Spectrophotometric Determination of Copper(II) using 2,2[O-Tolidine-4,4-bis azo]bis[4,5-diphenyl imidazole](MBBAI)

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Abstract:
Spectrophotometric method was developed for the determination of copper(II) ion. Synthesized (2,2[O-Tolidine-4,4-bis azo]bis[4,5-diphenyl imidazole]) (MBBAI) was used as chromogenic reagent at pH=5. Various factors affecting complex formation, such as, pH effect, reagent concentration, time effect and temperature effect, have been considered and studied. Under optimum conditions concentration ranged from (5.00-80.00) μg/mL of copper(II) obeyed Beer`s Law. Maximum absorption of the complex was 409nm with molar absorptivity 0.127 x 10 ^3 L.mol ^-1 .cm ^-1. Limit of detection(LOD) and Limit of quantification were 1.924 and 6.42 μg/mL, respectively. The stoichiometric composition of the chelate is 1:2 (Cu:MBBAI). Experimental results for studying some selected ions as interference were reported. The developed method was successfully applied to determine copper (II) ion in dental filling.

Key words: Azo dye(MBBAI), Copper (II), Dental filling and Spectrophotometry...

Introduction:
Copper (II) is a heavy metal which can pollute the environments widely when released from industry and agriculture for this reason, there has been an interest by the researcher in studying quantitative estimation methods of copper(II)(1). Spectroscopic methods are one of the most famous methods to determined trace amount of metal in their complexes. It is cheap, simple and has an excellent sensitivity (2). Copper is a fundamental follow supplement to plant and life of the creatures. It is found basically in blood stream and temperature of humans as a co-factor in different enzymes, generally, high measures of copper can be toxic and even fatal to life forms. It is exceedingly harmful if present in drinking water. Copper is likewise a fundamental component for hemoglobin amalgamation, rectify nerve working, and bone improvement, Copper insufficiency causes ischemic coronary illness, iron deficiency, and abnormal wool growth(3). Many techniques are used to determine copper including atomic absorption spectrometry (4-6), potentiometry (7-11), inductive coupled plasma-emission spectrometry (12), inductive coupled plasma-mass spectrometry(13).

Materials and Methods:
Reagent and Solutions:
All analytical reagents and solutions used in preparation are in high purity.
Preparation of Standard Solutions.
Copper (II) solution (100μg/mL) was prepared by dissolving 0.029 gm from Cu(NO3)2 in 100 mL distilled water.
Sodium hydroxide solution (0.1M) was prepared by addition of 50 mL distilled water to 0.2 g of sodium hydroxide .
Nitric acid solution (0.1M) was prepared by diluting 0.18 mL concentrated nitric acid(65%,1.41 g/cm ^3 ) in 50 ml distilled water.

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Reagent solution (MBBAI) (1000µg/ml) was prepared by dissolving appropriate weight (0.1g) in absolute methanol and complete the volume to100ml with methanol.

**Synthesis of Ligand 2,2[o-Tolidine-4,4-bis azo]bis[4,5-diphenyl imidazole](MBBAI)**

The reagent was prepared by conventional method of a diazotization aromatic amine (O-Tolidine)(22). The reagent 2,2[O-Tolidine -4,4-bis azo]bis[4,5-diphenyl imidazole] was prepared by reacting an amount of diazonium salt of O-Tolidine with (4,5,diphenyl imidazole) as starting materials(Alaa Frak et.al)(23 - 24).

**Interferences**

Cations solution of (Co²⁺, Cd²⁺, Ag⁺, Ni²⁺) ions (1 mg/mL) were prepared by dissolving (0.155g) of Co(NO₃)₂, (0.105g) of Cd(NO₃)₂, (0.078g) of AgNO₃, (0.155g) of Ni(CO₃)₂, respectively, in 50 mL distilled water for each.

**Dental Filling Application**

Preparation done by dissolving (0.10g) from dental filling in (10mL) nitric acid (65%), then the solution was filtered and stored in a dark 100 mL bottle. The filtrate acidified with 3M HCL to precipitate silver ion(Ag⁺) as silver chloride and separated by filtration. The filtrate which contained copper ion(II) was applied for spectrophotometric analysis.

**Preliminary Study**

2 mL from prepared solution of copper(II) (100µg/mL) were placed in test tube, then 1mL from prepared solution of ligand (MBBAI) (1000µg/mL) was added to the test tube drop by drop with shaking with observing the formation of color or precipitate, then drops of nitric acid (0.1M) were added to a portion of this mixture and drops of NaOH(0.1M) were added to the other portion to study the effect of acid function. It was found that color formed clearly in acidic media while there was no change in color in basic media.

