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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) $\mu\text{g/mL}$ of copper(II) obeyed Beer's Law. Maximum absorption of the complex was 409nm with molar absorptivity $0.127 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$. Limit of detection(LOD) and Limit of quantification were 1.924 and 6.42 $\mu\text{g/mL}$, 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 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),

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and flow injection catalytic photometric method (14).

Azo compounds, as well as imines, are usually used to obtain stabilized metals with low oxidation state through the expansion of delocalized π electron system leading to absorbed shorter visible light(15). Generally, azo compounds can be used as dyes and pigments due to the highly colored (16). Azo compound has many application in various fields in industries as well as in pharmaceutical preparations (17-19). Azo compound has thermal and optical properties and this leads to many important application in toner (20-21).

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 (100 $\mu\text{g/mL}$) was prepared by dissolving 0.029 gm from $\text{Cu}(\text{NO}_3)_2$ 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.

Reagent solution (MBBAI) (1000 μ g/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 (Co^{+2} , Cd^{+2} , Ag^{+} , Ni^{+2}) ions (1 mg/mL) were prepared by dissolving (0.155g) of $\text{Co}(\text{NO}_3)_2$, (0.105g) of $\text{Cd}(\text{NO}_3)_2$, (0.078g) of AgNO_3 , (0.155g) of $\text{Ni}(\text{CO}_3)_2$, 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) (100 μ g/mL) were placed in test tube, then 1mL from prepared solution of ligand (MBBAI) (1000 μ g/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 μ g/mL) with 1mL of prepared copper ion(II) solution (200 μ g/mL) at ambient temperature.

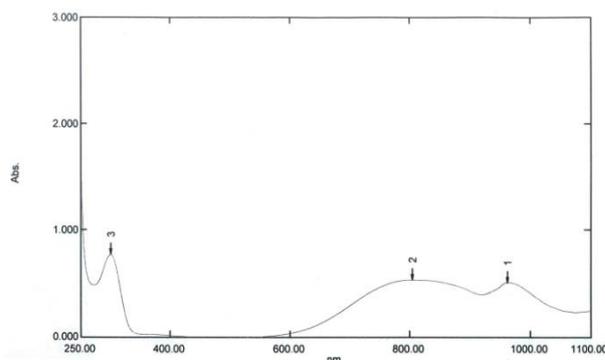


Figure1. The spectrum of reagent (MBBAI).

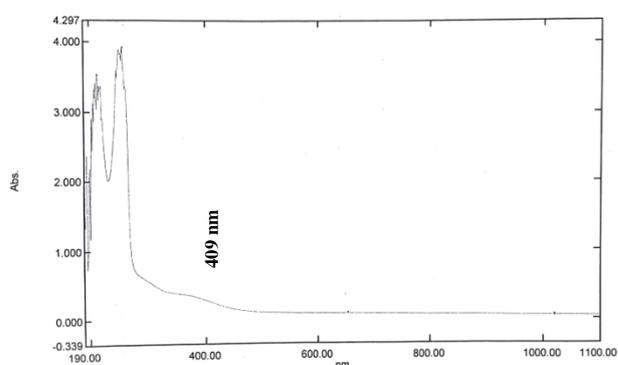


Figure 2. The spectrum of Cu(II) complex with (MBBAI) reagent.

Spectrums obtained in figs 1 and 2 showed that there are three peaks for the reagent at 300,800 and 900 nm in figure 1 and appearance of new peak in 409 nm in figure 2 due to the coordination between the ligand and copper (II) ion.

Optimization of Reaction Conditions

pH effect

A series of copper complex solutions were prepared by mixing the concentration (50 μ g/mL) of Cu^{+2} ion with the reagent solution at a concentration (600 μ g/mL), and the pH was adjusted at different values between (1-7) using HNO_3 (0.1M)/NaOH(0.1M) at 20C^o for 5 min.

