

## Analytical Potentiality of Computer - Controlled Background Correction with Electrothermal Atomic Emission Spectrometry : Determination of Copper in Milk Powder

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### ABSTRACT

A fully automatic electrothermal atomic emission spectrometry (ETA-AES) is described. This system is based on an echelle monochromator modified for wavelength modulation which is completely controlled by microcomputer . The advantages of the system in atomic spectrometry have been discussed . Aspects of the analytical performances such as calibration , detection limit , precision , and recovery for copper are considered . This system is applied for routine determination of copper in commercial powdered milk by slurry atomization versus aqueous atomization techniques .

### INTRODUCTION

During the last 25 years , several workers <sup>[1-6]</sup> , have attempted to develop such analytical systems based on commercial components to solve many problems associated with analytical atomic absorption spectrometry (AAS) . Such systems offer significant advantages in the measurement of AAS which allowed a high degree of improved operating automation , speed, real saving in analytical and data handling time , ease of use , and low costs . In addition , the capability of simultaneous multielement analysis by AAS

using a high speed PDP II computer was also designed <sup>[ 7 - 8 ]</sup> . The use of electrothermal atomiser as an emission source for trace element analysis has been extensively investigated by Ottawa's group at the university of starthcyld<sup>[9]</sup>. The progress in electrothermal atomic emission spectrometry (ETA-AES) was evident in the area of atomiser studies designed to improve excitation source <sup>[ 9 - 10 ]</sup> , development of automatic background correction <sup>[11-14]</sup> and introduction of the platform and probe atomization techniques as an

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efficient means for reduction of matrix interferences [15 - 18]. A system for the measurement of very sensitive ETA-AES signals has been designed based on single - channel spectrometrics SM III echelle spectrometer modified for wavelength modulation [13]. In this instrument the emission signals were measured via a lock - in amplifier on a chart recorder. The manual recording and subsequent processing of data creates a serious obstacle to efficient use of such a system, a side from the errors introduced by the analyst during the data manipulation which inevitably time consuming. In order to eliminate these problems the signal processing system in this instrument was replaced by an Apple IIe computer with little modification made on the previous system [13]. In this paper, some aspects of the operation and application of this system are described. In particular, the system was applied for the determination of copper in an infant milk powder using slurry sampling versus dry- ashing procedure. Copper was selected for two reasons, first it is the most interference - free element reported in atomic spectrometry, in order to evaluate the analytical performances of the present system [19]. Second, because of its importance as an essential nutrient for infants [20] and its toxicity when present at elevated level in milk which believed to cause many disease during early childhood [21-23] which is useful for the purpose of quality control.

## EXPERIMENTAL

### Instrumentation

The microcomputer - controlled ETA-AES used in this study is illustrated schematically. The system consists of an ET atomizer which is an HGA 500 graphite furnace and powder supply (Perkin- Elmer corporation) was used in conjunction with AS-1

autosampler. The spectrometer is a high resolution spectrometrics SMI III echelle monochromator modified for wavelength modulation for automatic background correction [13]. The specifications of these systems are given in table(1). The microcomputer is connected to the PMT output of the spectrometer via an A/D converter (type AI 13 instructive structure Inc) enabling the computer to record the analytical data automatically. The microcomputer is an Apple IIe system with 64k RAM and a double 5.25 in floppy disc unit for programme and data storage, and a dot matrix printer fitted with a Grappler interface. The operator communicates with computer via a keyboard and video unit. Data acquisition is achieved using an assembly language routine and the rest of the programme is written in BASIC language [24]. Wavelength selection at 324.75 nm was normally achieved by alignment using copper hollow cathode lamp. The instrumental parameters and optimised furnace programme are given in table (2).

### Reagents

Analar grade reagents were used throughout and all solutions were prepared in and diluted with doubly distilled water. A stock solution containing 1000 mg l<sup>-1</sup> copper was prepared by dissolving the appropriate amount of salt in dilute HNO<sub>3</sub>. The stock solution was acidified to a final acid concentration of 0.1 M and the solution was stored in an acid washed polyethylene container. Working Cu standard solutions were freshly prepared as required from this stock solution by dilution with water.

