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ESTIMATING PERFORMANCE PARAMETERS TO DETERMINE COPPER USING ATOMIC ABSORBTION SPECTROMETRY

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Performance parameters estimation is playing an important role in establishing the quality and accuracy of measurements taken using an analytical method for determining a parameter.

In this paper are studied the estimation of some performance parameters of an analytical method for the determination of copper by atomic absorption spectrophotometry. This method has proved to be linear, accurate and precise and can be used in any laboratory.

Keywords: copper, performance parameters, analysis methods, validation method

1. Introduction

Copper is a toxic, persistent and bio-accumulative heavy metal that cannot be easily metabolized, does not decompose and can affect human health [1]. In the human body, although copper is present in all body tissues, the brain, kidney, liver and heart contain the maximum amount. Excess of copper causes adverse effects on health, such as Wilson's disease, abdominal cramps, diarrhea, neurological and psychiatric defects, nausea, vomiting, headache, fatigue, while a its deficiency cause disturbances in raising children and anemia [2-5]. Main sources of exposure are inhalation (breathing air), ingestion (drinking water and food) and contact with skin (burning, itching and inflammation) [4].

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Copper is present in soil crust, rocks, water, air and food. It could be released into the environment both from industrial and natural sources. Industrial sources include: metallurgy, burning fossil fuels and wood, landfill and sewage. Among the known natural sources: forest fires, volcanoes, dust and decaying vegetation [6].

Due to its properties (corrosion resistance, malleability, ductility, electrical conductivity) it presents multiple industrial applications (transport, agriculture, medicine) and it is frequently used in electrical wires, switches, electroplating, pipes health, coins, metal alloys and fireworks [7].

Copper concentration could be determined by various analytical methods such as atomic absorption spectrometry (AAS), Inductively Coupled Plasma (ICP), ion selective electrode (ISE), emission spectrometry and anodic stripping voltammetry (ASV) [8].

The literature data indicates that these methods are dependent on the pH, laborious, time-consuming, applied on limited sample and with high interference. Among reagents used to determine copper applying spectrophotometric methods are known: 2-aminocyclopentenel – dithocaeboxylate; leucocrystal violet; Cysteine (RSH) by hexacyanoferrate; 3-methoxy-4-hydroxybenzaldehyed 4-bromophenyl hydrazine; 1,5-diphenylcarbazon (DPC); bromosulphonazo; Alizarin Red S; acetophenone-pchlorophenylthiosemicarbazone; 1,3-diaminepropen-3-propyl-anchored sillca gel; pyridylazo-4-phenyl-3-thiosemicarazone; DBH-PF; 2-(5-bromo-2-pyridylazo-5-diethylaminophenol; diantipyryl-(2-bromophenol) methan; 5-(2-benzothiazolylazo)-8-hydroxyquinoline; hydroxy-3-carboxy-5-sulfobenzene diazoaqminoazbenzene; 2-ketobutyric acid thiosemicarbazone; O-bromo phenyl fluorene; Salicylalde hidebenzoylhydrazone [3].

An alternative for mercury electrodes used for determining copper by voltammetric methods is the chemically modified electrodes (CMS). The substances used in obtaining chemically modified electrodes are: amoxicillin; cysteine; calix(4)arenes; diquinolyl-8,8'-disulfide; hexacyanoferrate; tetraethyl thio-ram disulfide; tetraphenylporphyrin; p-tert-Butylcalix(4)arenas; 1,2-methyl-bis (2-aminocyclopentene-carbodithioate) ethane; polypirrole pyrocatechol violet and 2-aminothiazole silica organo-functionalized.

Recent studies have developed a modified glassy carbon electrode that could be used for the determination of copper levels by anodic stripping voltammetry (ASV) [8].

Studies performed in time have shown that a number of techniques to remove the copper can be used with high efficiency, namely: reverse osmosis [9], adsorption [10], chemical precipitation [11], ion exchange [12], cementation [13], biosorption [14], electrodeionization [15], electrodialysis [16] and liquid membrane technique [17-19].

This study aims to establish some performance parameters for the determination of copper by atomic absorption spectrometry.

2. Experimental

2.1. Reagents

All analytical grade reagents have been used without further purification. Nitric acid (HNO_3, ρ = 1.4 g/mL) was purchased from Fluka, and standard copper solution 1000 mg/L Cu ($Cu\ (NO_3)_3$ in HNO_3 pure 0.5 mol/L) was purchased from Merck (Germany). The nitric acid of concentration 0.03 mol/L was prepared by diluting concentrated nitric acid (ρ = 1.4 g/mL). Reagents were prepared with distilled water.

2.2. Apparatus

Analytical control was achieved using an AAnalyst 800 (Perkin Elmer Instruments - US) atomic absorption spectrophotometer at 324 nm wavelength.

2. 3. Procedure

The principle of the method consists in nebulization by the flame of the atomic absorption spectrometer of the filtered and acidified sample to be analyzed. The direct determination of copper concentration is performed at 324 nm using the spectrometer facility of continuous background correction system.