**Results and Discussion**

**Absorption Spectra**

The absorption spectra of reagent (MBBAI) and the complex Co(II)-MBBAI with ratio of 50%(v/v) were scanned against methanol as blank by mixing 1 mL of prepared reagent solution (1000 µg/mL) with 1mL of prepared copper ion(II ) solution (200µg/mL) at ambient temperature.

**Optimization of Reaction Conditions**

**pH effect**

A series of copper complex solutions were prepared by mixing the concentration (50 µg/mL) of Cu²⁺ ion with the reagent solution at a concentration (600µg/mL), and the pH was adjusted at different values between (1-7) using HNO₃(0.1M)/NaOH(0.1M) at 20°C for 5 min.
Fig 3 explains that the maximum absorbance was around 5.00 after that the absorbance decreased, this may be due to the formation of precipitations(24).

**Effect of Reagent Concentration**

Solutions of copper(II) (50µg/mL) and various concentration of the ligand (100-900)µg/mL were mixed to study the effect of reagent concentration under optimum conditions. The pH adjusted to 5.00 and the $\lambda_{\text{max}}$ at 409 nm.

![Figure 4. Effect of ligand concentration.](image)

Fig 4 shows that the maximum absorbance of ligand obtained at the concentration of (600)µg/mL after that the absorbance decreased.

**Effect of Addition Sequences**

The effect of addition sequences were tested following the sequences summarized in table (1) which was followed to study the effect of sequences of addition under optimum conditions.

| NO. | Sequences of addition | Absorbance of Co(II)-MBBAI complex |
|-----|-----------------------|-----------------------------------|
| 1   | M+L+pH                | 0.146                             |
| 2   | L+M+pH                | 0.048                             |
| 3   | M+pH+L                | 0.095                             |
| 4   | L+pH+M                | 0.024                             |

M=Metal , L=Ligand

From results illustrated in Table (1) the first order addition was adopted due to the maximum absorbance obtained.

**Effect of Time**

Stability of copper(II)-MBBAI complex along period of time(5-80) min. was studied under optimum conditions.

Gradually high temperature affected the stability of complex due to the dissociation of the complex, this can be noticeable from Fig6. 20 °C was chosen for optimization.

**Calibration Curve**

Series of solutions with different concentration of copper(II) ranged from (5-80)µg/mL were mixed with the ligand (60)µg/mL under optimum conditions to obtain the calibration curve by plotting the absorbance against the concentration.
Fig 7 explains that the Copper(II) obeyed Beer’s law in concentrations (5.0-80.0)µg/mL.

### Accuracy and Precision of the Described Method

Accuracy and precision were determined for the applied method in term of recovery and relative standard deviation (RSD%), respectively. Results of recovery and RSD% were illustrated in table 2.

| Analyte | Concentration (µg/mL) | Measured concentration(µg/mL) | RSD% n=3 | Recovery % |
|---------|-----------------------|-------------------------------|----------|------------|
| Cu²⁺   | 30                    | 29.959                        | 0.230    | 99.863     |
|         | 50                    | 49.912                        | 0.016    | 99.824     |
|         | 70                    | 69.806                        | 0.140    | 99.722     |

Results in Table 2 explain that the developed method was precise as the value of relative standard deviation was <0.3%.

The analytical Parameters for the proposed method are list in table 3.

### Table 3. Analytical parameter for copper (II) determination

| Parameter                                | Value      |
|------------------------------------------|------------|
| \( \lambda_{\text{max}} \) (nm)         | 409        |
| Linearity range (µg/mL)                  | 5-80       |
| Molar Absorptivity (Lmol⁻¹cm⁻¹)          | 0.127x10⁻⁴ |
| Sandell’s Sensitivity (µg/cm²)           | 0.040      |
| Limit of detection\(^a\) (µg/mL)         | 1.924      |
| Limit of quantification\(^b\) (µg/mL)    | 6.415      |
| Regression Equation                      | \( Y=0.0020x+0.0297 \) |
| Slope                                    | 0.002      |
| Correlation coefficient (\( R^2 \))      | 0.9995     |

\(^a\) Limit of detection(LOD)=(SD/S)*3.3  
\(^b\) (Limit of quantification)LOQ=(SD/S)*10  
where SD is standard deviation , S is the slope of calibration curve

### Determination of Stoichiometry and Formation Constant

Mole ratio method in addition of Job’s method of continuous variations were chosen to study the composition of the complex formed, results illustrated in Figs 8 and 9. Both methods indicated that the ratio of metal ion to reagent molecules (M:L) was (1:2) at pH=5.0.