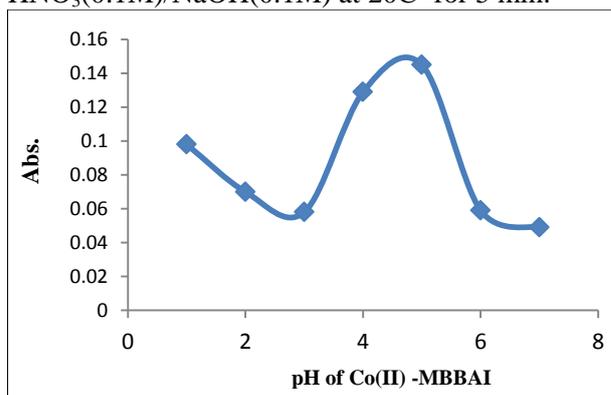


Figure 3. Effect of pH value

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

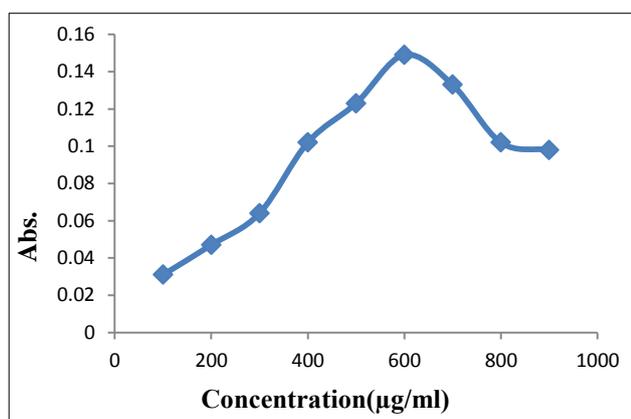


Figure 4. Effect of ligand concentration.

Fig 4 shows that the maximum absorbance of ligand obtained at the concentration of (600) $\mu\text{g}/\text{mL}$ 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.

Table 1. Effect of sequences of addition

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.

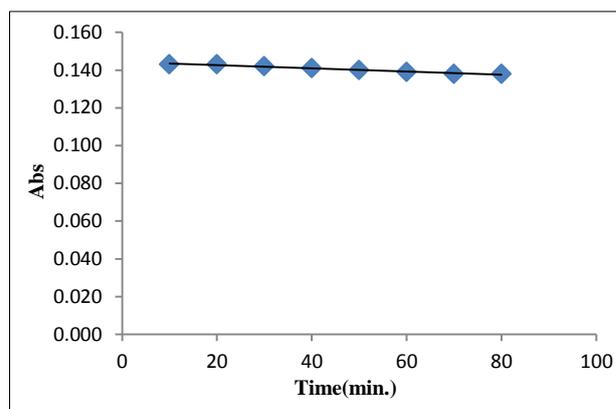


Figure 5. Effect of time.

Fig 5 explains there is excellent stability during long period(5-80)min. , this is due to the strong coordination between copper(II) and the ligand.

Effect of Temperature

Temperature may affect the stability of complex, for that, the effect of temperature (20-60) $^{\circ}\text{C}$ was studied under optimum conditions.

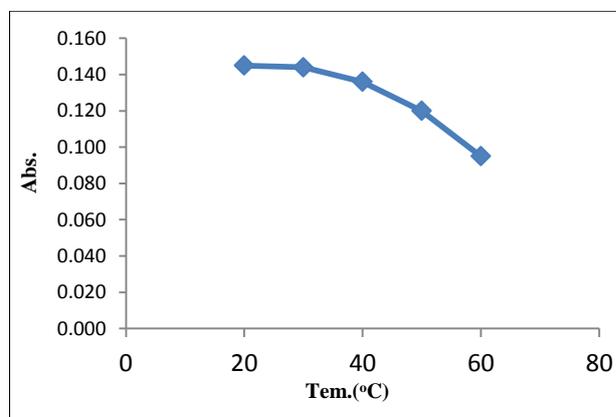


Figure 6. Effect of temperature.

Gradually high temperature affected the stability of complex due to the dissociation of the complex, this can be noticeable from Fig6. 20 $^{\circ}\text{C}$ was chosen for optimization.

Calibration Curve

Series of solutions with different concentration of copper(II) ranged from (5-80) $\mu\text{g}/\text{mL}$ were mixed with the ligand (60) $\mu\text{g}/\text{mL}$ under optimum conditions to obtain the calibration curve by plotting the absorbance against the concentration.

