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## Metal Chelates of Copper and Nickel with Murexide in Mixed Isopropanol: Water Solvent: Spectrophotometric Study

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### A B S T R A C T

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A sensitive, accurate, and quick spectrophotometric technique for determining Ni(II) and Cu(II) in analytical samples employing murexide reagent was investigated in a water-isopropanol mixed solvent. UV/Vis spectroscopy was used to characterize the produced complexes. In three mixtures of water-isopropanol mixed solvent, various experimental parameters affecting complex formation were examined. For Ni(II) and Cu(II), the resulting complex in a 3:7 water-isopropanol mixed solvent showed maximum absorbance at  $\lambda_{\max} = 458$  nm at pH 3.1 and 470 nm at pH 3.8, respectively. Beer's law was maintained in the concentration ranges of (0.2 to 3.5 ppm) for Ni(II) and (0.2 to 4.0 ppm) for Cu(II). The molar absorptivity ( $\epsilon$ ) and sensitivity values of the Ni(II) and Cu(II) complexes were determined to be (7800  $\text{l.mol}^{-1}.\text{cm}^{-1}$  and 0.32  $\text{ppm}^{-1}$ ) and (18700  $\text{l.mol}^{-1}.\text{cm}^{-1}$  and 0.20  $\text{ppm}^{-1}$ ), respectively. The continuous variation method was used to explore the structure of the prepared compound. The acquired results revealed that the complexes have a (1:2) (M:L) molar ratio, indicating that this method was more sensitive, precise, and accurate when the effect of  $\text{Cl}^-$ ,  $\text{NO}_3^-$ ,  $\text{CH}_3\text{COO}^-$ , and  $\text{SO}_4^{2-}$  was studied.

## 1 Introduction

Metal chelates are important compounds in numerous fields; i.e., they are used in the paint and dyes industry; such as nickel and copper complexes with some Schiff bases (Wahba et al., 2017; Elsherif et al., 2020a; Elsherif et al., 2020b), they are also used as catalysts in polymerization processes such as Ziegler-Nata catalysts (Cossee and Ziegler, 1964), and are also widely used in the nuclear industry to purify uranium and also in the treatment of nuclear waste (Flett et al., 1983). Furthermore, they play an important role in many biochemical reactions in the body, participate in the composition of many important natural biological compounds; i.e. hemoglobin, enzymes, vitamin B12 and chlorophyll in plants (Spessard and Miessler, 1996), also play a great role in diagnosis and physical therapy like: contrast agents in nuclear magnetic imaging, as active compounds in the treatment of cancer (Erkey, 2011; Elsherif et al., 2021; Elsherif et al., 2018). Large numbers

of complexes are prepared and tested from silver, copper, ruthenium, platinum, and gold, and used as antioxidants and antibacterial (Ndagi et al., 2017; Esmaeili et al., 2019; Lum et al., 2013; Warra, 2011). They are also used in the development of solar energy storage technologies (Erkey, 2011).

Complex formation reactions are also the basis of various analytical methods, such as: solvent extraction, chromatography, gravimetric analysis, UV-VIS molecular absorption and emission spectroscopy (Jeffery, 1981; Elsherif et al, 2020c; Muneer et al., 2020). They are also used in different techniques for determination of metal ions or their compounds in environmental and pharmaceutical samples. There are many analytical techniques that include the determination or extraction of metal ions in the form of chelates such as: molecular absorption methods in the visible and ultraviolet region, molecular fluorometric methods, complex formation titrations, solvent extraction methods, solid phase extraction methods, cloud point

extraction methods (Nasir Uddin et al., 2013; Birghila et al., 2008). However, there are other methods that may or may not use complexes such as voltammetry, polarography, ion exchange methods, and chromatography (Alabidi et al., 2021).

Murexide (Figure 1) is a colorimetric indicator used in the detection of metal ions and is widely used in conventional EDTA titrations (Elsherif et al., 2020c). It is widely used in the determination of calcium ions, and other metal ions like copper, nickel, cobalt, thallium, and alkaline earth elements (Masoud et al., 2006). Murexide dissolves in water and forms complexes with most metal ions with different oxidation states, in aqueous and non-aqueous solutions. The stability constants of these complexes are usually estimated by spectroscopic methods, and are often not high in aqueous solutions causing some limitations when using murexide as a colorimetric agent. The stability of the murexide complexes can be increased by using solvents with a lower dielectric constant than water (Huang et al., 2018; Shamsipur et al., 1989; Shamsipur and Alizadeh, 1992).

