



Investigation of Murexide-Zn(II) Complexes in Water-Isopropanol Mixtures: Spectroscopic Analysis and Stability Studies

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ABSTRACT

This study investigates the complexes formed between murexide and Zn (II) ion in water-isopropanol mixtures using spectroscopic analysis. The impact of varying solvent ratios on the absorption spectrum of murexide and its complexes with Zn (II) ion is examined. Furthermore, the influence of pH, time, and interfering ions on the stability of the complexes is investigated for three different isopropanol-water ratios with varying polarities (8:2, 6:4, and 3:7). The molar ratio and stability constants of these complexes are determined using the continuous variations method. The results indicate that complexes with a molar ratio of 1:2 (ML₂) are formed at the ratios 8:2 and 6:4, while a molar ratio of 1:1 (ML) is observed at the ratio 3:7. The formation constants (K_f) for the complexes are found to be 7.32×10^{10} , 1.10×10^{11} , and 3.98×10^5 for the three ratios, respectively, suggesting an inverse relationship between complex stability and water content in the solvent mixture. Additionally, the spectroscopic method employed is evaluated based on sensitivity (0.0406 ppm^{-1}), detection limit (0.016 ppm), and quantification limit (0.054 ppm). The linear range of concentration, as determined by Beer-Lambert's law, spans from 0.2 ppm to 3.93 ppm.

دراسة معقدات موركسيد-Zn(II) في خليط من الماء-أيزوبروبانول: تحليل طيفي ودراسة الاستقرار

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الكلمات المفتاحية:

طيف UV-VIS
Zn(II)
ثابت التكوين
حد الاكتشاف
الحساسية

الملخص

تضمن هذا البحث دراسة معقدات الموركسيد مع أيون Zn (II) في مخاليط من الماء والأيزوبروبانول بالطريقة الطيفية. حيث تم دراسة تأثير النسب المختلفة من المذيبات على طيف امتصاص الموركسيد وعلى معقداته مع أيون Zn (II). كذلك تم دراسة تأثير pH والزمن وتأثير الأيونات المتداخلة على استقرار المعقدات المتكونة في ثلاث نسب مختلفة من الأيزوبروبانول: الماء متدرجة في القطبية (8:2، 6:4 و3:7). النسبة المولية وثوابت الاستقرار لهذه المعقدات في النسب المختارة من المذيبات تم تقديرها باستخدام طريقة التغيرات المستمرة، وقد تكون المعقد بنسبة مولية 1:2 (ML₂) عند النسبتين 8:2 و6:4، وبنسبة مولية 1:1 (ML) في النسبة 3:7. ثوابت الاستقرار K_f للمعقدات في النسب الثلاث على التوالي كانت كالتالي: 7.32×10^{10} ، 1.10×10^{11} ، 3.98×10^5 . أي أن هناك تناسباً عكسياً بين استقرار المعقدات المتكونة وكمية الماء في خليط المذيبات. أيضاً تم تقييم الطريقة الطيفية المستخدمة بتقدير كل من الحساسية (0.0406 ppm^{-1}) حد الكشف (0.016 ppm) وحد التقييم (0.054 ppm). المجال الخطي للتركيز وفقاً لقانون بيير-لامبرت كان ضمن التراكيز من 0.2 ppm إلى 3.93 ppm.

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Introduction

Complexes hold significant importance across various fields. For instance, they find applications in the paint and dye industry, such as nickel and copper complexes with Schiff bases [1]. Additionally, complexes, including Ziegler–Natta catalysts, serve as catalysts in polymerization processes [2]. These compounds are widely utilized in the nuclear industry for uranium purification and nuclear waste treatment [3]. In biochemical reactions, complexes play pivotal roles in permeating the structure of essential biological compounds such as hemoglobin, enzymes, vitamin B12, and chlorophyll in plants [4]. Furthermore, these complexes have crucial implications in medical diagnosis and treatment, serving as contrast agents in magnetic resonance imaging and as active compounds in cancer therapy [5]. Extensive research has been conducted on the preparation and evaluation of complexes containing elements such as silver, copper, ruthenium [6], platinum [7], and gold [8]. They have also been explored as antioxidants, such as copper complexes with amino acids [9], and as antibacterial agents [10]. Moreover, these complexes are instrumental in developing solar energy storage technologies [5]. Complex formation reactions involve various analytical methods, including solvent extraction, chromatography, gravimetric analysis, and spectroscopic analysis, enabling the estimation of metals and compounds in environmental, pharmaceutical, and other samples [11]. In the realm of chemical analysis and spectroscopy, murexide is a notable colorimetric indicator for metal ion detection. This approach has garnered substantial interest for its application in conventional EDTA titrations. Murexide is classified as an ammonium purpurate or the ammonium salt of purpuric acid. Murexide is denoted by its systematic name ammonium 2,6-dioxo-5-[(2,4,6-trioxo-5-hexahydropyrimidinylidene)amino]-3H-pyrimidin-4-olate. The structural formula of murexide is depicted in Fig. 1 [12].

