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Simultaneous Determination of Iron and Copper in Aqueous Solution Using Spectrophotometry

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Abstract Iron and copper mixtures were quantitatively determined in aqueous solutions for the first time using the analytical method in this approach with the aid of T60U Spectrophotometer. The method is so simple, fast, economic, and can be carried out easily on a bench. The absorbencies for iron, copper and iron-copper mixtures in aqueous solution were measured using T60U adopted with UVWin6 software UV -VIS Spectrometer (pg instruments United Kingdom) by filling two 5 ml quartz cells of dimensions 1*1*5 cm, the first is filled with the blank solution which is a solution containing all the constituents of the sample except iron and copper and the other quartz cell is filled with the stock solution iron and/or copper under investigation. Absorbency of iron was determined by setting T60U spectrometer wavelength at 504 nm, meanwhile for copper absorbency measurements the apparatus is set at 304 nm. Iron, copper, and iron-copper mixture calibration curves were of very high accuracy with the least linear regression value of ≤ 1, i, e the measured data were of relative standard deviation value (RSD) of ≤ 1.5%.

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Keywords: *iron assessment, coper assessment, plasma, serum, solvent extraction, spectrophotometry.*

1. INTRODUCTION

Spectrophotometry is a method to measure how much a chemical substance absorbs light by measuring the intensity of light as a beam of light passes through sample solution. The basic principle is that each compound absorbs or transmits light over a certain range of wavelength. This measurement can also be used to measure the amount of a known chemical substance. Spectrophotometry is one of the most useful methods of quantitative analysis in various fields such as chemistry, physics, biochemistry, material and chemical engineering and clinical applications.

Spectrophotometers are of **high clinical importance** in almost all branches of medicine. Their ability to measure concentrations of metabolically important substances in body fluids, such as **blood**, **cerebrospinal fluid**, **urine** and **amniotic fluid**, also for correct diagnostic findings and **continuous monitoring of patients**. The substances that can be quantitatively analyzed by spectrophotometers are numerous, **they include hemoglobin, erythrocytes, hematocrit, amylase, bilirubin, cholesterol, glucose, urea, creatinine, lipase, triglyceride, albumin, alcohol, ammonia, copper, magnesium, lactate, calcium, iron, magnesium, aluminum, sodium carbonate, carbon monoxide and even certain enzymes**.

The following example showing the high vital role for spectrophotometry: At least 1,000,000 such measurements per day on rather diverse equipment are made in this country.

lambert-beer's law, states that the relationship between absorbance and concentration exists as a straight according to the following equation:

$$A = \varepsilon\, b\, c$$

where **b** is the length of the path traveled by the monochromatic light through the sample, **C** is the concentration and **ε** is a molar absorptivity constant that depends on both wavelength and substance. This measurement can also be used to measure the concentration of a known chemical substance within the wavelengths of the maximum absorption (λ max.) or the minimum absorption (λ min.). When monochromatic light passes through a substance in a solution, the ratio of the transmitted light intensity (I) to the incident light intensity ($\mathbf{I_0}$) is called transmittance (**T**) as seen by equation - 1, while the common logarithm of the reciprocal of transmittance is called absorbance (**A**) equation – 2.

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$$T = \frac{I}{I_0} \quad \ldots\ldots\ldots\ldots\ldots \quad (1)$$

$$A = \log \frac{I_o}{I} = -\log T \quad \ldots\ldots\ldots\ldots \quad (2)$$

$A = \varepsilon\, b\, C$, $b = 1$ cm , hence

The spectrometer measures the intensity of the monochromatic light that passes through the solution to calculate the amount of light absorbed and the amount light transmitted, thus enabling us to determine the concentration of a substance sample taking in consideration the length of the cuvette equals 1 as seen from equation 3. **From the absorbance and transmittance, we can identify certain substances that we placed in the cuvette [1].**

$$\mathbf{A = \varepsilon\, C} \quad \ldots\ldots\ldots\ldots\ldots \quad \mathbf{(3)}$$

There are two major classes of devices:

Single beam spectrophotometer (Fig. 1):

Compares the light intensity between two light paths, one path containing a reference sample and the other the test sample. A single-beam spectrophotometer measures the relative light intensity of the beam before and after a test sample is inserted [1].