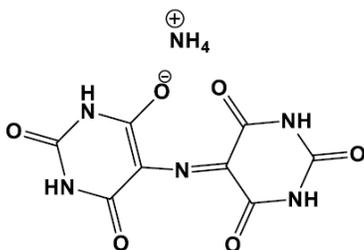


Figure (1). Chemical structure of murexide

In the present paper, a spectrophotometric investigation of the murexide complexes with copper and zinc ions in binary isopropanol-water mixtures at 25°C has been reported.

## 2 Materials and Methods

### 2.1 Chemicals and reagents

Murexide was purchased from Merck chemicals and was of ACS reagent grade. Isopropanol ( $\geq 99.5\%$ ) was also from Merck and of ACS reagent grade. Copper (II) sulphate pentahydrate (ACS reagent,  $\geq 98.0\%$ ), Zinc chloride (reagent grade,  $\geq 98\%$ ), Hydrochloric acid (ACS reagent, 37%), and Sodium hydroxide (reagent grade,  $\geq 98\%$ , pellets (anhydrous)) were all purchased from Sigma-Aldrich. All the reagents and solvents were of analytical grade and chemically pure and were used as received without any further purification.

### 2.2 Instrumentation

UV-visible spectra of free and complexed murexide were recorded using Cary-60 model UV-visible spectrophotometer from Agilent. Optimum pH monitoring of complex formation was done using 3505 pH meter from Jenway.

### 2.3 Standard solutions

The stock solutions ( $5 \times 10^{-3}$  M) of murexide, copper sulphate pentahydrate, and zinc chloride were prepared by dissolving the compounds in deionized water. Sample dilutions were carried out by taking the appropriate aliquots from the stock solutions followed by dilution with proper quantity of mixed solvent (isopropanol: water). To investigate the effect of various anions on absorption spectra of prepared complexes, stock solutions (1.0 M) of potassium chloride, sodium nitrate, sodium sulphate, and sodium acetate were prepared by dissolving these salts in deionized water.

### 2.4 The absorption spectra of murexide in different mixtures of isopropanol and water

A series of standard solutions of equal concentration of murexide; contains different proportions of water and isopropanol start from 10% to 100% water, was prepared and then the absorption spectra of these solutions were measured in the UV-VIS region (200-800 nm). The absorption maxima ( $\lambda_{\max}$ ) of each complex in different proportions of water and isopropanol were determined. Various parameters that affect complex formation were investigated which include: time and pH.

The pH value of the complex solutions was adjusted using 0.1 M solutions of hydrochloric acid and sodium hydroxide. The absorption of the formed complexes was measured till 3 hours to determine the stability of these complexes with time

### 2.5 Determination of complex stoichiometry by method of continues variation

Nine solutions were prepared by mixing various proportions of  $5.0 \times 10^{-3}$  M solutions of both metal ion and murexide. The solutions contain various mole (or volume) ratios of both reactants, and these ratios are as follows:

$$1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1$$

The absorbance of the solutions is measured and plotted against the mole (or volume) fraction of the murexide.

### 2.6 Determination of complex stoichiometry by method of continues variation

In the linear range of Beer-Lambert Law, solutions with various metal ion concentrations were prepared, the absorbance of these solutions was measured, the linear relationship between the metal ion concentration and the absorbance was plotted, and the sensitivity was calculated as the slope of the straight line. The standard deviation of ten measurements of Blank's solution was used to evaluate the detection limits. The detection and quantification limits were calculated using the following equations:

$$DL = \frac{3xSD}{S} \quad (1)$$

$$QL = \frac{10xSD}{S} \quad (2)$$

Where: DL and QL detection and quantification limits, respectively,  $S_D$  standard deviation, S slope.

Different concentrations of interfering ions such as chloride, nitrate, sulfate, and acetate were also investigated.

### 3 Results

#### 3.1 Absorption spectra of murexide at different proportions of water: isopropanol

Figure 2 depicts the UV-visible absorption spectra of murexide in the 300-700 nm range. As seen in the graph, the presence of two absorption groups is indicated by the presence of two peaks, one at 515-520 nm and the other at 320-330 nm. The higher intensity absorption peak is generated by the  $\pi \rightarrow \pi^*$  transition, and the lower peak is caused by the  $n \rightarrow \pi^*$  transition, according to the murexide structure (Figure 1). The formation of hydrogen bonds between the solvent and murexide ions causes the absorption peak at 520 nm to shift blue as the solvent polarity decreases (Elsherif et al., 2018). The absorption maxima at each water: isopropanol proportion are shown in Table 1.