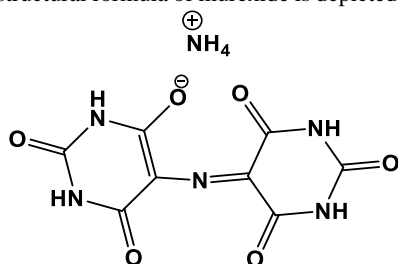


Fig. 1. Structure of murexide

Murexide has been extensively applied in the estimation of calcium ions and is also utilized for estimating copper, nickel, cobalt, thallium, and alkaline earth elements [13]. It has been widely employed in complex formation reactions with 3d and 4f ions, leading to a notable color change from purple to orange–red in either aqueous or organic solvents [14]. Murexide is soluble in water and can form complexes with diverse metal ions in various oxidation states, encompassing aqueous solutions, nonaqueous solutions, and mixed solvent solutions [13]. The binding ratios observed for these complexes are typically 1:1 (ML) [16,15] or 1:2 (ML₂) [17], and in certain cases, both ML and ML₂ complexes can coexist within the same solution [18]. Spectroscopic methods are commonly employed to estimate the stability constants of these complexes [19]. However, the stability constants in aqueous solutions are often modest, which limits the use of murexide as a colorimetric indicator [16]. For instance, to mitigate murexide decomposition, which is visually observed as a gradual fading of the purple colour until the solution turns transparent, murexide solutions must be maintained within a pH range of 4.5 to 9.0 [20]. The stability of murexide complexes can be enhanced by employing solvents with lower dielectric constants and solubilities than water [16].

In their scientific research article, Shamsipur and Alizadeh [15,16,21] conducted a spectroscopic investigation on the murexide complexes formed with various metal ions (Co(II), Ni(II), Cu(II), Zn(II), Cd(II), and Pb(II)) in a solution of dimethyl sulfoxide (DMSO). The researchers determined the binding ratio of these complexes using the mole ratio method, which was consistently found to be 1:1 for all the cases. Additionally, they calculated the equilibrium constants of the complexes and observed that they

adhered to the Irving–Williams rule. Furthermore, a comparison of the complex stabilities between DMSO and aqueous solutions revealed that the murexide complexes exhibited greater stability in DMSO solution than in aqueous solution.

Kargosha et al. [22] introduced a sensitive and selective method for quantifying Ni(II) ions in environmental samples using murexide based on fluorescence measurements. This method involved analysing the emission spectrum of the murexide complex with Ni(II) at different concentrations while considering the effects of pH, time, murexide concentration, and interference from various ions on the estimation process.

In a separate investigation, Elsherif et al. [17] performed a spectroscopic examination of the formation of murexide complexes with Co(II) and Ni(II) ions in aqueous solutions. The authors studied the absorption spectra of murexide and its complexes with Co(II) and Ni(II) in aqueous solution, investigating the impact of time and pH. The researchers observed that the absorption of the complexes decreased over time. They also found that the Co(II) complex exhibited the highest absorption in the pH range of 7.5–8.5, while the Ni(II) complex displayed the highest absorption in the pH range of 9.5–10.5. Moreover, the binding ratios of the complexes were determined using both the continuous variation method and the mole ratio method, resulting in a ratio of 2:1 (M:L). The formation constants were calculated, and the results indicated that the order of stability was Co(II) > Ni(II). Additionally, the molar absorption coefficients of the complexes were calculated. Additionally, Elsherif et al. [23] developed a spectrophotometric method for the determination of zinc(II) and copper(II) ions using murexide ions. The mixture reagent interacted with Zn(II) and Cu(II) instantaneously at pH 7 and pH 5.5, respectively, and the absorbance of the solution was stable for 220 and 120 minutes, respectively. The stoichiometry of the complexes was found to be a 1:2 metal-to-ligand ratio for Zn and Cu. The continuous variation approach was used to estimate the stability constant (K_{stab}) values, which were found to be on the order of 1.35×10^{16} and 2.30×10^{17} for the Zn and Cu complexes, respectively.

This study aimed to investigate the stability of the murexide complex with zinc(II) ions in a mixed solvent of water and isopropanol using absorption spectroscopy in the visible region. The effects of pH and time on the stability of the complex were examined. The stoichiometry, molar absorption coefficient, and stability constant were estimated using the continuous variations method. Additionally, the impact of certain interfering ions on complex stability was studied.

