: Repeatability of zinc determination by FAAS

Absorbance				Zinc concentration found (ppm)	SD	RSD%
Measure value 1	Measure value 2	Measure value 3	average value			
0.372	0.372	0.372	0.372	1.059	0.02	1.9%
0.370	0.372	0.371	0.371	1.056		
0.367	0.365	0.368	0.366	1.042		
0.354	0.353	0.352	0.353	1		
0.355	0.354	0.357	0.355	1.011		
0.356	0.356	0.357	0.356	1.014		
0.373	0.373	0.372	0.372	1.062		
0.361	0.36	0.359	0.36	1.025		
0.367	0.369	0.368	0.368	1.048		
0.371	0.375	0.373	0.373	1.062		

Accuracy: Concentrations of (0.5, 1, 1.5) ppm have been used to study the accuracy, **Table 3** shows the necessary data for obtaining the absolute error and the relative error.

Sensitivity: Results show that the limit of detection (LOD) of the method is 0.02 ppm and the limit of quantitation (LOQ) is 0.05 ppm.

Polarographic method:

Linearity: Linearity was evaluated through graphical representation of the measured peak current (Ip) at pH 4.6 depending on total zinc concentration of standard solutions and depending on linear regression of the obtained calibration data.

Concentration of (0.4, 0.8, 1, 1.2, 1.6, 1.8, 2, 2.2, 2.4, 2.6) ppm have been used to study the linearity. The linear equation was y=2120x with a correlation coefficient of 0.9992.

Precision: The solution 1ppm has been measured ten times. Standard deviation and relative standard deviation of the response have been calculated and the results were illustrated in **Table 4**.The RSD for repeatability of sample preparation is 1.78%.

Accuracy: Concentration of (0.4, 1.2, 2) ppm have been used to study the accuracy, **Table 5** shows the necessary data for obtaining the absolute error and the relative error.

Table 3: Accuracy of zinc determination by FAAS

Conc. ppm	Absorbance				conc. found ppm	Aver. conc. ppm	Absolute error	Relative error	Average relative error
	Meas. Value 1	Meas. Value 2	Meas. Value 3	Average value					
0.5	0.161	0.160	0.161	0.161	0.46	0.48	0.02	4%	3.1%
	0.189	0.188	0.189	0.188	0.53				
	0.155	0.158	0.157	0.157	0.45				
1	0.33	0.34	0.33	0.33	0.95	0.96	0.04	4%	
	0.342	0.345	0.343	0.343	0.98				
	0.336	0.338	0.335	0.336	0.96				
1.5	0.512	0.511	0.513	0.512	1.46	1.48	0.02	1.3%	
	0.53	0.528	0.531	0.529	1.5				
	0.520	0.519	0.519	0.519	1.48				

Table 4: Repeatability of zinc by polarographic method

NO.	peak current				Zinc concentration found (ppm)	SD	RSD%
	Measure value 1	Measure value 2	Measure value 3	Average Value			
1	2105	2105	2106	2105	0.993	0.017	1.78%
2	2099	2100	2101	2100	0.991		
3	2107	2107	2108	2107	0.994		
4	2092	2093	2095	2094	0.988		
5	2183	2182	2186	2184	1.03		
6	2142	2142	2141	2141	1.01		
7	2102	2104	2105	2103	0.992		
8	2163	2162	2161	2162	1.02		
9	2110	2109	2108	2109	0.995		
10	2161	2162	2164	2162	1.02		

Table 5: Accuracy of zinc by polarographic method

Conc. ppm	peak current				conc. found ppm	aver. conc. ppm	Absolute error	Relative error	Average relative error
	Meas. value1	Meas. value2	Meas. value3	Average value					
0.4	851.9	849.2	855.5	852.2	0.402	0.420	0.02	5%	2.63%
	916.6	918	919.1	917.9	0.433				
	896.3	896.5	910.2	901.0	0.425				
1.2	2651	2643	2656	2650	1.25	1.23	0.03	2.4%	
	2567	2576	2552	2565	1.21				
	2610	2612	2599	5607	1.23				
2	4293	4291	4293	4292	2.02	2.01	0.01	0.5%	
	4249	4252	4248	4249	2				
	4291	4289	4287	4289	2.02				

Sensitivity: Results show that the limit of detection (LOD) of the method is 0.004 ppm and the limit of quantitation (LOQ) is 0.04 ppm.