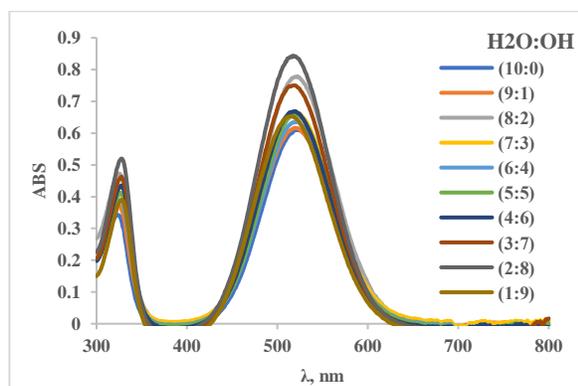


Figure (2). Absorption spectra of murexide at various water: isopropanol proportions

Table (1).  $\lambda_{max}$  values of murexide at various water: isopropanol proportions

Isopro.:H <sub>2</sub> O	$\lambda_{max}$ (nm)
0:10	521
1:9	521
2:8	521
3:7	520
4:6	520
5:5	519
6:4	519
7:3	518
8:2	517
9:1	515

#### 3.2 Absorption spectra of metal-murexide complex at different proportions of water: isopropanol

Figure 3 depicts the relations between the absorbance and wavelength of Ni(II) and Cu(II) complexes with murexide in three different mixed solvent ratios (8:2, 6:4, and 3:7 - H<sub>2</sub>O: Isopropanol). Blue shifts of complexes absorption peaks are seen, resulting in new, distinct absorption peaks specified to complexes absorption. The reason for these displacements is that when the complex is formed, the contribution of the  $n \rightarrow \pi^*$  transitions in the murexide decreases due to the participation of n electrons in the formation of coordination bonds with metal ions (Elsherif et al, 2020c). The blue shifts in the complexes were as follows: 7 nm and 6 nm for the nickel and copper complexes, respectively.

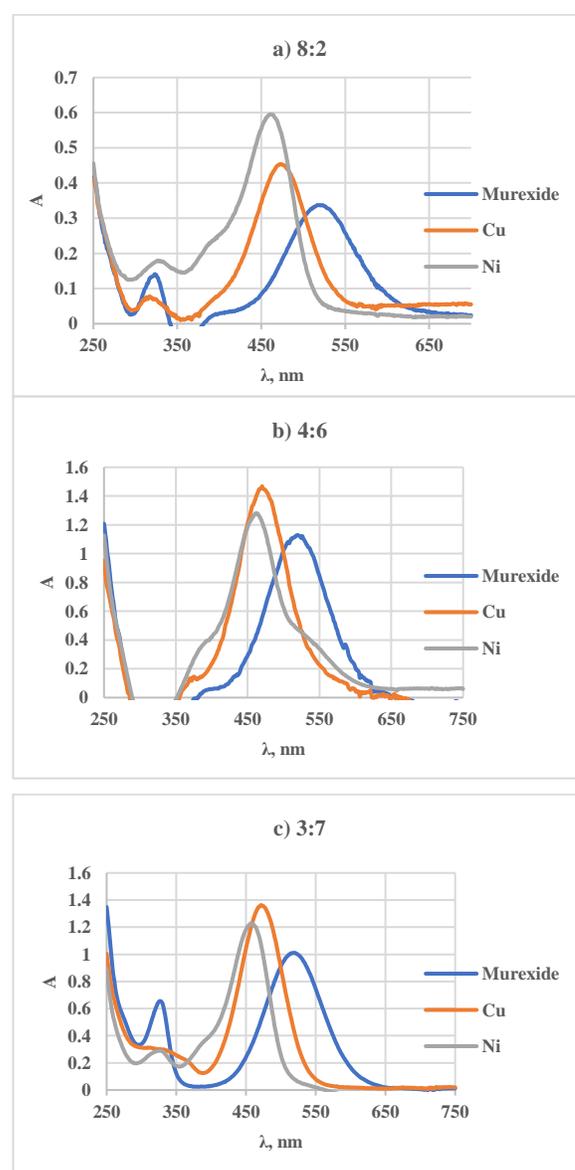


Figure (3). Absorption spectra of Cu-Murexide and Ni-Murexide complexes at various solvent proportions

### 3.3 Effect of pH on absorption maxima of metal-murexide complex at different proportions of water: isopropanol

One of the most critical factors influencing complex formation and stability is pH. Some complexes are stable in an acidic medium, some in a basic one, and still others in a neutral medium. Furthermore, all ligands are either weak acids or weak bases that ionize depending on the medium's pH. Murexide is classified as a weak acid since it contains four protons, as illustrated in Figure 4 (with very weak ionization constants, the protons ionize entirely in basic medium ( $pK_{a1}=9.2$ ,  $pK_{a2}=10.5$ ) (Winkler, 1972). Murexide dissociates into uramil and alloxan in a high acidic media (Ramaiah and Gupta, 1956; Viesca and Gómez, 2019).