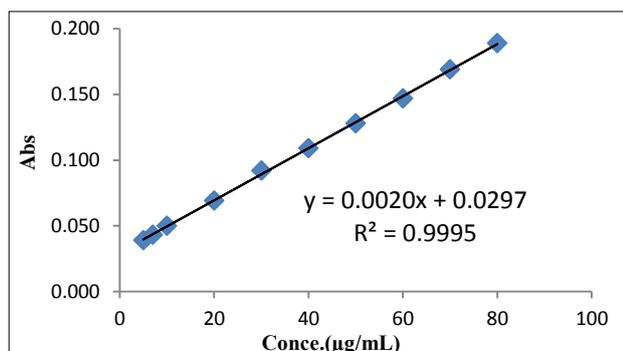


Figure 7. Calibration curve for Spectrophotometric determination of copper(II).

Fig 7 explains that the Copper(II) obeyed Beer's law in concentrations from (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.

Table 2. Accuracy and precision studies

Analyte	Concentration (µg/mL)	Measured concentration(µg/mL)	RSD% n=3	Recovery%
Cu ⁺²	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

λ_{max} (nm)	409
Linearity range (µg/mL)	5-80
Molar Absorptivity (Lmol ⁻¹ cm ⁻¹)	0.127x10 ⁴
Sandell's Sensitivity (µg/cm ²)	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 (Cu⁺²) and ligand were mixed.

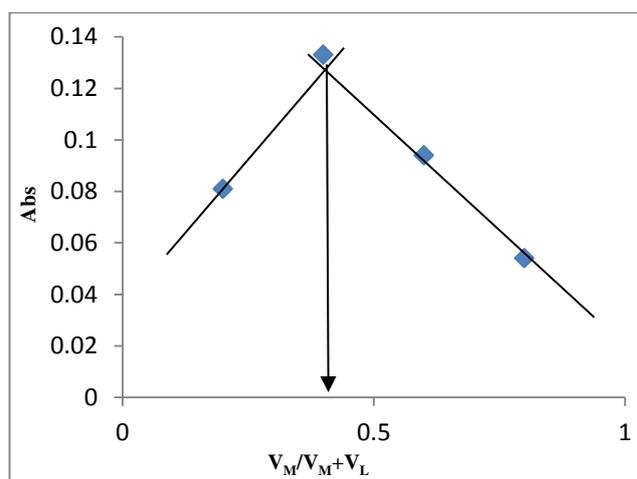


Figure 8. Job's method of continuous variations.

Mole ratio Method ligand

Carried out by mixing known concentration of Cu(II) ion (3.1x10⁻⁴M) with increasing concentration of (MBBAI) (1.55x10⁻⁴-12.4x10⁻⁴M)

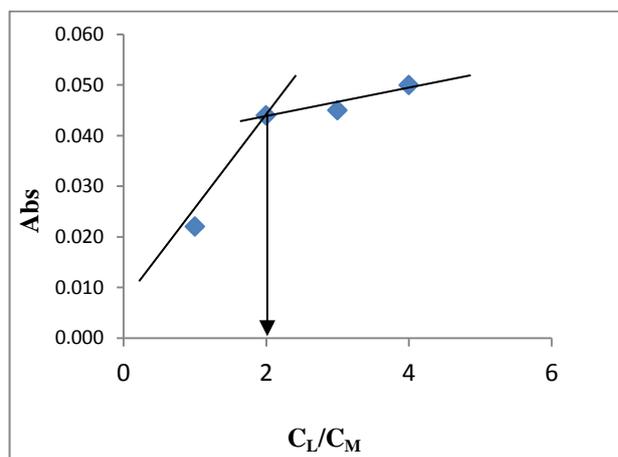


Figure 9. Mole ratio method

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).

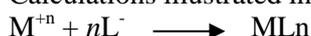
Table 4. Effect of foreign ion on the determination of Cu(II) ion.