The sample acidified with nitric acid (ρ = 1.4 g/mL), containing between 0.2 - 1 mg/L, is inserted into a 100 mL volumetric flask and completed then with distilled water.

In the same working conditions the water blank samples have been run.

The determination of copper concentration in a sample could be achieved by nebulization sample in a burner flame and measuring the absorbance at 324nm. The nebulizer was washed by aspiration of 0.03 mol/L nitric acid [20].

3. Results and discussion

According to the Eurachem Guidelines [21], the validation method is defined as "the process by which laboratory check it the method is suitable for the application for which will be used" and "the process by which laboratory determine its characteristics for performance and limitations of the method and by identifying the factors that influence these characteristics and the degree of influence of these factors. "

The validation method is performed using a set of performance parameters, namely: selectivity and specificity; linearity (the work concentration);

precision (repeatability, reproducibility and intermediate precision); accuracy; limit of detection; limit of quantification and robustness [22, 23].

In this paper the following performance parameters were evaluated: linearity; limit of detection [LOD]; limit of quantification [LOQ]; precision and accuracy.

Linearity

The ability of an analytical method to get results proportional to the concentration of the analyte in the sample defines linearity [24].

Linearity was demonstrated on the absorbance measured for six samples of copper standard solution different concentration - Table 1.

Table 1

Calibration data for copper

	Cu Concentration (mg/L)	A_1	A_2	A_3	A_4	A_5	$A_{med.}$
0	0.00	0	0	0	0	0	0
1	0.20	0.052	0.053	0.054	0.053	0.054	0.0532
2	0.40	0.107	0.105	0.105	0.106	0.105	0.1056
3	0.60	0.161	0.162	0.159	0.161	0.161	0.1608
4	0.80	0.209	0.213	0.211	0.209	0.210	0.2104
5	1.00	0.261	0.261	0.256	0.257	0.257	0.2584
6	1.20	0.317	0.315	0.313	0.315	0.316	0.3152

Then, the calibration curve for absorbance against concentration has been plotted - Figure 1. On this basis, some parameters of the linear regression analysis were calculated – Table 2.

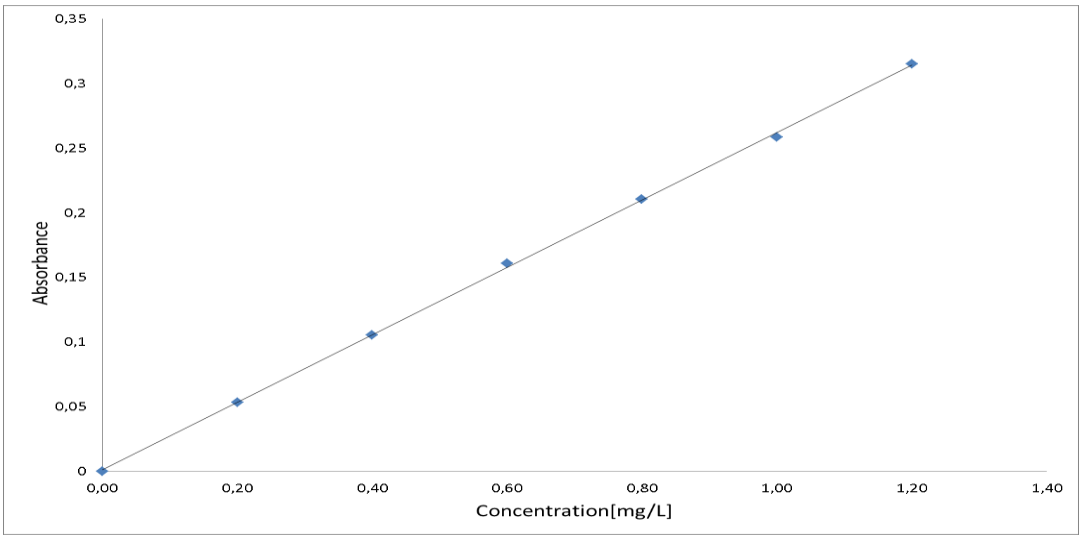

Fig. 1. Calibration curve for Copper at 324 nm

Table 2

Calculated parameters of the linear regression analysis

Statistic parameters	Value
Regression line equation	$y = 0.2609\ x + 0.0011$
Slope of the regression line, b	0.2609
Standard deviaton of the slope, s_b	0.0021
Intersection to the origin, a	0.0011
Standard deviation of intersection, s_a	0.0015
Correlation coefficient, R	0.9997
Determination coefficient, R^2	0.9994
Standard deviation, s	0.0000
Relative standard deviation, RSD	0.0000
Number of freedom degrees, v	5
Residual sum of squares	0.0000256
Regression sum of squares	0.0762
Standard deviation of estimate y	0,0023
Function F	14911,79

The limit of detection (LOD)

The lowest concentration or amount that can be detected against the blank and can be different from zero represents the limit of detection [21, 24].