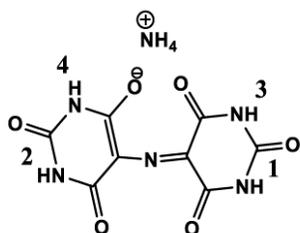


Figure (4). The four protons of murexide as a weak acid

Figure 5 displays graphical curves derived from studies investigating the effect of pH on metal ion complexes with murexide in the three investigated ratios. For Ni complex, it is noted that the complex in the three ratios have low absorption in the strong acidic medium ( $pH < 3$ ) and high absorption in the range of (4-9). However, in the ratio (8: 2) Ni complex has low absorption in the strong alkaline medium ( $pH > 9$ ) due to metal ion precipitation in the form of hydroxide. Otherwise; for Cu complex, it should be remarked that in the ratios (4:6) and (8:2), the complex exhibited weak absorption in acidic medium ( $pH < 4$ ), whereas in the ratio (7:3), the complex displayed considerable absorption in strong acidic medium ( $pH < 2$ ). However, in the case of the ratio (3:7), the complex was relatively stable over a wide pH range (2-8). At the ratio (8:2), where the Cu (II) ion precipitates as hydroxide, there is also a decrease in absorption in weak acid media ( $pH > 6$ ). Table 9 shows the optimal pH values for the production of two complexes.