### PROCEDURES

Two procedures for sample preparation were investigated for the determination of Cu in commercial powdered milk. The initial step in each



procedure involved drying the milk sample in an oven at 110 °c for six hours .Slurry – Sampling Procedure : Approximately 1g of milk sample was slurred with 10ml of a 1:2:1 mixture of H<sub>2</sub>O : NH<sub>4</sub>OH : 1.4 dioxane [25]. The dispersion solution was then diluted to 100 ml with water . A 20 µl aliquot of this dispersion was injected into the atomiser via AS-1 autosampler for the direct analysis of Cu . Different concentrations of standard Cu were added to 5 ml portions of this slurry for the purpose of the standard additions method .

#### Dry – Ashing Procedure

1g of dried milk powder was mixed with 0.5 ml of 40% m/v magnesium nitrate ( purified ) as ashing aid and 1 ml of water in a fused silica crucible using a platinum wire . A cover was placed on the crucible and was dried overnight at 105-110 °c . The crucible was then placed in front of the open door of a muffle furnace at 450°c . The crucible was carefully observed to ensure that the sample was charred slowly . It was essential to ensure that the smoke was not allowed to catch fire . The evolution of smoke was controlled by moving the crucible gradually into the muffle furnace . When all the smoke had been evolved the crucible was moved to the back of the muffle furnace and incinerated at 450°c for one hour . The content of the crucible was then cooled and moistened with a few (3-5) 5µl aliquots of concentrated HNO<sub>3</sub> ( Aristar ) and were then re- ignited at 450°c for one hour . The ash was cooled and dissolved in 5ml of 2M HNO<sub>3</sub> and the solution transferred to a 100ml volumetric flask and diluted to mark with water .

#### Computer Programme

The data acquisition is achieved using an assembly language routine

and the remainder of the programme is written in BASIC language [24] . Figure (2) shows the sequence of operation involve in the use of microcomputer – controlled background correction system for ETA-AES . The AEFNSQ 5 programme consists of a series of a routine steps which are required for the measurement of emission signal from ET atomiser

( figure 2) . these steps are:

- Enter the experimental details such as experimental title , date , element , and wavelength.
- Select the integration limit (1-500) . The whole interval from 1 to 500 is equivalent from 0-10 s which is sufficient for most transient furnace signals.
- Select the X and Y axis scales for plotting the signal on the graphic display { Y-axis ( 1-33000) and X-axis (1- 5) }
- Print atomization data on the printer automatically and plotting for the graphic display can be printed optionally for individual signals by pressing (P) after each atomization.

After all experimental options are selected , the computer waits for the atomization trigger from the furnace . This signal is provided by programming the *Read function* on the furnace programmer for the start of the atomization cycle after the atomization data collected ( for a period of 10 s ) . The computer stores the number and reduces them to produce the atomization number , peak , location , peak height and peak area which printed on the display and on the printer . The system will then be ready to accept another atomization trigger . If any function is to be changed , this can be done via the AEFNSQ5 menu by pressing the ES-CAPE key on the computer keyboard . At the end of the atomization , the appropriate changes to the condition of



measurement may be made and (N) key is present to tell the computer that the next atomization is about to occur. The computer programme [24] provides a choice of analyte intensity measurement positions using 5-step square-wave modulation waveform. The instrument can compute the relative intensities at the center and in the wings of the line profile (figure .3) with reference to background intensity. Thus two sets of corrected peak height and area emission intensities can be generated for each atomization which allow calibration in a high and a low sensitivity mode using either or both peak height and peak area data [26].

## RESULTS AND DISCUSSION

### Optimization of Integration limits

Since a peak picking routine is employed for the measurement of peak height, this value is the same irrespective the integration limits selected. Integration limits (in the range 1-500 = 10 s) were optimized by measuring an emission signal for 100  $\mu\text{g l}^{-1}$  copper solution. An integration start of 35 and integration stop of 55 were found to give optimum peak area as shown in the figures (4) and (5). Using wide integration limits, the peak area was found to decrease markedly to a greater extent than might be anticipated from noise consideration. After correction of peak areas, it was shown that the optimum integration start time was 0.35 second (the peak areas obtained were multiplied by the number of points of integration ( $\text{NP}=\text{IE}.\text{IS}+1$ ) where IE the integration end and IS the integration start. Similarly, an integration stop time of about 1.1 second was found to be optimum after correction of peak areas

( $\text{NP}=\text{IE}$ ), but worse noise was obtained at higher integration stop times (i.e after 1.1 second) and if this compensated, a bigger integration time (i.e

more than 1.1 sec.) may give the same peak area.

