A Rapid and Sensitive Colorimetric Sequential Injection System for Cu(II) ion Determination Using New Azo Reagent

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ABSTRACT

A sensitive and accurate Sequential Injection Analysis technique for determining Cupper (II) ion in different samples, using a new azo derivative reagent of 8hydroxy quinoline (HQDABA). The reagent and Cu(II) complex diagnosis included Infrared spectroscopy, Ultraviolet-Visual radiation, Elemental Analysis (CHN), Energy dispersive X-ray spectroscopy (EDX), nuclear magnetic resonance spectroscopy ¹HNMR and mass spectroscopy techniques. The method depends on the reaction of Cupper (II) with the (Azo derivative of 8-hydroxy Quinoline) reagent in a neutral medium to form a Brown complex which gave a maximum absorbance at 420 nm. The optimum conditions such as flow rate, Copper (II) volume, reagent volume; pH, reagent concentration, and reaction coil length are thoroughly examined. A Copper (II) curve is constructed, and the suggested method obeys Beer's law over ration range of 1-10 mg/ L. The limits of detection (LOD) and on (LOQ) are determined to be (0.361) mg/ L and(1.204) mg/ L, while Sandell's sensitivity and molar absorption coefficient are to be (0.120) μ g/ cm² and (527.05) L/ mol. cm respectively. The recommended method has given good accuracy with recovery rates ranging from (77.82% - 115%).

Keywords: Sequential Injection Analysis, cupper, different samples, determination, Azo derivative Of 8-hydroxy Quinoline

INTRODUCTION

The sequential injection technique relies on injecting materials sequentially into the loading coil, then pulling them in the reverse direction and then pushing them in the forward direction for the purpose of reducing dispersion after the best mixing is obtained and the reaction process in the loading coil is completed. This is done by controlling the rapid change in the direction of the carrier current flow to Backwards, then forwards until a complete reaction is obtained. This technology is characterized by the consumption of microscopic quantities of materials, and the selective or selective valve replaces the injection valve[1-5]. The method of choice is typically different models and flow currents, as well as the branched flow pipes that are simple and a suitable injection valve linked to the branched pipes. All these features [3, 4, 6]. Making SIA technology flexible and easy to adapt to the chemical systems to be worked on. In this work, the suggested approach used a reagent derivative of 8-hydroxy Quinoline to give a simple, fast and sensitive way to estimate cupper ions and to identify the optimal reaction conditions to achieve the best results [1, 3, 5, 7-9]. sequential injection methods were used with several methods as spectroscopic method[10-12] and chromatography methods [13-16], to determination copper.

Copper is a trace element that is essential to the health of all organisms Living things (humans, plants, animals and microorganisms) in humans. Copper is essential for organ function and metabolism. It contains the human body has complex mechanisms to maintain homeostasis Trying to ensure a constant supply of copper as available[17-19], while get rid of excess copper whenever this occurs. However, like all Essential Elements and Nutrients Dietary ingestion of too much or too little copper can lead to adverse results of excess or deficiency Copper in the body, each has its own set of health effects Harmful, but at the

same time excess copper is considered toxic to the human body and other living **organisms[20-24].**

EXPERIMENTAL

APPARATUS

All absorbance measurements were tested using the Manual sequential flow injection system. the pH value was measured using the Oakton 2100 Series pH/mV/Ion/0C/0F Meter. All samples were weighed using an Ohaus PA214 Pioneer Analytical Balance. The maximum wavelength was selected by using Shimadzu UV-1700 spectrophotometer. Flame atomic absorption spectrometry was used to calculate the real value of cupper samples. Infrared spectroscopy I.R, Ultraviolet-Visual radiation, Elemental analysis (CHN), Energy dispersive X-ray spectroscopy (EDX), Proton nuclear magnetic resonance spectroscopy (¹HNMR) and Mass spectroscopy (Shimadzu LCMS 2010 A) techniques were used to diagnose the reagent and cupper (II) complex.

MATERIALS

Except of the HQDABA reagent all chemicals were analytical grade and were used without additional purification. The solutions in this study were prepared using distilled water.