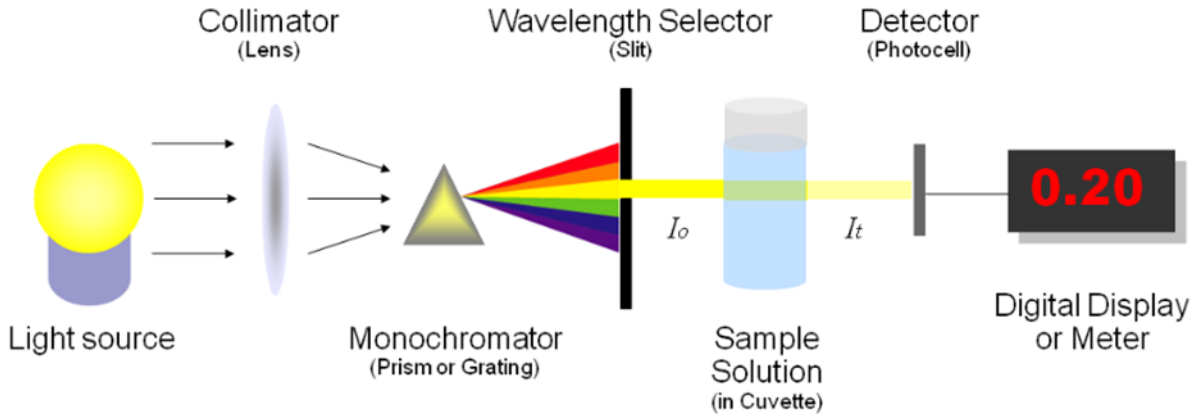

Fig.-1 *Schematic diagram of Single beam spectrophotometer.*

Although comparison measurements from double-beam instruments are easier and more stable, single-beam instruments can have a larger dynamic range and are optically simpler and more compact. Additionally, some specialized instruments, such as spectrophotometers built onto microscopes or telescopes, are single-beam instruments due to practicality (Fig. 2) [1].

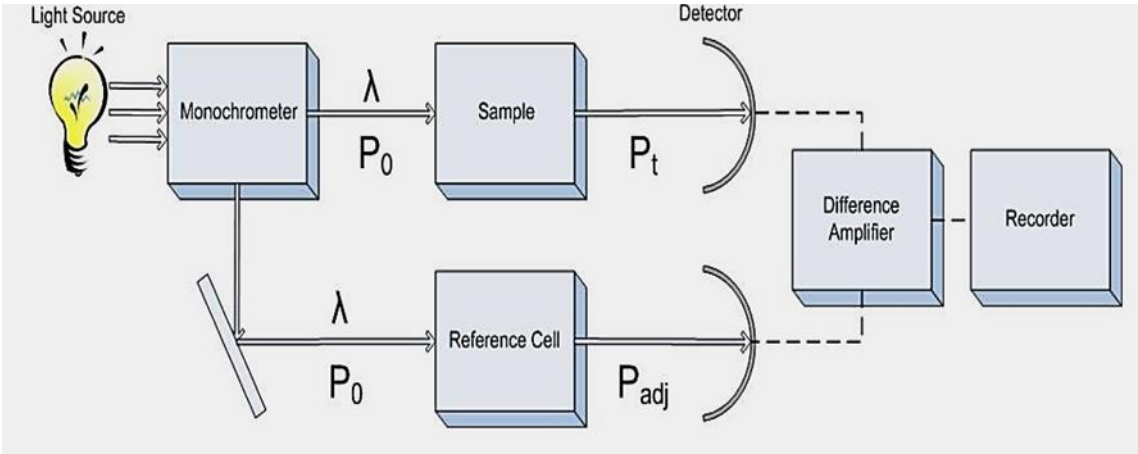

Fig.-2 *Schematic diagram of Double beam spectrophotometer.*

Iron level in blood is a reliable diagnostic indicator of various diseasestates. **Increased levels of iron concentration** in blood are associated with blood loss via increased destruction of red blood cells (e.g., hemorrhage) or decreased blood cell survival, acute hepatitis, certain sideroachresticanemias, ingestion of iron-rich diets, defects in iron storage (e.g. pernicious anemia). **Decreased levels of blood iron** may result from insufficient iron ingestion from diets, chronic blood loss pathologies, or increased demand on iron storage as during normal pregnancy.Simple, direct, and automation-ready procedures for measuring ironconcentrations find wide applications in research, drug

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discovery and environmental monitoring. Iron analysis was done using traditional trade kits, furthermore, Blood levels of iron and copper have been associated with a wide range of clinical illnesses.