### Effect of the HGA 500 Graphite Furnace On Emission Signal of Cu

It is known that the radiation emitted from the tube wall increases the signal noise in ETA-AES. The majority of this radiation is scattered by the furnace window furthest from the spectrometer. It was observed that by replacing this window by a carbon disc, the amount of noise on the signal was reduced. Therefore, the removal of both front and rear windows was found to further improve the S/N ratio. An enhancement in the signal was observed in addition to a reduction in the noise when both windows were removed. This signal enhancement may be due to an increase in residence time of atoms in the optical path since the vapor may leave the furnace along the length of the tube instead of through the central injection hole. No blanks were observed with both windows out.

### Optimization of ETA program

It was shown that a period of 35 s (5 s ramp) at 130°C was suitable for drying the aqueous copper solution (20  $\mu\text{l}$ , 100  $\mu\text{g l}^{-1}$ ), but the time was extended to 45 s for drying the milk samples. The windows of the furnace should not be removed to avoid the spreading of the solution along the graphite tube due to the gas flow out of the ends of the tube. Figure (6) shows the effect of temperature on the magnitude and precision of AE signals of 20  $\mu\text{l}$  of 50  $\mu\text{g l}^{-1}$  copper solution in which the front and rear windows were left in and then out. In the case when both windows are out, it is shown that RSD% of 8 injection at each temperature setting indicated a precision of approximately 1.8-3.9% with the optimum occurring at 2800°C. When both windows were in the precision was poorer at 2800°C. In addition, the magnitude of peak



height and peak area with windows in was a factor of two lower in magnitude than with windows out at each condition. The effect of increasing ash temperature to the copper AE signal was investigated and shown that it is optimum at 900°C for a period of 20 s with a time ramp of 1 s. The effect of gas flow on AE signals of Cu was examined for both windows in and out. It can be seen from figure (7) that the gas flow had an insignificant effect when windows of the furnace were removed. However, when both windows were left in, the sensitivity of Cu decreased markedly with increasing gas flow and was optimum in the gas stop mode. Thus removal of windows appears to have a similar effect to employing the gas stop mode in the furnace programme. This observation lends support to the idea that the increase in sensitivity with windows removed is related to atom residence time.

### Calibration Graphs for Copper

This system was used to construct calibration graphs for copper using a wide range of concentrations. One of the first group was in the range 20-800  $\mu\text{g l}^{-1}$  (fig. 8). It is evident that the linearity of the calibration graph is not effected using peak height 1 (i.e linear up to 100  $\mu\text{g l}^{-1}$  copper, curve A) and is similar to that observed with conventional ETA-AES<sup>[27]</sup>. However, using peak area 1, the linear portion is extended up to 200  $\mu\text{g l}^{-1}$  (curve B), and better precision is obtained at concentration between 100 and 200  $\mu\text{g l}^{-1}$  (RSD=1.3–2.3%) compared with peak height which gave a precision of 5.7–8%. The calibration graphs for higher concentration of Cu of 1000–10000  $\mu\text{g l}^{-1}$  were also constructed (fig. 8) using both peak height 2 (curve C) and peak area 2 data (curve D). Whilst the secondary graphs (less sensitive curve) deviate from the slope of unity, they are con-

siderably more linear than those on the primary graphs (more sensitive curves) and extend the effective working range of the techniques.