Comparing the previous results, both methods are selective, sensitive, and give satisfactory accuracy and precision as RSD is not more than 2.0% (**Table 6**).

Table 6: Comparing the results of FAAS and polarographic method

Method	FAAS	Polarographe
RSD%	1.9%	1.78%
Relative error%	3.1%	2.63%
LOD	0.02	0.004
LOQ+	0.05	0.04

Application: Both methods were further applied to determine zinc concentration in infant milk samples, the results obtained are shown in **Table 7**.

The voltammetric curve and the resultant standard curve for one milk sample by polarographic method determination are shown in **Fig. 1**.

Results in **Table 7** indicate that all studied milk gave similar results with the values written on the labels expect for samples B_1, E_1 and G_2 which gave results higher than values written on the labels.

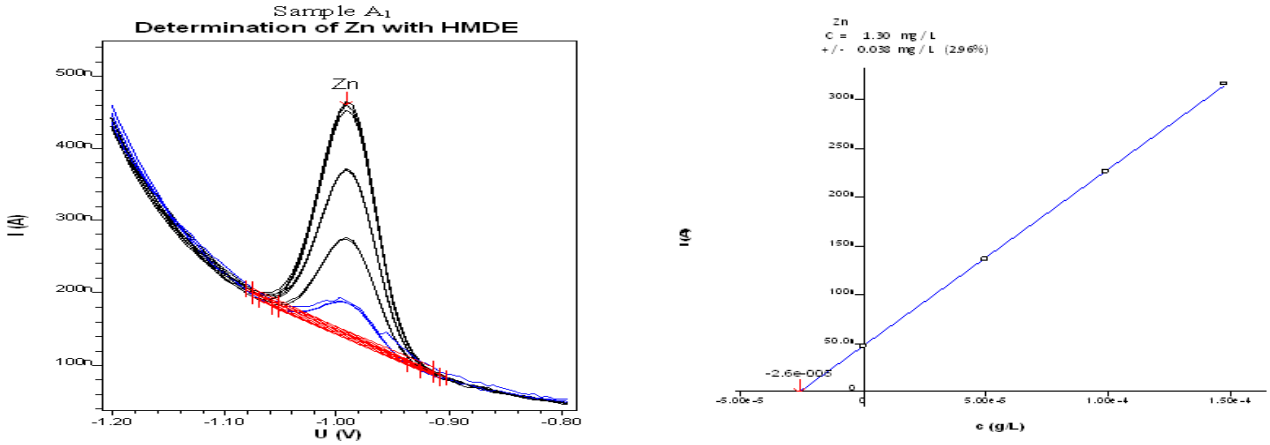

Figure 1: Voltammetric curve and standard curve of milk sample by polarographic method determination.

Table 7: zinc concentration in milk samples

Sample	Average of zinc conc. found by FAAS method (mg /100g)	Average of zinc conc. found by polarographic method (mg /100g)	Zinc conc.on the milk package label (mg /100g)
A_1	3.86	4	3.9
B_1	4.22	4.35	3.7
E_1	5.01	5.16	3.8
E_2	5.1	5.16	5
A_2	5.55	6.63	5.8
G_2	5.11	5.20	3.4
H_2	4.12	4.29	4.5
C_2	6.39	6.42	5.8
D_2	4.03	4.14	3.9
F_2	5.87	5.64	5.4

CONCLUSION

Results of this study showed similarity in precision, accuracy and selectivity between FAAS and polarographic methods for zinc concentration determination in infant milk samples, especially after applying some modifications which help canceling interferences. Despite the high sensitivity of the polarographic method, FAAS method was better due to the ease of its application. All the studied infant milk samples were within the recommended daily intake of zinc (5 mg) according to the recommendations of the Committee on Nutrition of the American Academy.

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