### Job Method

In this method mixture of different volumes of the solution in equal concentration (1x10⁻⁴ M) from both ion (Cu²⁺) and ligand were mixed.
Effect of Foreign Ions

Definite amount of cations and anions were used as a foreign ions to study the possibility of the interferences with determination of Cu(II) ion, results are explained in table (4).

Table 4. Effect of foreign ion on the determination of Cu(II) ion.

| Foreign ions | Formula structure of ions | Absorbance without interferences | Cations | E% |
|--------------|---------------------------|----------------------------------|---------|----|
| Co²⁺        | Co(NO₃)₂                  | 0.146                            |         |    |
| Cd²⁺        | Cd(NO₃)₂                  | 0.053                            | -63.96  |    |
| Ag⁺        | AgNO₃                    | 0.146                            | 0.00    |    |
| Ni²⁺        | Ni(NO₃)₂                  | 0.087                            | -38.73  |    |
| Cl⁻        | KCl                      | 0.146                            | 0.00    |    |
| NO₃⁻        | KNO₃                    | 0.153                            | 4.97    |    |
| SO₄²⁻        | K₂SO₄                  | 0.227                            | 55.48   |    |
| I⁻        | KI                      | 0.176                            | 20.54   |    |

Some ions were selected to study the effect of the interferences with Cu(II) ion (Table 4), it was found that some of the ions increased the absorbance while the others decreased the absorbance, this was due to the competition of these ions with Cu(II) to form the complex with the ligand which decreased the competition and increased the sensitivity of this method towards Cu(II) ion. The reaction was specific and sensitive for Cu(II). Selectivity of reaction can be confirmed by using suitable masking agents.

Complex Stability Study

Mole ratio method was used to determine the stability constant of the colored complex depending on the equilibrium reaction for the complex(25). Calculations illustrated in Table 5.

Table 5. Value of stability constant for Cu(II) complex.

| Complex       | Am | As | α    | K(stability) |
|---------------|----|----|------|--------------|
| Cu[MBBAI]₂    | 0.050 | 0.043 | 0.140 | 0.818x10⁹   |

The results in Table 5 explain that the complex has high stability, for that it is possible to use the ligand(MBBAI) in the spectral estimation of copper ion.

The effect of temperature on the stability constant for the Cu-MBBAI complex.

The values of stability constant of Cu(II) with the reagent (MBBAI) were studied at various temperatures ranged from (10-60)℃. The results are illustrated in Table (6).

Table 6. The effect of temperatures on the stability constant for Cu(II) complex.

| T(K)  | Am | As | α | Kx10⁹ |
|-------|----|----|---|-------|
| 10    | 283.150 | 0.177 | 0.053 | 0.700 | 2.266 |
| 20    | 293.150 | 0.171 | 0.0510 | 0.702 | 2.245 |
| 30    | 303.150 | 0.165 | 0.0490 | 0.703 | 2.223 |
| 40    | 313.150 | 0.156 | 0.0460 | 0.705 | 2.188 |
| 50    | 323.150 | 0.150 | 0.0440 | 0.707 | 2.162 |
| 60    | 333.150 | 0.130 | 0.0380 | 0.708 | 2.145 |

Results obtained in Table 6 explained that there is a limited effect of temperatures on the stability of complex.

Thermodynamic Function of the Complex.

Thermodynamic function ∆H, ∆G and ∆S were calculated, results were illustrated in Fig 10 and Table 7.

Table 7. The effect of temperature on thermodynamic function for Copper(II) complex.

| T(K)  | Log. K | ∆H(KJ/mole) | ∆G(KJ/mole) | ∆S(KJ/mole, K) |
|-------|--------|-------------|-------------|----------------|
| 283   | 3.532  | -34.449     | 0.201       |                |
| 293   | 3.411  | -35.643     | 0.190       |                |
| 303   | 3.299  | -36.834     | 0.179       |                |
| 313   | 3.193  | -38.008     | 0.170       |                |
| 323   | 3.095  | -39.189     | 0.161       |                |
| 333   | 3.002  | -40.381     | 0.152       |                |

Negative value of enthalpy explained that the reaction was exothermic for that, it can be noted by decreasing the temperature the possibility of
complex formation will increased, in addition to that the reaction was spontaneous according to the negative sign of free energy. The stability of the complex was confirmed due to the value of entropy which approach to zero (less random and spontaneous).