PREPARATION OF THE HQDABA

Diazonium chloride solution was prepared by dissolving 1.3714 g,0.01 mol from 4amineo benzoic acid in 20 mL of water and 3 mL rated HCl. The solution was treated with 5 mL of aqueous 0.69g,0.01mol sodium nitrite drop wise and stirred for 30 min at 0°C(the diazonium salt formation stage show in scheme (1). This diazonium chloride solution was added drop wise with stirred to alkaline solution of 8-hydroxy quoline1.4516g,0.01mol which dissolved in 150 mL of ethanol, and 5 mL of 10% sodium hydroxide was added. After the mixture had been stirred for 1h at 0-5°C. The product was filtered, air dried and recrystallized twice from hot ethanol and then dried in the oven at 50°C for two hours, m.p 190-195 °C as shown in Scheme 1.



Scheme (1): Formation stage of HQDABA

Preparation Of Standard Stock Solutions

Copper (II) ion Solution 100 mg L⁻¹: A stock solution was prepared by dissolving 0.0268 g of copper chloride in 100 mL of distilled water, and working solutions were obtained by further dilution.

- New organic reagent solution (HQDABA) 1 ×10⁻⁴ molL⁻¹: A stock solution was prepared by dissolving 0.0293g of the organic reagent in 100 mL of ethanol. more dilute solutions of the reagent were prepared as required.
- Interference solutions: All interference ions solutions are prepared in 100 mgL⁻¹ concentration, by dissolving amounts (0.0404,0.0403,0.0231,0.0484,0.022,0.013,1.63,0.0137) gram of the substance

NiCl₂.6H₂O, CoCl₂.6H₂O, Pb(CH₃COO)₂, FeCl₃.6H₂O, NaCl, NaF, CH₃COONa and NaHCO₃ respectively in distilled water (pH=7) and completing the volume to 100 mL. to get on Ni⁺², Co⁺², Pb, Fe⁺³, [•]F⁻, CH₃COO⁻, Cl⁻and HCO₃⁻¹ as interference ions .Each foreign ion was tested in two concentrations. The first test was in low concentration of interference ion at 10 mg L⁻¹, whereas, the second test was in a high concentration of interference ion terference ion at 50 mg L⁻¹.

• Masking agent: All masking agents have been prepared in 100 mg L⁻¹ concentration, by dissolving the right quantities of the chemical in distilled water and bringing the total volume to 100 m L. The masking Tartare sodium weight is 0.01 g. The masking agent for each interfering ion was added as drop by drop until reach the appropriate amount needed to remove the interfering effect.

SAMPLES COLLECTION

The suggested method was applied on different samples. six samples were including Foods (sesame, Aqueous, metal wire and medicine sample. All samples were prepared using the wet digestion method and the concentration of copper on these samples was determined by Flame Atomic Absorption Spectrometry (FAAS) as standard method, and this value was considered as a real value of copper in samples for comparison with the suggested method.

PREPARATION OF SAMPLES

The wet digestion method was used to prepare the samples in this study and procedural show as follows[25]:

10g of sample was weighed and then transferred to a 200ml beaker.20 mL from concentrated nitric acid HNO_3 was added and the mixture was heated up by using

a boiling water bath until the total remnant volume of the mixture became 2-3mL.The mixture cooled down.20mL from concentrated HNO_3 ,10mL from concentrated H_2SO_4 and 8mL from H_2O_2 were added and the mixture was heated up for another time.After completing the digestion process, 20 mL of distilled water is added and the mixture is heated up to discard all extra acids. (Repeat this step as needed until remove all extra acids).Finally the mixture cooled down, then filtered in a 100 mL volumetric flask, and the solution was diluted to the mark with distilled water.

RESULTS AND DISCUSSION

The proposed Sequential Injection Analysis method in this study was depending on the complexation of copper (II) ion with Of Azo derivative Of 8-hydroxy Quinoline which gave Brawn complex having an absorbance maximum at 420 nm. The laboratory-prepared reagent Azo derivative Of 8-hydroxy Quinoline and copper (II) complex were diagnosed by using many techniques including ¹HNMR, mass spectroscopy, elemental analysis (CHN), Energy Dispersive X-ray spectroscopy (EDX)), infrared and ultraviolet-visual spectroscopy. The optimum conditions for this reaction were studied carefully in order to increase the accuracy of the results.