LOD can be determined by measuring 10 samples blank, once each face blank and standard deviation of the sample blank.

The detection limit is calculated using the relation below:

$$LOD = x_{m(blank)} + 3\sigma$$

where: $x_{m(blank)}$ - the average concentration of the 10 blank samples [mg/L];

σ - standard deviation of the blank sample;

3 - reliable parameter;

$$LOD = 0 + 3 * 0$$
$$LOD = 0$$

The detection limit was calculated using the blank standard deviation and the slope of the calibration curve, varying only constant F.

The limit of quantification (LOQ)

The lowest concentration or amount can be determined with an acceptable level of repeatability and accuracy is the limit of quantification [21, 24].

LOQ can be determined by measuring ten blank samples, each one against blank and standard deviation recorded for each sample is calculated.

The limit of quantification is determined using the relation below:

$$LOQ = x_{m(blank)} + 10\sigma$$

where: $x_{m(blank)}$ - the average concentration of the 10 blank samples [mg/L];

σ - standard deviation of the sample blank;

10 - reliable parameter;

$$LOQ = 0 + 10 * 0$$

$$LOQ = 0$$

The limit of quantification was calculated using the blank standard deviation and the slope of the calibration curve, varying only constant F.

Precision

The degree of dispersion of a set of values obtained after independent measurements of multiple samples derived from a homogeneous sample under the same conditions of work represents precision [21, 24].

Under the rules drawn up by ICH (International Council for Harmonisation of Technical Requirements for Registration Guidance), precision is made up of three components: repeatability, intermediate precision and reproducibility [24].

Repeatability

Repeatability is independent results obtained from measurements performed on identical samples, using the same procedure in interworking laboratory by the same operator and in a short time [21, 24].

In this study, repeatability was demonstrated by measuring of six samples of 0.6 mg/L concentration each, in the same day, by the same operator and under the same conditions. The results recorded are presented in Table 3.

Table 3

Experimental results obtained from the analysis of samples performed to demonstrate the repeatability of the copper determination method using atomic absorption spectrometry, λ = 324 nm

Replicated measurements number	Measured signal, A (y_i)	x_i	$(x_i - \bar{x})^2$
1	0.1610	0.612495	4.7174×10^{-6}
2	0.1582	0.601763	7.3276×10^{-5}
3	0.1606	0.610962	4.0808×10^{-7}
4	0.1630	0.620161	9.6781×10^{-5}
5	0.1590	0.604829	3.0182×10^{-5}
6	0.1608	0.611729	1.9751×10^{-6}
		$\bar{x} = 0.610323$	$\sum (x_i - \bar{x})^2 = 0.000207$
Standard deviation, s	0.006440 mg/L		

RSD	0.0106
RSD%	1.06 %

Intermediate precision

Intermediate precision represents independent results obtained by measurement of identical samples, using the same procedure in interworking laboratory by different operators and different days [21, 24].

In this study, the intermediate precision was demonstrated by measuring the concentration of six samples of 1.0 mg/L concentration each in three different days by six operators, under the same conditions. The results obtained are presented in Table 4.

Tabel 4

Experimental results obtained from the analysis of samples performed to demonstrate the intermediate precision of the copper determination method using atomic absorption spectrometry, λ = 324 nm

Statistic parameters	Day 1	Day 2	Day 3
Average concentration [mg/L]	0.995528	1.003194	1.000224
The average absorbance	0.26083	0.262833	0.262058
The standard deviation, s	0.007359	0.002803	0.006181
RSD	0.007392	0.002794	0.006179
RSD%	0.7392	0.2794	0.6179

Accuracy

Approximation of actual value/reference value is accepted and found value is accuracy [21,24].

In this study, accuracy was demonstrated by comparing the result obtained from measurements of a sample of known concentration (0.4 mg/L Cu) with actual value measurement.

Accuracy is calculated using the relation below:

$$A\ (\%) = ((x_m - x) / x) * 100$$

where: A = accuracy, %;

x_m- average value of concentration obtained in the laboratory, mg/L;

x - the real value of the reference material concentration, mg/L;

The experimental data determined are presented in Table 5.

Table 5

Experimental results obtained from the analysis of samples performed to demonstrate the accuracy of the copper determination method using atomic absorption spectrometry, λ = 324 nm

Replicated measurements number	Measured signal, A, (y_i)	x_i
1	0.1056	0.400153
2	0.1058	0.400920
3	0.1058	0.400920
4	0.1050	0.397854
5	0.1068	0.404753
6	0.1046	0.396320
		$\bar{x}$ = 0.400153
Accuracy (A(%))	0.038	

4. Conclusion

In this study, emphasis was placed on performance parameter estimation in order to validate the method of copper determination by atomic absorption spectrometry.

The experiments have demonstrated that the method present a good linearity over the range 0 - 1.2 mg/L. This method has a good detection limit and limit of quantification. The method proved to be precise and accurate.

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