Determination copper in an aqueous solution containing a mixture of iron, cobalt and nickel using spectrophotometry after treatment with coloring agents such as 1,5-bis (di-2-pyridyl methylene), DPTH, 2,4,6-tripyridyl-3,5-triazine, 2,9-dimethyl-1,10-phenanthroline hydrochloride, 1-nitro-2-naphthaline (with a nonionic surfactant), 2-2´-bipyridine and 1,10-phenanthroline [2-8]. Such procedures need a solvent extraction process which may not extract the elements under study quantitively.

The traditional technique for the determination of Cu &Fe as well as any elements mixtures is atomic absorption [9&10].

In this project a new analytical method depends on UV-VIS spectrometry using T60U spectrophotometer was tested and verified to determine the exact concentrations of iron and copper simultaneously in slightly acidic solutions looking forward applying the method blood analysis. Iron and copper mixtures were quantitatively determined in aqueous solutions for the first time using the analytical method in this research using T60U Spectrophotometer. The method is so simple, fast, economic, and can be carried out easily on a laboratory bench. The absorbencies for iron, copper and iron-copper mixture solutions were measured using **T60U UV -Vis Spectrometer adopted with UVWin6 software** (pg instruments United Kingdom) by filling two 5 ml quartz cells of dimensions 1*1*5 cm, the first with the blank solution which is a solution containing all the constituents of the sample except iron and copper and the other quartz cell is filled with the stock solution under study, the measuring the absorbencies for the solutions. **Absorbency of iron was determined by setting T60U spectrometer wavelength at 504 nm, meanwhile for copper absorbency measurements the apparatus is set at 304 nm.** Iron, copper, and iron-copper mixture **calibration curves were of very high accuracy with the least linear regression value of ≤ 1, i,e the measured data of relative standard deviation value (RSD) of ≤ 1.5%.**

2. PROCEDURE

2.1. Instruments

The instruments used were: T60U adopted with UVWin6 software UV -Vis Spectrometer – pg instruments United Kingdom as seen by figure -3, Cuvette, Quartz cuvettes, Centrifuge. Thermostatic shaker bath, digital pH meter, Sartorius 4-digit digital balance, Oven as well as variety of Pyrex glassware tools suck conical flasks, volumetric flasks, filtration funnel, beakers, filter papers, glass pipettes, measuring cylinders, watch glasses, spatula, and Eppendorf pipets.

Fig.-3 *T60 UV vis Spectrophotometer*

2.2. Materials

Chemicals: Ferric chloride pure and copper sulphate pentahydrate from BDH, England.

2.3. Experimental:

Preparation of stock solutions: - 3.443 g/dl Fe+3 primary stock solution was prepared by dissolving 16.2212 gm of pure ferric chloride in distilled water, then the solution was diluted to 100 ml in a Pyrex 100 ml (1 dl) volumetric flask at room temperature, then preparation of 1.5 g/dl, 0.75 g/dl, 0.1 g/dl, 0.5 g/dl, 0.025 g/dl and 0.01 g/dl stock solutions by dilution of the primary stock solution with distilled water in

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50 ml Pyrex volumetric flask at room temperature according to the formula and the pH of each solution brought to 2 by acidifying with the relevant acid according to the following relationship:

N1V1=N2V2

The absorbance was measured for each stock solution using T60U adopted with UVWin6 software UV -Vis Spectrometer (pg instruments United Kingdom) by filling the 5 ml quartz cells of dimensions 1*1*5 with the blank solution which is a solution containing all sample constituents except iron and copper, and the other quartz with the stock solution under study, (table-1). Absorbency of iron was determined by setting T60U spectrometer at 504 nm meanwhile for copper absorbency measurements the apparatus is set on 304 nm.

Table-1 *Absorbencies of different iron concentrations in aqueous solutions.*

Sample No.	Sample code	pH	g/dl	A
1	**1F**	2	0.01	0.036
2	**2F**	2	0.025	0.277
3	**3F**	2	0.05	0.34
4	**4F**	2	0.1	0.39
5	**5F**	2	0.75	1.11

- 1.273 g/dl Cu+2 primary stock solution was prepared by dissolving 12.48 gm of copper sulphate pentahydrate (CuSO4.5H2O) by distilled water in a 100ml volumetric flask at room temperature, then 0.5 g/dl, 0.1 g/dl, 0.05 g/dl, 0.02 g/dl and 0.01 g/dl by dilution the primary stock solution with distilled water in 50 ml volumetric flask at room temperature, the absorbencies were measured for each standard solution (Table-2).