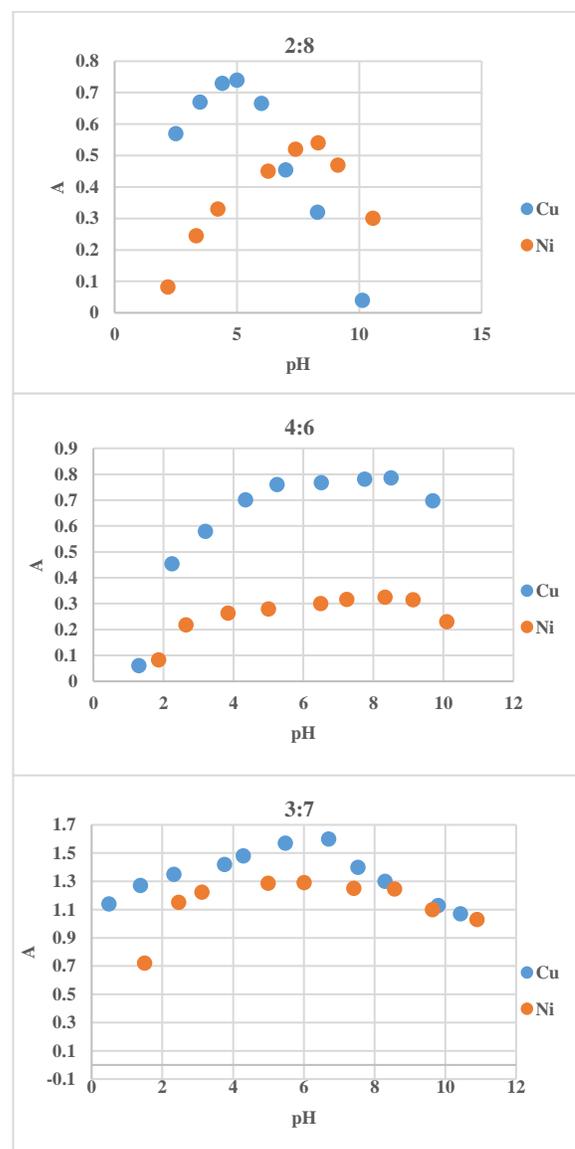


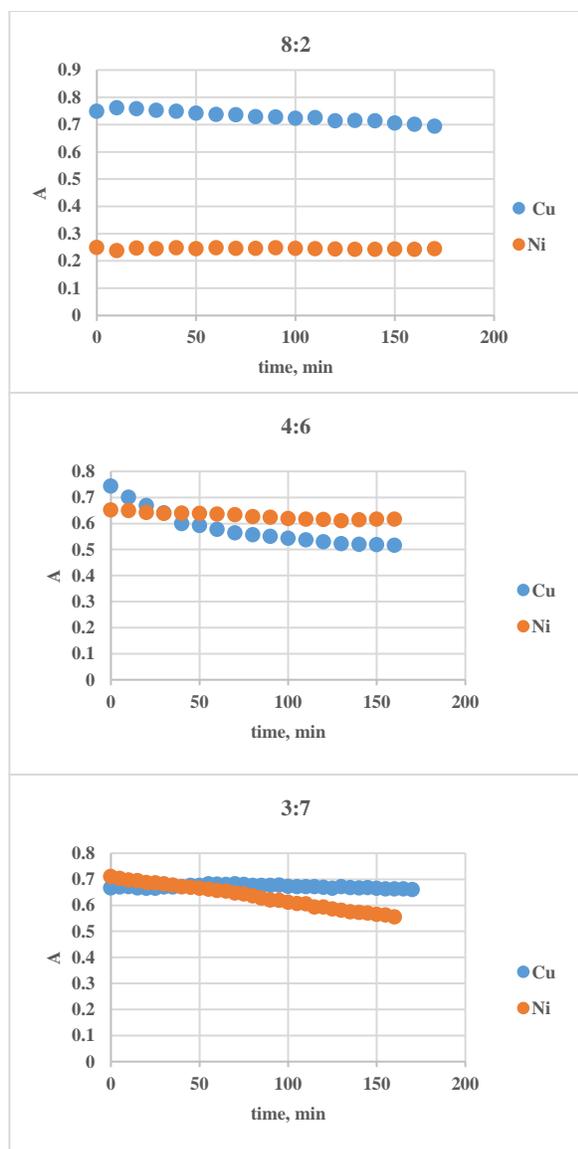
Figure (5). Effect of pH on metal-murexide complexes at different water: isopropanol proportions

### 3.4 Stability of metal-murexide complex over time at different proportions of water: isopropanol

Investigating the effect of time on complex stability have been gained more concern in order to recognize whether the complex reached its maximum stability or not, and to distinguish the stability duration of complex before it dissociates (Muneer et al., 2020). To demonstrate the effect of time on investigated murexide complexes stability; the change in absorbance of the complexes was measured for a period of 3 hours.

Figure 6 shows the change in absorbance versus time for Ni(II) complexes with murexide in the three mixed solvent proportions. It is observed that these complexes form quickly (the color changes immediately after addition), and they are still stable over the whole studied

time and it does not undergo any dissociation (except for 3:7 proportion which undergoes a slight dissociation). The same observed in the case of copper complexes in three studied proportions, where the complex is formed quickly and there is no remarkable dissociation (a slight dissociation is observed with the proportion of 4:6).



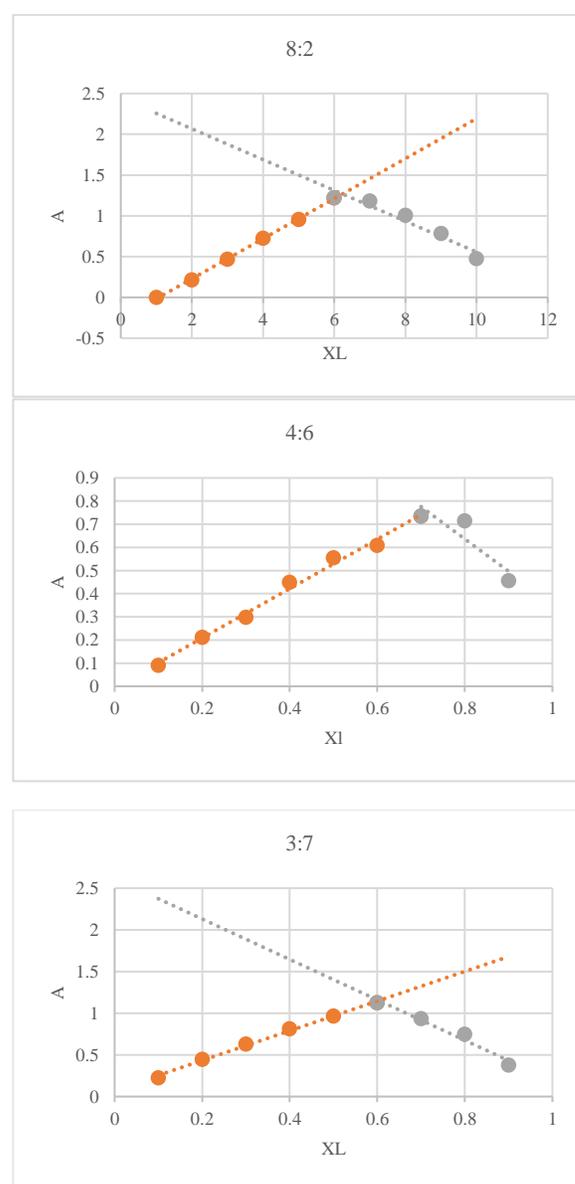
**Figure (6).** Effect of time on metal-murexide complexes at different water: isopropanol proportions