Foreign ions	Formula structure of ions	50µg/mL	E%
		Absorbance after addition of ions	
Cations			
Absorbance without interferences		0.146	
Co ⁺²	Co(NO ₃) ₂	0.053	-63.96
Cd ⁺²	Cd(NO ₃) ₂	0.011	-92.46
Ag ⁺¹	AgNO ₃	0.146	0.00
Ni ⁺²	Ni(NO ₃) ₂	0.087	-38.73
Anions			
Cl ⁻¹	KCl	0.146	0.00
NO ₃ ⁻¹	KNO ₃	0.153	4.79
SO ₄ ⁻²	K ₂ SO ₄	0.227	55.48
I ⁻¹	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 this 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 determin the stability constant of the colored complex depending on the equilibrium reaction for the complex(25). Calculations illustrated in Table 5.



$$K_{\text{stability constant}} = \frac{[ML^{+n}]}{[M^{+n}][L^{-}]^n}$$

$\alpha = \frac{A_m - A_s}{A_m}$ Where A_m is the greatest absorption and A_s is Absorption at the stoichiometry

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

Complex	Am	As	α	K(stability)
Cu[(MBBAd) ₂]	0.050	0.043	0.140	0.818x10 ⁹

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

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

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

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

T(C ^o)	T(K)	Am	As	α	Kx10 ⁹
10	283.150	0.177	0.0530	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 ΔH , ΔG and ΔS were calculated, results were illustrated in Fig 10 and Table 7.

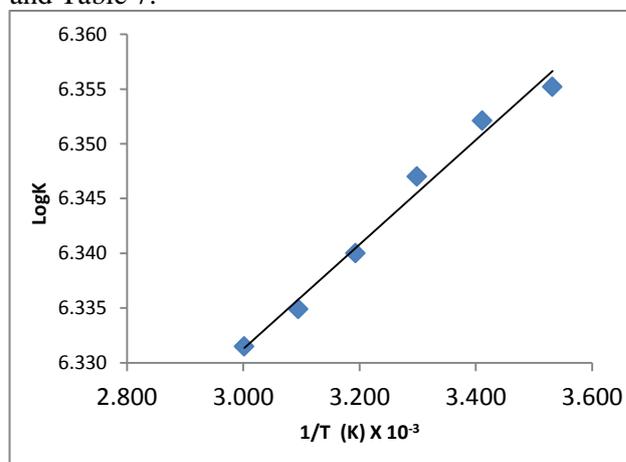


Figure 10. Relation between Log K and 1/T values for copper(II) complex.

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

T(K)	1/T x10 ⁻³ (K ⁻¹)	Log. K	ΔH	ΔG (K.J/mole)	ΔS (K.J/mole. K)
283	3.532	6.355		-34.449	0.201
293	3.411	6.351		-35.643	0.190
303	3.299	6.347	91.3320	-36.834	0.179
313	3.193	6.340		-38.008	0.170
323	3.095	6.335		-39.189	0.161
333	3.002	6.332		-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.