Table-2 *Absorbencies of different copper concentrations in aqueous solutions*

Sample No.	Sample code	pH	g/dl	A
1	**1C**	**2**	**0.01**	**0.251**
2	**2C**	**2**	**0.02**	**0.236**
3	**3C**	**2**	**0.05**	**0.263**
4	**4C**	**2**	**0.1**	**0.305**
5	**5C**	**2**	**O.5**	**0.849**
6	**6C**	**2**	**1.273**	**1.981**

Stock solutions of iron and copper mixtures were prepared by mixing equal volumes of iron and copper stock solutions (10 ml, each) as seen in table 3.

Table-3 *Absorbencies of different iron and copper concentrations in aqueous solutions.*

Sample No.	Sample code	Fe			Cu		
		pH	g/dl	A	pH	g/dl	A
1	**1m**	**2**	**1.722**	**0.219**	**2**	**0.637**	**3.594**
2	**2m**	**2**	**0.375**	**0.078**	**2**	**0.05**	**2.897**
3	**3m**	**2**	**0.05**	**0.044**	**2**	**0.025**	**2.613**
4	**4m**	**2**	**0.025**	**0.032**	**2**	**0.01**	**2.502**

3. RESULTS AND DISCUSSION:

Absorbencies of iron and copper stock solutions were measured using T60U adopted with UVWin6 software UV -Vis Spectrometer.

A graph plots for absorbencies versus concentration (*gm/dl*) were done using anew version of graph plotter 4,4,1 as seen by figures 4&5. The plots are straight lines which verify the straight-line equation:

y= mx + b, where m is the slope ($\Delta y/\Delta x$) which is the extinction coefficient (ϵ) of each case:

ϵ=1.2302 *dl/g* for Fe as seen by figure 4.

, ϵ=1.3894 *dl/g* for Cu (Fig. 5).

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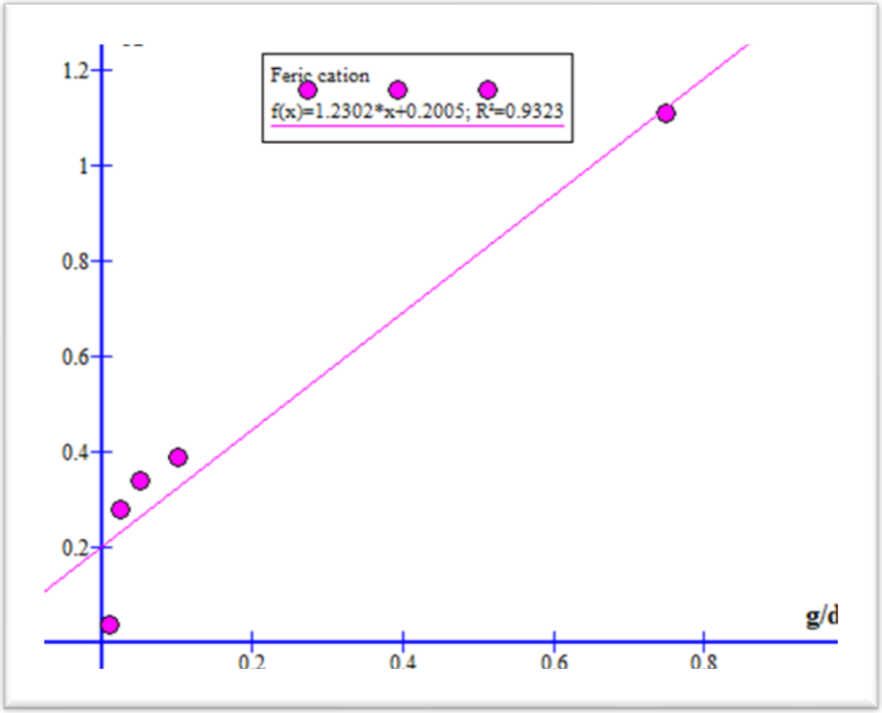