Study of FT-IR Spectra for Ligand and Complex

Figs 11-12 and Table 7 explain the FT-IR study and the absorption frequencies for reagent and the reagent-MBBAI.

![Figure 11. FT-IR spectrum of ligand](image)

![Figure 12. FT-IR spectrum of complex](image)

**Table 8. FT-IR absorption frequencies for reagent and the reagent-MBBAI.**

| Compound | MBBAI  | [Cu(MBBAI)] |
|----------|--------|-------------|
| v(N-H)   | 3057w  | 3421        |
| v(C-H)Ar | 2895m  | 3061        |
| v(N≡N)   | 1477m  | 1454        |
| v(C≡C)   | 1543m  | 1541        |
| v(C≡N)   | 1633m  | 1649        |
| (M-O)    | -----  | 455         |
| (M-N)    | -----  | 540         |

w=weak, m=medium

**The Suggested Figure for the Complex**

Suggestion of the complex structure as shown in figure 13 is due to FT-IR spectra and the stoichiometry obtained from Job and Mole ratio methods.

![Figure 13. The suggested structure for the complex.](image)

**Application**

A sample of dental filling provided by Nordiska dental company, Sweden was prepared to apply the developed method. The composition of alloy is Ag 69.9%, Sn 18.8%, Cu 10.2% and Zn 1.1%. The preparation was done by dissolving (0.10 g) from dental filling in (10mL) nitric acid (65%), then the solution was filtered and stored in a dark 100mL
bottle. The filtrate was acidified with 3M HCL to precipitate silver ion (Ag⁺) as silver chloride and separated by filtration. The filtrate which contains copper ion(II) was applied for Spectrophotometric analysis. A comparison between the labelled concentration and measured concentration of the dental Filling was illustrated in Table 9.

Table 9. Result of the application for copper(II) in sample of dental Filling .

| Container | Spectrophotometric method | E% (n=3) | Rec.% (n=3) |
|-----------|---------------------------|----------|-------------|
|           |                           | 10.2%    | 10.3%       | 0.1         | 100.1       |

Results obtained in Table 9 show that the method has high sensitivity towards copper(II) ion and can be applied for the colorimetric determination(26).

Conclusion:
Simple, fast and inexpensive method was developed for the determination of copper(II). Validation studies and application explained that copper(II) can determined quantitatively by using this developed method. Results obtained showed that the reagent is specific for the determination of copper(II) in pharmaceutical and water samples. Analytical parameters such as specify, limit of detection, accuracy and recovery indicate that this method can be applied successfully for the determination of copper(II).

Conflicts of Interest: None.

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التقدير الطيفي للنحاس الثنائي باستخدام 2,2-O-توليدين-4,4-بيس أزو (MBBAI) (أيميدازول) 

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الخلاصة:
تم تطوير طريقة طيفية لتقدير النحاس الثنائي باستخدام الكاشف اللوني 2,2-O-توليدين-4,4-بيس أزو (MBBAI) (أيميدازول) عند دالة حامضية تساوي 5. تأثير الدالة الحامضية على تركيز الimin جامع، احتمال التغيرات على تركيز النحاس الثنائي، تم تطبيق هذه الدراسة باليورونكيرن لمتداولة تأثيرات، تم دراستها حسب القوانين المطلوبة في دراسة الطاقة تأثير درجة الحرارة وتأثير الزمن، تم أن تثير أكسيد النحاس الثنائي عند درجة حرارة لا تتجاوز 5.00 °C. L mol⁻¹ cm⁻¹) = 0.127х10⁴، واتنافسة مولارية (Cu: MBBAI) عن محتوى كميات ملليجرام (µg/mL) = 80.00 عالى التوالي. احتمالات الحديد الكيميائي كانت 1.924 و 6.415 µg/mL (1:2) تم دراسة تأثير بعض الايونات كمبتلات مع أيون النحاس وتم تطبيق هذه الدراسة في دراسة النحاس الثنائي في نموذج حشوة السن.

الكلمات المفتاحية: التقدير الطيفي، النحاس الثنائي، حشوة السن، وصبغة الأيزور.