1. Materials and Methods

2.1 Chemicals and solvents

The study used chemicals and solvents of ACS grade purity from Merck and Sigma–Aldrich without further purification. Chloride salts were chosen because of their high solubility, which yielded clear and pure solutions. The salts included potassium chloride, sodium acetate, zinc chloride, sodium nitrate, and sodium sulfate. The reagents used were the murexide indicator (C₈H₄N₅O₆NH₄), sodium hydroxide, and hydrochloric acid.

2.2 Instruments

The ultraviolet–visible (UV–VIS) absorbance of the murexide solutions and their complexes with Zn(II) were measured using a Cary 60 UV–VIS spectrophotometer from Agilent. A pH 3505 device from Jenway, UK, was used to adjust the pH of the complex solutions.

2.3 Standard solutions

Stock solutions (5×10^{-3} M) of murexide and ZnCl₂ were prepared by dissolving the specified weight in distilled water. To investigate the effect of interfering ions, solutions with a concentration of 1.0 M were prepared by dissolving KCl, NaNO₃, Na₂SO₄, and CH₃COONa salts in distilled water.

2.4 Spectra of murexide absorption in various mixtures of water and isopropanol

A series of equal-concentration standard solutions of murexide were

prepared, containing varying ratios of water and isopropanol ranging from 10% to 100% water. The absorbance of these solutions was measured in the ultraviolet-visible region (300-700 nm).

2.5 Determination of the λ_{\max} of murexide complexes with Zn(II)

A standard solution of the murexide complex with zinc ions was prepared that contained equal volumes of murexide and zinc ions, after which the volume was adjusted to the mark using a mixture of water and isopropanol at the studied ratio. The absorbance of these solutions was measured in the ultraviolet-visible region (300-700 nm).

2.6 The effect of pH on the absorption Spectra of the complexes

A series of standard solutions of the murexide complex with zinc ions in water and isopropanol were prepared, and the pH was adjusted using HCl solution for the acidic medium and NaOH solution for the basic medium. The pH values of the solutions ranged from 2 to 11. Subsequently, the absorbance of these solutions was measured in the ultraviolet-visible region at λ_{\max} corresponding to each complex. Based on the obtained results, the optimal pH for each complex was selected.

2.7 The effect of time on the absorption Spectra of the complexes

A standard solution of the murexide complex with the target element ion was prepared in water and isopropanol, and the pH was adjusted to the optimal value obtained from the previous experiment. The absorbance at the λ_{\max} was subsequently measured every 5 minutes for 3 hours for each complex.

2.8 Determination of complex stoichiometry by Job's method

A series of equal-concentration standard solutions were prepared containing varying volumetric (or mole) ratios of murexide and Zn ions, with the following changing ratios: 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9. The concentrations of both murexide and Zn ions should be equal (5×10^{-3} M). The pH of each solution was individually adjusted, and the absorbance of each complex was measured at λ_{\max} . The relationship between the absorbance and the volumetric (or mole) ratio of Zn ions (or murexide) is plotted.

2.9 Determination of detection limits and quantification limits

A series of standard solutions were prepared for each complex containing different volumes of Zn ions at a concentration of 5×10^{-3} M (20, 40, 80, 150, 200, 250, 300 μ L), while maintaining a constant volume of murexide in all the solutions (2 mL) and at the same concentration (5×10^{-3} M). The pH was adjusted to the optimal value for each complex, after which the absorbance was measured at λ_{\max} for each complex.

2.10 Effect of interfering ions

The effect of interfering ions on the absorption spectra of the complexes was studied using previously prepared CH_3COOH , NaCl, NaNO_3 , and Na_2SO_4 . A series of standard solutions for each complex was prepared, to which different concentrations of the salts were added. The pH of the solutions was adjusted to the optimal values specific to each complex, after which the absorption was measured at λ_{\max} for each complex. The concentrations of the salts used were as follows: 0, 0.01, 0.02, 0.05, 0.1, 0.2, and 0.5 M.

2. Results and Discussion

3.1 Absorption spectra of murexide in various mixtures of water and isopropanol

Fig. 2 shows the UV-VIS absorption spectra of the murexide obtained in the range of 300 nm to 700 nm. Two absorption peaks are observed, one at λ_{\max} of 515 nm to 520 nm and the other at 320 nm to 330 nm, indicating the presence of two absorption chromophores. According to the murexide structure, the peak with the highest intensity corresponds to the $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions in the two pyrimidine rings. Additionally, blueshifts are observed in the absorption peaks, proportional to the decrease in solvent polarity, due to the formation of hydrogen bonds between the solvents and murexide [24,25].

Table 1 presents the absorbance values and corresponding wavelengths obtained. Based on these results, three different ratios of isopropanol to water were chosen for further studies because they exhibited high absorption values and varying degrees of polarity. These ratios are 8:2, 6:4, and 3:7.