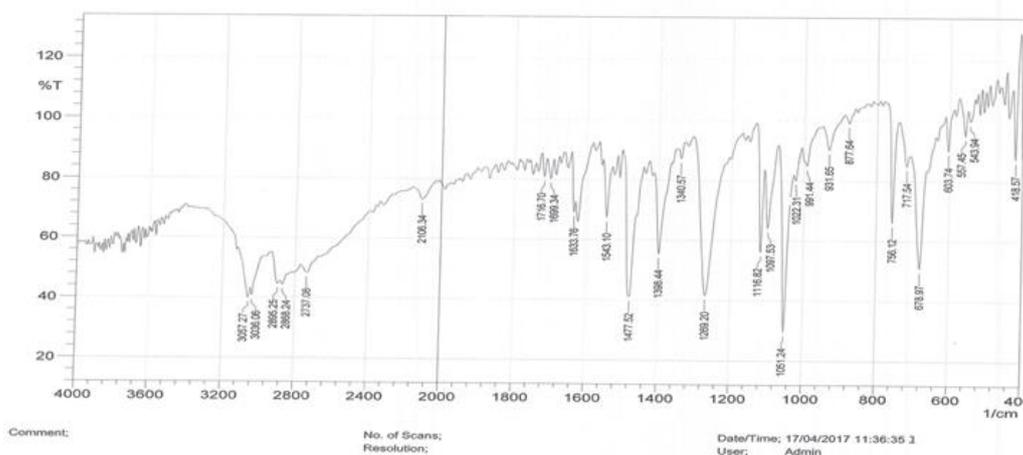


Figure 11. FT-IR spectrum of ligand

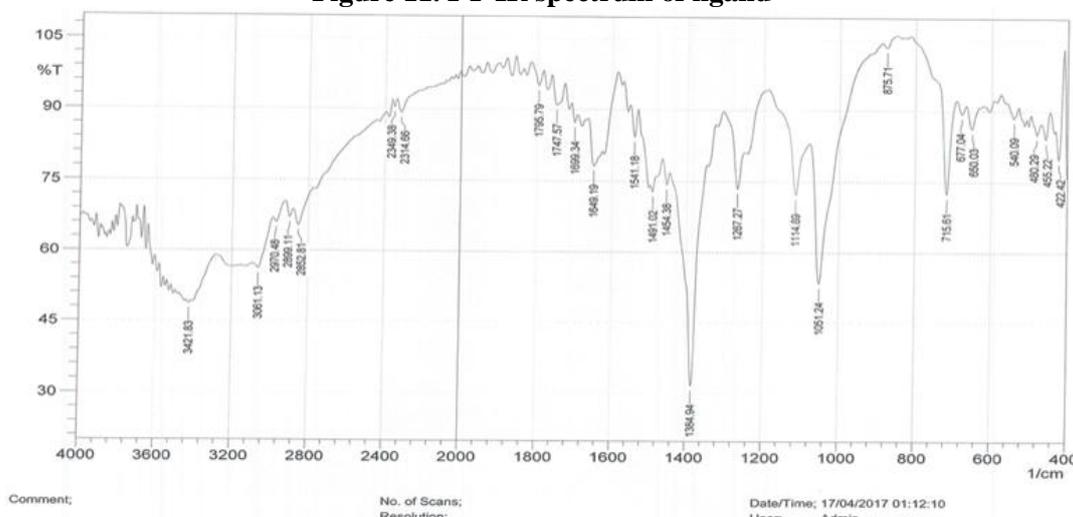


Figure 12. FT-IR spectrum of complex

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

Compound	MBBAI	[Cu(MBBAI)]
$\nu(\text{N-H})$	3057w	3421
$\nu(\text{C-H})_{\text{Ar}}$	2895m	3061
$\nu(\text{N=N})$	1477m	1454
$\nu(\text{C=C})$	1543m	1541
$\nu(\text{C=N})$	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.

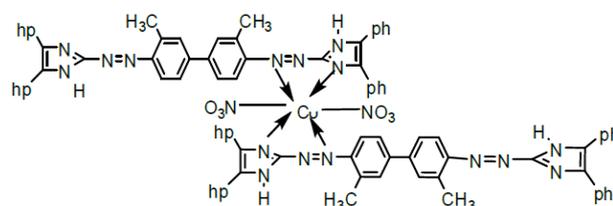


Figure13. The suggested structure for the complex.

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^{+1}) 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 .

Contain	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 [O]-2,2- توليدين-4,4- بس أزو] بس [4، 5- داي فنيل ايميدازول] (MBBAI)

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² قسم الكيمياء، كلية العلوم، جامعة كربلاء، كربلاء، العراق.

الخلاصة:

تم تطوير طريقة طيفية لتقدير النحاس الثنائي باستخدام الكاشف اللوني [O]-2,2- توليدين-4,4- بس أزو] بس [4، 5- داي فنيل ايميدازول] (MBBAI) عند دالة حامضية تساوي 5. عوامل مختلفة تؤثر على تكوين المعقد، مثل، تأثير الدالة الحامضية، تركيز الكاشف، تأثير الوقت وتأثير درجة الحرارة تم أخذها بنظر الاعتبار ودراستها كانت الدراسة مطاوعة لقانون لامبرت بير عند تراكيز تتراوح بين 5.00- 80.00 µg/mL من النحاس الثنائي. الامتصاص الأعظم للمعقد كان عند 409 nm وامتصاصية مولارية $0.127 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$. حدود الكشف التحليلية والكمية كانت 1.924 و 6.415 µg mL⁻¹ على التوالي. اتحادية المعقد هي (Cu: MBBAI)(1:2). تمت دراسة تأثير بعض الايونات كمتداخلات مع ايون النحاس وتم تطبيق هذه الطريقة بنجاح في تقدير النحاس الثنائي في نموذج حسوة السن.

الكلمات المفتاحية: القياس الطيفي، النحاس الثنائي، حسوة السن و صبغة الايزو.