Figure 1. Proposal reaction between copper (II) and HQDABA reagent.

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Elemental Analysis (Chn) Of The (Hqdaba)Reagent And Copper (Ii)

Complex.

When the experimentally collected information is compared to the theoretically estimated data in Table 1, a high degree of convergence is seen, demonstrating the correctness of the HQDABA reagent composition.

 Table 1. Analytical and Physical properties of Reagent and its copper(II)complex using CHN analysis

Compound	Color	m.p	Yiel	M.f		(Calc.)%		
		°C	d	(M.wt		Fou	nd %	
			%	g/mol)	С	Η	Ν	Μ
Reagent	Red	190-	80	293.3	(61.71	(4.4	(14.08	
$C_{16}H_{13}N_3O_3$		195)	1))	
					65.41	4.43	14.31	
$[Cu(C_{16}H_{13}N_{3}O_{3})_{2}]$	Brow	224	78	739.146	(50.57	(3.2	(11.70	(7.67)
Cl ₂].H ₂ O	n)	2))	8.59
					51.95	3.36	11.36	

Energy dispersive X-ray spectroscopy (EDX) for Cu (II) complex

The EDX result proved the existence of C, O, Cu, N and Cl in the chemical structure of the Cu (II)complex, as shown in the SEM-EDX in Table 2. This result indicates the formation of the complex.

Table (2):- EDX analysis of elements percent for Reagent and its copper complex

Compound	(Theoretical)% Practical %								
	С	Cl	Ν	Cu	0				
Reagent	(65.41)	()	(14.31)	()	(16.3)				
	58.33		11.67		19.00				
Complex	(51.57)	(9.60)	(11.36)	(8.58)	(15.15)				
	56.89	9.32	10.18	7.67	15.50				

Infrared Spectrum

The FTIR spectra of the reagent and copper complex were analyzed and allocated based on a detailed comparison in terms of their spectra in order to clarify the mode of bonding and the effect of the metal ion on the reagent Table 3. The FTIR spectrum of the Reagent (Fig.2 and Fig. 3) exhibited broad band at (3441 cm⁻¹) has been assigned to the stretching vibration of v(OH) of carboxyl group, disappearance of this band in the spectra of copper(II) complex indicated the deprotonation of carboxyl prior to and indicated the coordination with the copper(II) ion. The band at (3074 cm⁻¹) in the spectrum of the reagent refereed to v(OH) of phenol. Strong band in the reagent spectrum has been observed at (1687 cm^{-1}) ascribed to the v(C=O) for the carboxyl group. Significant change in the position to higher frequency has been also observed on complexation with copper(II) ion. The strong band in the free reagent spectrum at (1589 cm⁻¹) due to v(COO) asymmetric vibration, significant change in the intensity and in position to lower frequency was observed on complexation with metal ion (Fig3). The band at (1506 cm^{-1}) in the spectrum of the reagent was assigned to the v(COO) symmetric, suffered a great change to higher frequency on complexation with copper(II) ion. Bands characteristic of the azo bridge vibration at (1463 cm⁻¹) and (1402 cm⁻¹), on complex non shiften without change in shape was observed indication not the engagement of this group in the coordination with the copper(II) ion. The absence of new band around 526 and 441 cm⁻¹ due to metal nitrogen and oxygen respectively in reagent spectrum and which appearance in complex spectrum.[26-30]

Compounds	v(OH)carboxyl	v(C=O)	v as(COO)	v s(COO)	v(-N=N-)	v (M-	v (M-
	+ v(OH)phenol					0)	N)
Reagent = $C_{16}H_{13}N_3O_3$	3433 br.	1687	1589 sh.	1591sh.	1463	-	-
	3074 br.	s.			sh.		
					1402		
					sh.		
$[Cu(C_{16}H_{13}N_3O_3)_2Cl_2].H_2O$	3441br.	1689	1591sho.	1506sh.	1463sh.	526w.	441w.
		sh.			1398sh.		

 Table (2):
 The Main Frequencies of the Reagent and its copper(II) complex.

br = broad, s= strong, , sh = sharp, sho = shoulder, w = weak , as= asymmetric, s=



symmetric





Figure 3. FTIR spectrum of Azo derivative copper (II) Complex.