### 3.5 Stoichiometry and stability constant of metal-murexide complex at different proportions of water: isopropanol

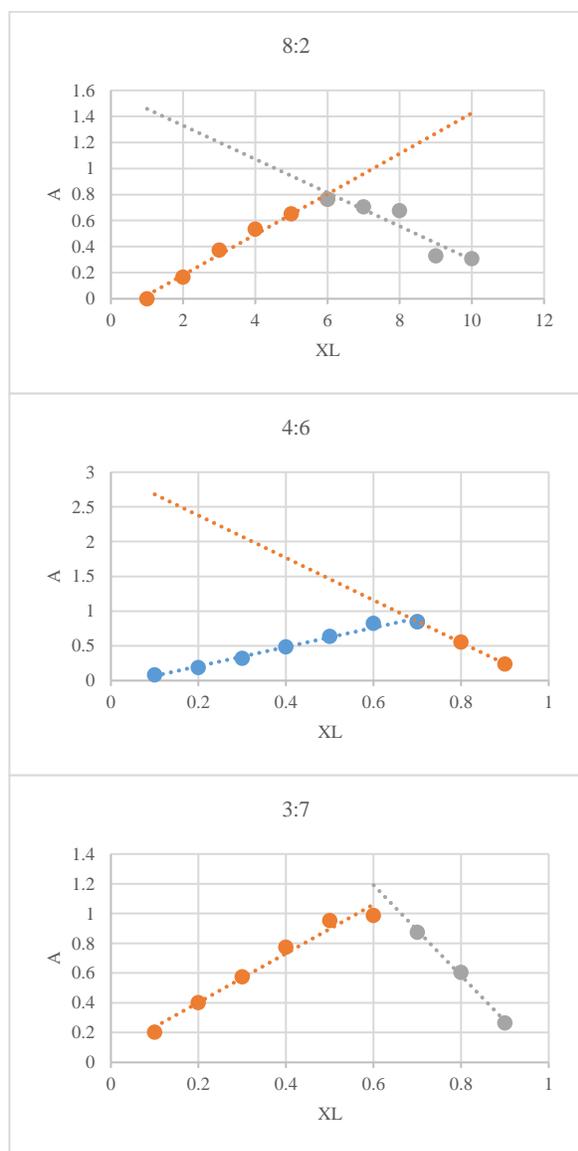
Spectroscopic methods are one of the most important methods that are used to determine the stoichiometry and stability constant for complexes, especially if they are colored; as these complexes show clear and distinct absorption peaks in UV-VIS region. There are various spectroscopic methods, including the continues variation

method presented by Job and modified by Copper and Vosbury (AL-adilee and Hessoon, 2019).

The graphical Job curves developed for Ni(II) and Cu(II) complexes with murexide in the three examined proportions are shown in Figures 7 and 8. The absorbance versus mole fraction of the ligand relationship was displayed. The stoichiometry ratios for Ni (II) and Cu (II) complexes were determined from the Figures 7 and 8 as follows: The stoichiometry ratios were 1:1 (ML) for the (8:2) proportion and 1:2 ( $ML_2$ ) for the (4:6 and 3:7) proportions. As the number of water molecules increases, they compete with ligand molecules for coordination with metal ions; however, as the number of water molecules decreases, the competition between water and the ligand decreases, allowing the metal ion to bind to more than one ligand.