Fig.-4 *Calibration curve of Ferric ion at 504 nm.*

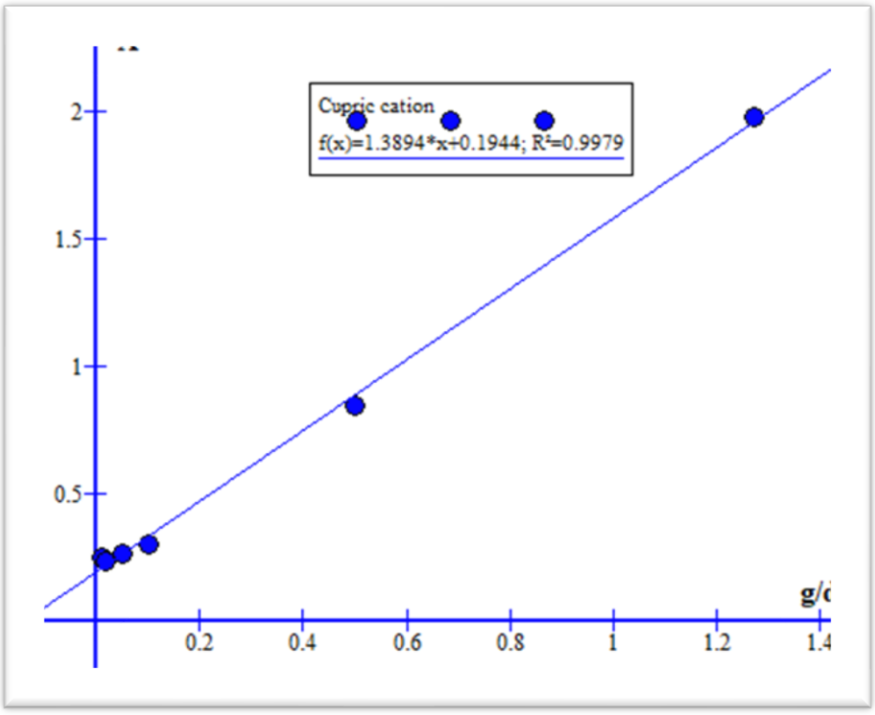

Fig.-5 *Calibration curve of cupric cation at 304 nm.*

Figure 6 is showing the simultaneous determination of iron and copper in the mixture solution by inserting lines parallel to x-axis through absorbency point value, then a perpendicular line on the x-axis (parallel to y-axis), the intercept point with the x-axis indicating the concentration of element under investigation. Blue intercepting lines indicating copper values and brown lines indicating iron values. Also, it is possible to calculate each concentration by the following formula, according to Lambert -Beers law relationship (3):

$$A = \varepsilon C \qquad \ldots\ldots\ldots\ldots\ldots \qquad (3)$$

hence, **$C = A/\varepsilon$, where A and ε values are known as discussed earlier.**

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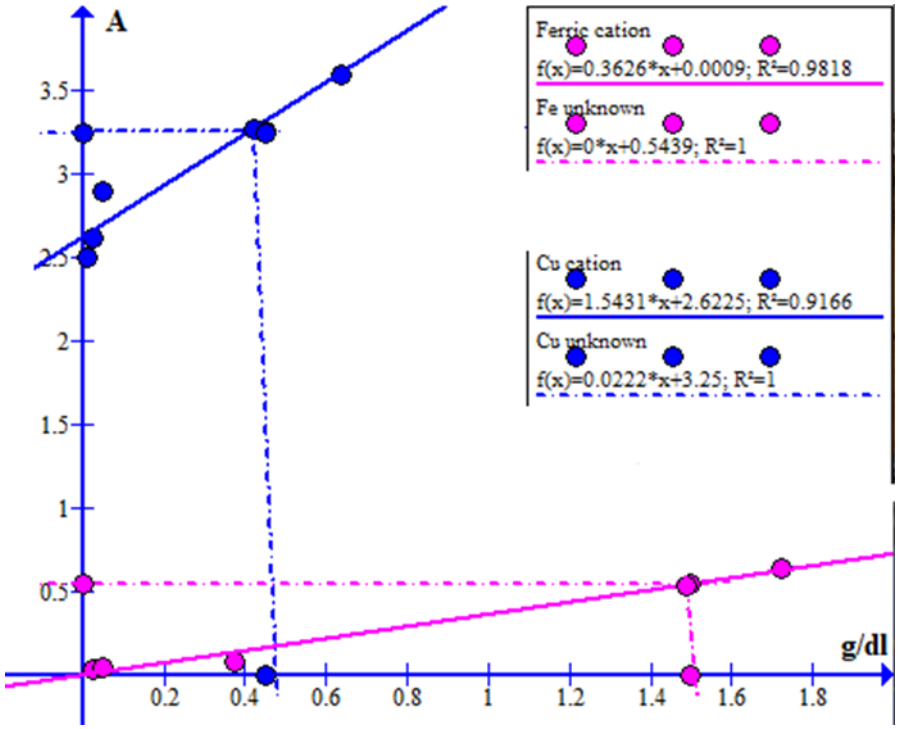

Fig. 6 *Calibration curves of iron and copper.*

The authors like to suggest applying this practicable, easy, accurate, and fast method to the analysis of blood components instead of using many valuable traditional kits that contain highly aromatic compounds with healthy side effects.

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