¹HNMR Of Reagent

The 1HNMR spectrum of the reagent in DMSO (Fig 4) shows multiplet signals at $(\delta=7.2315-8.0127 \text{ ppm})$ refers to aromatic protons .On the other hand, the signal at $(\delta=7.630 \text{ ppm})$ due to proton of phenol. Whereas, the signal at $(\delta=12.6411 \text{ ppm})$ is assigned to proton of carboxylic group and the signal peak at $(\delta=2.500 \text{ ppm})$ referred to DMSO-d6.



Figure 4 ^{.1}HNMR spectrum of reagent

Mass Spectroscopy For Reagent

The mass spectrum of the HQDABA reagent are recorded at room temperature, (Fig 5) and showed a molecular ion peak M+ at m/z+ = 293.3 attributed to the main molecular weight of reagent (293.3), and the spectrum showed several peaks assigned to the molecular ions at (m/z+), 293.3, 144.2, and248 were due to various fragments ions $[C_{16}H_{11}N_3O_3]+$, $[C_9H_6N_3]+$, $[C_{15}H_{10}N_3O]+$. respectively. This data is in good contract with the corresponding molecular formula.



Figure 5: Mass spectrum of the Reagent

The Wavelength Of The Maximum Absorbance Of Azo Reagent And It's Complex

Using a Shimadzu UV-1700 spectrophotometer, the maximum absorbance of HQDABA and its complex with copper (II) was tested in different mediums in order to achieve the greatest sensitivity, results showed that the best spectra were in neutral medium. The maximum absorbance of the copper(II) complex appeared at420 nm and the maximum absorbance of the reagent was found at 499nm. As seen in the Fig. 6. According to these results; the 420 nm was chosen as a maximum wavelength (λ) in this study.



Figure 6. The absorption spectra of copper (II) complex (Red line) and the reagent (Black line) in neutral medium And ethanol (98%) as solvent of reagent.

Studying the Optimum Conditions Affecting the Flow Injection System for Copper (II) Determination by (HQDABA)

The best design

The effect of the system design on absorbance was also evaluated by testing two designs, the first design in which the Reagent load in first loop and ion load in second loop, while the second design has the Reagent second loop and ion load in first loop as Fig. 7. It was noted that the first design is the best in terms of the highest values of absorbance, so The first design was chosen in study the result shown in Table 4.

Table 4. The best designs of the flow system; Cu (II) concentration = 10 mg / L, The Reagent concentration = 8×10^{-5} mol/L , Volume of the ion = 94.20 µl (12 c,m)and Reagent volume = 125.6 µl (16 cm), length of Holding coil = 20 cm, Flow rate = 7=ml.min⁻¹,Reaction coil length = 30 cm and pH=7.

No. of design	Abs.					
1	0.012	0.012	0.012			
2	0.009	0.008	0.006			



Figure 7. The effect of Designs on the absorption value of Copper (II) complex

The Effect of the flow Rate for copper (II) Determination

The flow rate is considered one of the important variables that must be studied because it affects the formation of the complex. Different flow rates were chosen (2-7 ml.min⁻¹), from result notice that the sensitivity will increase at a Flow rate 5 ml.min⁻¹ because it give enough time for the complication process to occur while decreases after that as Fig.8 and Table 5.

Table 5. The Influence of Flow rate on the absorbance value; Cu (II) concentration = 10 mg / L, The Reagent concentration = $8 \times 10^{-5} \text{ mol/L}$, Volume of the ion = 94.20 µl (12 cm)and Reagent volume = 125.6 µl (16 cm), length of Holding coil = 20 cm, Reaction coil length = 30 cm and pH=7.