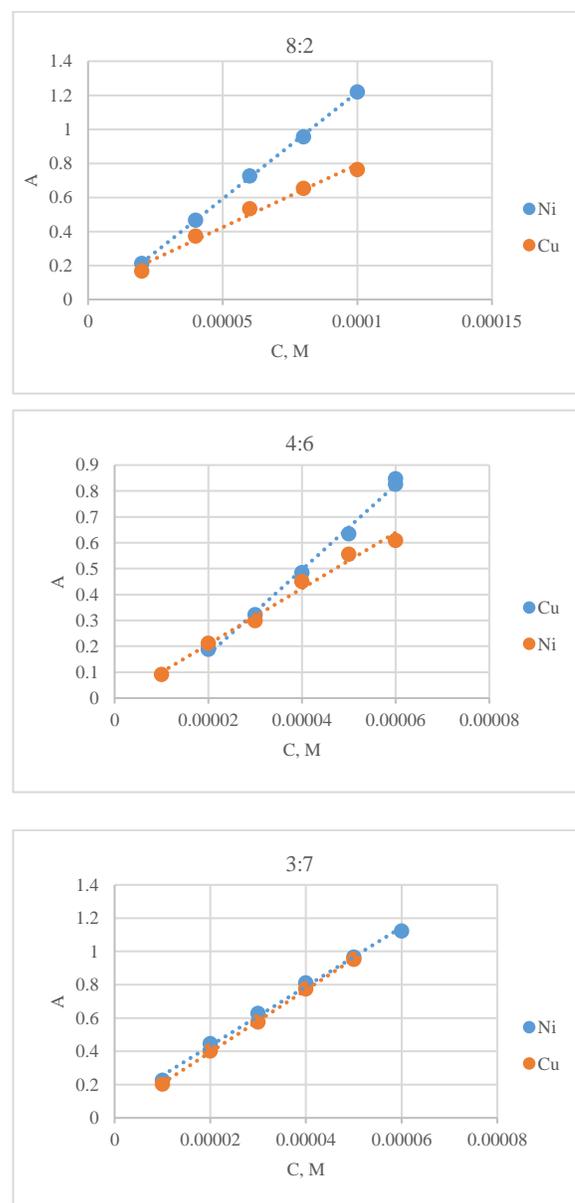
**Figure (7).** Job curves for Ni-murexide complexes in three mixed solvent proportions



**Figure (8).** Job curves for Cu-murexide complexes in three mixed solvent proportions

Tables 2 and 3 present the values of the molar absorptivity and stability constants of the produced complexes in the three tested ratios. According to the values of the stability constants, the complexes generated in the ratio 8:2 were the least stable, while they were the most stable in the ratio 3:7, i.e. The percentage of water in the solvent mixture has an inverse relationship with stability (Fat'hi and Shamsipur, 1993).

The calibration curves of Ni(II) and Cu(II) complexes with murexide are shown in Figure 9, from which the molar absorbance values were derived.



**Figure (9).** Calibration curves for murexide complexes in three mixed solvent proportions

**Table (2).** Parameters values of Ni-murexide complexes in three mixed solvent proportions

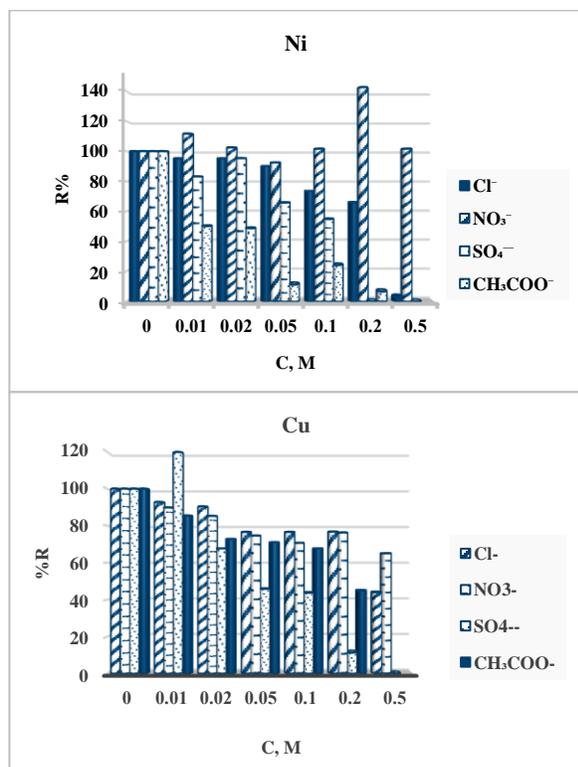
Ratio (w:Isopro.)	$\lambda_{\max}$ (nm)	pH	L:M	$\epsilon$ $\text{l.mol}^{-1}\text{cm}^{-1}$	$K_f$
2:8	465	8.3	1:01	11720	$5.06 \times 10^6$
4:6	462	10.1	2:01	10780	$2.26 \times 10^{12}$
7:3	458	3.1	2:01	17800	$4.33 \times 10^{12}$