### Analysis of Cu in Milk Sample

Slurry – sampling method was employed in the determination of Cu in powdered milk and compared with dry – ashing method using peak area measurements to calculate the sample concentrations and both aqueous calibration and standard additions were used. The results are shown in table (3) and (4). The recoveries obtained were acceptable for both sample treatment method. However, in neither case was the standard addition graph found to be parallel to aqueous calibration graph indicating that an interference was present. The standard additions method gave results which were in good agreement with those obtained by probe atomization<sup>[27]</sup>, indicating the validity of the method. It is worth noting that the recovery of 130% (Table 4) statistically rejected. This high value may be due to the contamination of sample. The detection limits of copper in milk sample were found to be 4.3  $\mu\text{g l}^{-1}$  using dry-ashing and 6.6  $\mu\text{g l}^{-1}$  using slurry sampling method. These values are about 2-3 times as high as those obtained with aqueous Cu solution (2  $\mu\text{g l}^{-1}$ ). If better detection limit is required for Cu in milk solution, the sample must be diluted to 10 times instead of 100. The precision obtained for Cu in milk sample with dry-ashing was 3.9% which is very similar to that obtained with aqueous Cu solution (RSD = 3.8%). However the slurry solution gave a precision of 6.6% which is 1.5 times greater than that obtained with aqueous solution. This may be the spreading of the dispersion solution along the graphite tube during the drying stage.



### CONCLUSION

In this work, it has been shown that the calibration graphs can be extended by measurement of intensity of the center of the line profile. Thus two overlapping calibration graphs can be produced, for low & high concentration measurements, which exhibit an over-all linear dynamic range of 4-5 order of magnitude

In this system, peak area gives better linearity than peak height. This may be influenced by the method of integration. If correct integration limits are not chosen, the peak area appears to be reduced to some form of time averaging effect. The peak height is not affected. It is fully-automatic, but for the best sensitivity the windows of the HGA500 graphite furnace must be removed prior to atomization stage. This disadvantageous in the routine analytical situation. However, this system offers advantages in removing error sources arising from operator bias and reduce the time devoted to tedious data processing. The application of slurry-sampling method for Cu analysis in milk powder gives similar performances to that obtained with dry-ashing method and found to be practical, simple, rapid, less susceptible to contamination and less chemical reagents are consumed.

Table 1: Technical Specifications for Spectrametrics SMI III echelle monochromator System for measurement of atomic emission signals.

Component	Specification
Monochromator	Spectrametrics SMI III echelle Focal length: 0.75m Slit width : vertical 200 µm Horizontal 500µm Band-pass : at 400nm is 0.025nm Reciprocal linear: 0.062nm/mm at 200 nm Dispersion: to 0.25nm/mm at 800nm
Lens	F= 157mm
Detector	Hamamatsu R292 photomultiplier tube spectral response 185-650nm
Current preamplifier	Setting x10 Ortec Brookdeal
Lock-in amplifier	Ortec Brookdeal 950 3D
Wavelength modulation	Frequency at 60Hz, modulation interval = 0.144nm at 400 nm

Table 2: Instrumental Parameters and Optimized Furnace Programme for the Determination of Cu in Milk Powder

Parameter	Settings		
Wavelength, nm	324.75 nm		
Slit width, nm	200 vertical 500 horizontal		
Modulation frequency	At 60Hz		
Signal mode	Peak height & peak area		
Injected volume, (µl)	20		
Purge gas	N <sub>2</sub> (O <sub>2</sub> free)		
Furnace Programme	temp. °c	Ramp(s)	Hold time(s)
Dry	130	5	35
Ash	900	1	20
Atom	2800	0	5(gas stop)
Clean	2800	1	3

Table 3. Determination of copper in a milk powder sample by computer-controlled ETA-AES. Using Aqueous Calibration.

Procedure	Mean conc. Of Cu (µg g <sup>-1</sup> ) N=4	Mean recovery for addition of 2.5 or 5.0 µg g <sup>-1</sup> (%)
Dry-ashing	5.21± 0.75	112
Slurry-sampling	5.14± 0.43	108

Table 4. Concentration and Recovery of Cu in Milk Powder by Computer-controlled ETA-AES. Using Standard Additions Method