Flow rate ml.min ⁻¹		Abs.		Mean	SD	RSD %
2	0.009	0.009	0.009	0.009	0.000	0.000
3.2	0.014	0.014	0.014	0.014	0.000	0.000
5	0.035	0.034	0.035	0.035	0.001	2.85
7	0.012	0.012	0.012	0.012	0.000	0.000



Figure 8. The effect of flow rate on the absorption value of Copper (II) complex

The Effect of pH Value on Copper (II) Determination

After choosing the optimum values of flow rate and reaction coil length, the pH effect was studied due to the acidic function effects on the Copper complex formation. The values of the pH which studied in this test ware (3-9) using NaOH and HCl to identify the ideal complexity pH. As seen in Figure 3.23 and Table 3.24 the optimum value of pH for the Copper complex formation was 6.8. At this test, the Copper (II) concentration is 10 mg L⁻¹ in a volume of 0.093 μ L, whereas the reagent concentration 3×10^{-5} mol.L⁻¹ in a volume of 0.117 μ L.

Table 6. The effected of pH on the absorbance value ; Cu (II) concentration = 10 mg / L, The Reagent concentration = $8 \times 10^{-5} \text{ mol/L}$, Volume of the ion = $94.20 \mu \text{l} (12 \text{ cm})$, Reagent volume = $117.75 \mu \text{l} (15 \text{ cm})$, Flow rate = 5 ml.min^{-1} , Reaction coil length = 40 cm and length of Holding coil = 40 cm.

pH		Abs.		mean	SD	RSD%
3	0.045	0.046	0.047	0.046	0.001	2.174
4	0.067	0.068	0.069	0.068	0.001	1.706
5	0.08	0.080	0.08	0.080	0.000	0.000
6	0.082	0.082	0.083	0.082	0.001	0.701
7	0.088	0.088	0.088	0.088	0.000	0.000
8	0.086	0.086	0.087	0.086	0.001	0.669
9	0.085	0.085	0.086	0.085	0.001	0.677



Figure9 The effect of pH on the formation of the Copper (II) complex

The effect of Holding coil Volume on the absorption Value of Copper (II) complex

The effect of the Holding coil length on the absorption values shown in Fig 9 and Table 6 of copper complex was studied, where different lengths were chosen, starting from 20 -50 cm. noticed from result that: the best length was 40 cm, as it achieves the highest absorption of the copper complex.

Table 7. The Influence of Holding coil length on the absorbance value; Cu (II) concentration = 10 mg / L, The Reagent concentration = 8×10^{-5} mol/L, Volume of the ion = $94.20 \ \mu l$ (12 cm)and Reagent volume = $125.6 \ \mu l$ (16 cm), Flow rate = $5 \ ml.min^{-1}$, Reaction coil length = $30 \ cm$ and pH=7.

H.C.L cm		Abs		Mean	SD	RSD%
20	0.035	0.034	0.035	0.035	0.001	2.857
30	0.047	0.046	0.047	0.047	0.001	2.127
40	0.088	0.088	0.088	0.088	0.000	0.000
50	0.029	0.028	0.029	0.029	0.001	3.448



Figure 10. The Reaction Coil Length on the production of Copper (II) complex

The Effect of Reagent Concentration Value on Copper (II) Determination

The effect of reagent concentration on the analytical signal is studied between 1×10^{-5} - 3×10^{-4} mol.L⁻¹ at pH 7. The analytical signal increased with the increases of reagent concentration up to 3×10^{-5} mol.L⁻¹. Due to the results, reagent concentration at 3×10^{-5} mol.L⁻¹ is selected for further experiments as an optimal value as shown in Fig. 11 and table 8.

Table 8. The Influence of Reagent conc. on the absorbance value ; Cu (II) concentration = 10 mg / L, Volume of the ion = 94.20 µl (12 cm), Reagent volume = 117.75 µl (15 cm), Flow rate = 5 ml.min⁻¹, pH= 7, Reaction coil length = 40 cm and length of Holding coil = 40

Reagent conc. mol 1 ⁻¹	Abs.			Mean	SD	RSD%
1×10 ⁻⁵	0.059	0.06	0.059	0.059	0.001	1.694
3×10 ⁻⁵	0.095	0.095	0.095	0.095	0.000	0.000
5×10 ⁻⁵	0.089	0.090	0.090	0.089	0.001	1.123
8×10 ⁻⁵	0.088	0.088	0.088	0.088	0.000	0.000
1×10 ⁻⁴	0.046	0.046	0.046	0.046	0.000	0.000
3×10 ⁻⁴	0.044	0.043	0.044	0.044	0.001	2.272



Figure 11. The effect of reagent concentration on the production of Copper (II) complex