**Table (3).** Parameters values of Cu-murexide complexes in three mixed solvent proportions

Ratio (w:Isopro.)	$\lambda_{max}$ (nm)	pH	L:M	$\epsilon$ L.mol <sup>-1</sup> cm <sup>-1</sup>	$K_f$
2:8	476	5.0	1:01	7370	$3.24 \times 10^6$
4:6	472	8.5	2:01	14911	$9.45 \times 10^{11}$
7:3	470	3.8	2:01	18700	$9.80 \times 10^{11}$

**3.6 Effect of interferences on metal-murexide stability at 3:7 water-isopropanol proportion**

At the 7:3 ratio, the effect of some interfering ions on the stability of the synthesized complexes was investigated. The presence of particular ions enhances the probability of the ligand being displaced by a foreign ion (Nasir Uddin et al., 2013). Figure 10 depicts the influence of different salt concentrations (CH<sub>3</sub>COONa, KCl, NaNO<sub>3</sub>, and H<sub>2</sub>SO<sub>4</sub>) on the complex recovery (unaffected concentration by the presence of interfering ions). The figure shows that the complex recovery is uninfluenced by these salts at low concentrations levels, but falls dramatically at large concentrations; that is, these complexes are not stable at elevated concentrations of these salts.



**Figure (10).** The effect of some interfering ions on complex stability

**3.7 Determination of detection limits, quantification limits, and sensitivity**

The ratio 3:7 was chosen for the evaluation of the spectrophotometric approach for the determination of Ni (II) and Cu (II) ions. The proposed spectroscopic method's sensitivity, detection limits, and quantification limits were all determined for this purpose. The sensitivity was calculated by plotting metal ion concentration in ppm versus absorbance (see Figure 11). The slope of the linear relationship is represented by the sensitivity. Table 4 shows the estimated linear range of concentrations at which Beer's law applies, as well as their correlation coefficient.

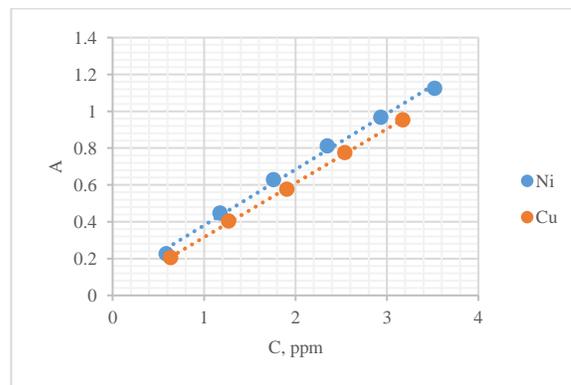
The detection limit (DL) and the quantification limit (QL) were estimated using the following equations (Elsherif et al., 2022):

$$DL = 3 \times SD \quad (1)$$

$$QL = 10 \times SD \quad (2)$$

Where: SD standard deviation of 10 Blanck's solution runs.

The obtained detection limits and quantification limits values are shown in Table 4.



**Figure (11).** Sensitivity of the proposed method at 3:7 proportion

**Table (4).** Some calculated parameters for the proposed method

Metal ion	S (ppm <sup>-1</sup> )	DL (ppm)	QL (ppm)	Linear rang (ppm)	R <sup>2</sup>
Ni	0.32	0.01	0.03	0.2-3.5	0.958
Cu	0.20	0.05	0.10	0.2-4.0	0.994

## 4 Conclusions

The coloring-developing reaction between murexide reagent and (Ni(II) and Cu(II)) was thoroughly explored in different proportions of water-isopropanol combination, as was the process for assaying Ni(II) and Cu(II) utilizing the complex coloring reaction in a 3:7 water-isopropanol mixture. When utilizing a UV-Vis. Spectrophotometer, maximum absorbance values were obtained at 458 nm and 470 nm for Ni-murexide and Cu-murexide complexes, respectively, and optimum circumstances at pH 3.1 & 3.8 and 0.2-3.5 & 0.2-4.0 ppm concentration for Ni and Cu, respectively. In comparison to other spectrophotometric approaches, this novel analytical method was comparatively simple, quick, and sensitive. In addition to the low detection limit of Ni and Cu, the analysis of a wide range of concentrations follows the Lambert- Beer's Law.

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**Conflict of Interest:** The authors declare that there are no conflicts of interest.

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