Procedure	Known increase conc. Of Cu adding to milk µg g <sup>-1</sup>	Measured conc. µg g <sup>-1</sup>	Conc. Of copper µg g <sup>-1</sup>	Recovery %	Mean Recovery %
Dry-ashing	0.00	5.8	5.8		92.5
	1.00	6.8		100	
	2.00	7.5		85	
	4.00	9.4		90	
	6.00	11.5		95	
Slurry	0.00	5.5	5.5		101
	1.00	6.8		130	
	2.00	7.6		105	
	4.00	9.8		107	
	6.00	11.0		91	

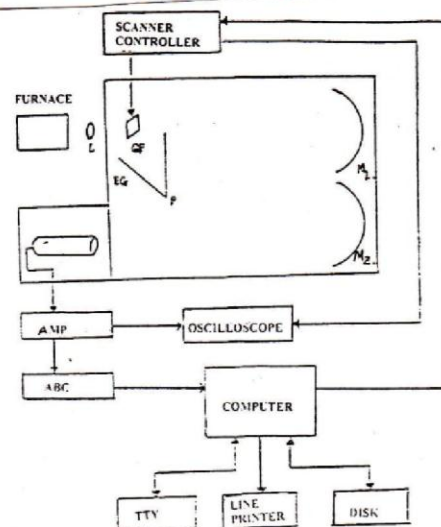


Fig.1: A block diagram of microcomputer-controlled ETA-AES. (L) Lens; (QP) quartz plate; (EG) echelle grating; (P) prism; (M<sub>1</sub> & M<sub>2</sub>) mirrors; (AMP) amplifier; (ADC) analog / digital convertor; (TTY) keyboard unit.

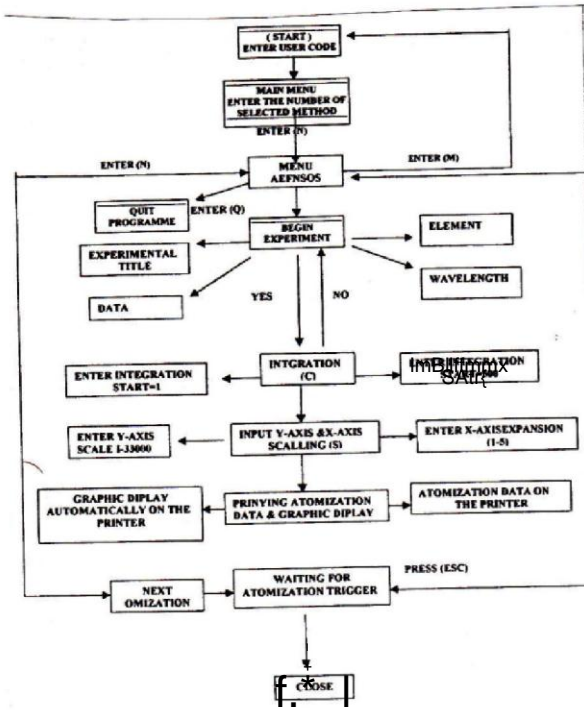


Fig (2)

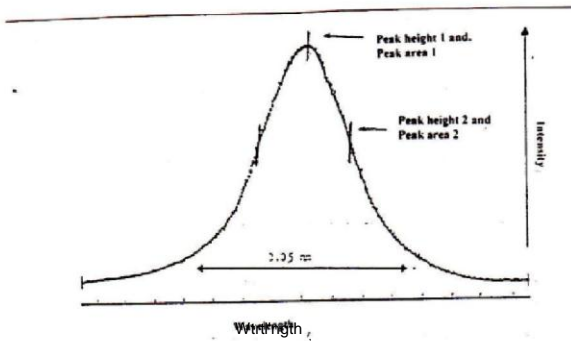


Fig.3: Emission intensity profile as observed on the oscilloscope using 5-step square wave wavelength modulation

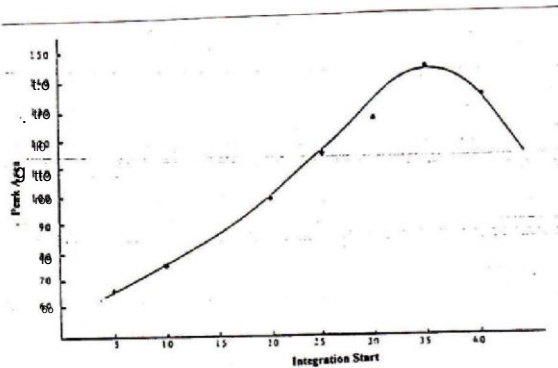


Fig.4: Variation of signal magnitude of Cu (20 pg; 400 pg l<sup>-1</sup>) with value of integration start at constant integration stop