Calibration Curve of Copper (II) Complex

After examining the various conditions and their impact on the absorbance value of Copper (II) complex, calibration curves of copper complex are constructed under optimal conditions using distilled water at pH 7 and a series of copper ion (II) solutions ranging from 0.5 to 50mg.L⁻¹. Beer's law is observed by measuring the Copper (II) complex absorbance at 420 nm over the concentration range of 0.5-10 mg L⁻¹ as illustrates in Fig. 11 and Table 8. The Sandell's sensitivity, limit of detection (LOD), and limit of quantification (LOQ) are founded to be (0.120) μ g cm⁻², (0.361) mg L⁻¹, and (1.204) mg L⁻¹, respectively.

Table 9. The Influence of the Cu(II) concentration on the absorbance value : The Reagent concentration = 3×10^{-5} mol/L , Volume of the ion = 94.20 µl (12 cm), Reagent volume = 117.75 µl (15 cm), Flow rate = 5 ml.min⁻¹ ,pH=7 at λ max= 420 nm ., Reaction coil length = 40 cm and length of Holding coil = 40 cm .

Cu (II)	Abs.			Mean	SD	RSD%
conc. mg/l						
1	0.018	0.019	0.017	0.018	0.001	5.556
3	0.035	0.035	0.035	0.035	0.000	0.000

5	0.050	0.050	0.050	0.050	0.000	0.000
7	0.066	0.065	0.067	0.066	0.001	1.515
9	0.083	0.083	0.083	0.083	0.000	0.000
10	0.095	0.095	0.095	0.095	0.000	0.000



Figure 13. Calibration curve of Copper (II) complex

Repeatability

In order to measure the precision of the SIA unit, the experiment is repeated ten times for Copper (II) at a concentration of 5 mg L^{-1} . The results are shown in Table 10 and Figure 13. And the result show that ,the SIA system in this study give good precision for Cu(II) ion determination using azo reagent .

Table 10. The Repeatability of the Cu (II) concentration on the absorbance value: Cu (II) concentration = 5 mg L⁻¹, The Reagent concentration = 3×10^{-5} mol/L , Volume of the ion = 94.20 µl (12 cm), Reagent volume = 117.75 µl (15 cm), Flow rate = 5 ml.min⁻¹ ,pH=7 at λ max= 420 nm ., Reaction coil length = 40 cm and length of Holding coil = 40 cm .

Samples	1	2	3	4	5	6	7	8	9	10	Mean	SD	%RSD
Readings													
Absorbance	0.050	0.050	0.050	0.050	0.050	0.049	0.05	0.050	0.050	0.050	0.050	0.0002	0.400



Figure 14. Repeatability of Copper (II) at a concentration of 5mg L⁻¹ in SIA unit

Dispersion Coefficient

The dispersion coefficient (D) used widely as experimental factor for determining the degree of sample dilution from the injection point to the detector. Due to the outcomes displayed in Table 11, the type of dispersion in this study is limited dispersion.

Table 11. The dispersion coefficient (D)

Cu(II) Conc. mg L ⁻¹	Res	Dispersion D = A°/A_{max}	
	A°	A_{max}	1.042
10	0.099	0.095	

A°: Absorbance with absent of dilution (outside the SIA system).

 $\mathbf{A}_{\text{max}}\!:$ Absorbance with presence of dilution (inside the SIA system).

Applications

various samples are tested to determine Copper (II) using the Aqueous suggested method. According to FAAS, the concentration of Copper (II) in the samples of sesame, Copper wire, Aqueous and medicine is located at the linearity range of 1- 10mg L^{-1} , thus this sample is measured directly. The recommended method gives good accuracy in comparison with the FAAS method, recovery rates range from 77.82% to 115% as indicates in Table 12.

 Table 12. Accuracy of suggested method compared with FAAS technique for evaluation of

 Copper (II)

Sample	True value (FAAS)	Measured value (suggested method)	Е%	Recovery %
	mg.L ⁻¹	mg.L ⁻¹		
Sesame	2.3	1.79	-22.17	77.82
Copper wire	2.6	2.99	15	115
Aqueous solution	1.8	1.8	0.00	100
Medicine	1.02	1.2	1.06	101

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