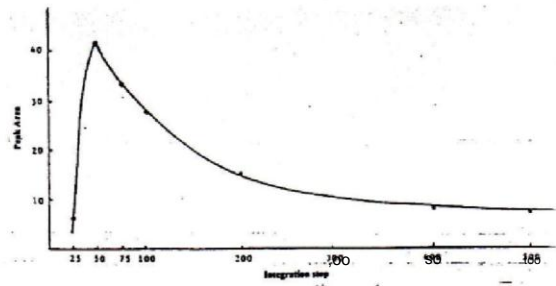


Fig.5: Variation of signal magnitude of Cu (20 pg; 100 pg l<sup>-1</sup>) with value of integration stop at constant integration start

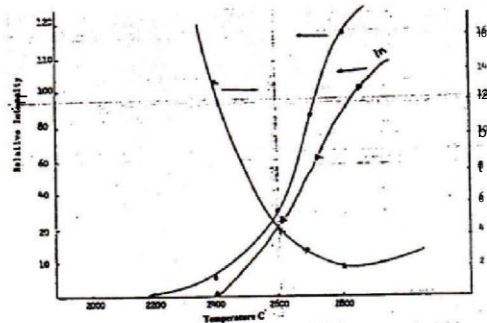


Fig.6: Effect of temperature on the signal of Cu when (A) both windows out (B) both windows in and (C) what is the position with respect to both windows out

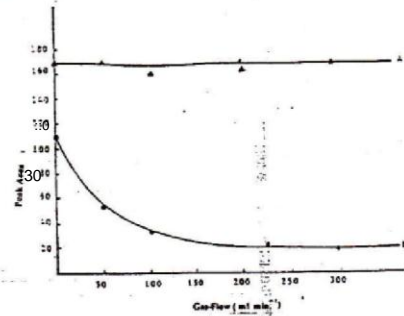


Fig.7: Effect of gas flow on Cu signal magnitude when (A) both windows out and (B) both windows in.

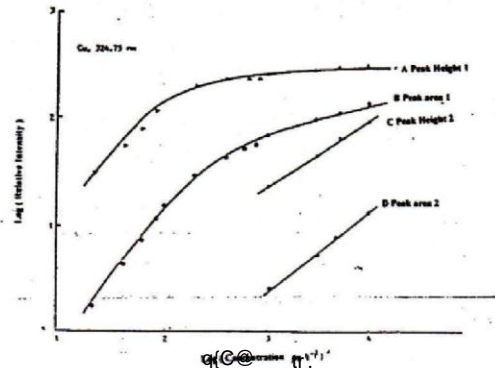


Fig.8: Emission signal modulation in ETA-AES using three modulating waveform. Upper curves- high sensitivity. A (A) peak height, B (B) peak area. Lower curves- low sensitivity. C (C) peak height, D (D) peak area.



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## دراسة الكفاءة التحليلية لمطيافية الانبعاث الذري الكهروحراري باستخدام الحاسوب : تقدير النحاس في حليب الاطفال

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### الخلاصة

لقد تمت دراسة أتمتة مطيافية التذرية الانبعاثية الكهروحرارية (ETA-AES) وذلك باستعمال موحد اللون ذي الميز العالي نوع echelle الذي تم تحويله لغرض تعديل الطول الموجي . جرت عملية مراقبة المعلمات التشغيلية للمنظومة باستخدام الحاسوب المايكروبي. لقد تم اختبار كفاءة هذه الطريقة من خلال دراسة المعلمات التحليلية مثل منحنيات المعايرة وحد الكشف والدقة والاستردادية بالمئة للنحاس حيث وضف النحاس الموجود في مسحوق حليب الاطفال لهذا الغرض . لقد استعملت تقنية التذرية بالردغة وتمت مقارنتها بالتذرية التقليدية.