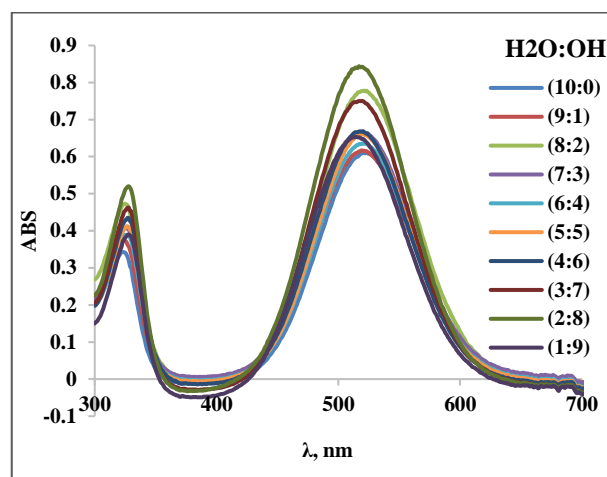


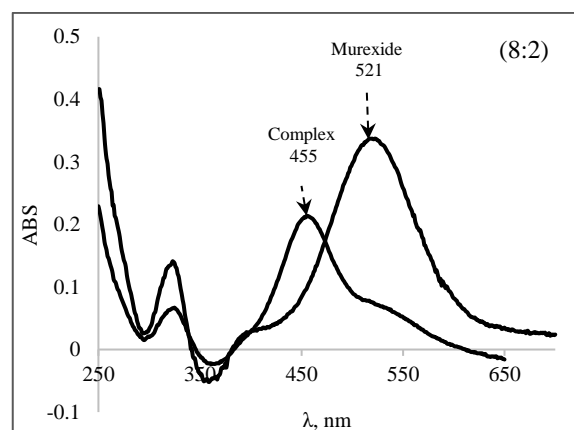
Fig. 2. Absorption spectra of murexide

Table 1. Absorbance values and λ_{\max} of murexide

ABS	λ_{\max}	H ₂ O:OH
0.610	521	10:0
0.617	519	9:1
0.778	521	8:2
0.675	520	7:3
0.636	520	6:4
0.660	519	5:5
0.669	520	4:6
0.750	518	3:7
0.844	517	2:8
0.635	515	9:1

3.2 Absorption spectra of the murexide-Zn complex

The relationship between the absorption and wavelength of Zn(II) complexes with murexide was plotted separately for the three selected ratios, as shown in Fig. 3. From the obtained spectra, blue shifts are observed in the murexide absorption peaks, giving rise to distinct new absorption peaks specific to each complex formed. These shifts occur due to the reduced contribution of $n \rightarrow \pi^*$ transitions in murexide, as the n electrons participate in the formation of coordination bonds with Zn(II) ions [26,27]. Notably, the absorption peaks for these complexes shift by different amounts, with absorption peaks occurring at 8:2, 6:4, and 3:7 at 460 nm, 455 nm, and 455 nm, respectively, while murexide absorption peaks occur at 521 nm, 520 nm, and 518 nm, respectively.



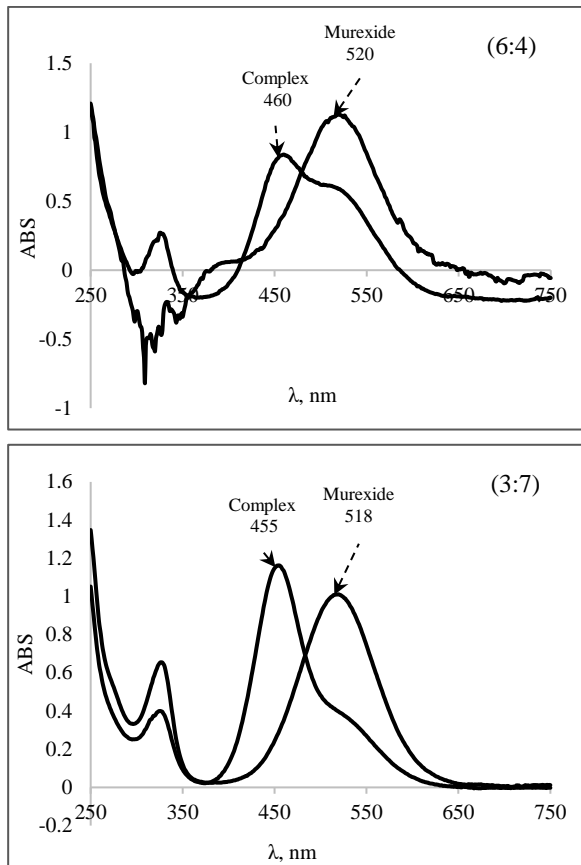


Fig. 3. Absorption spectra of Zn-murexide complexes at various water: isopropanol ratios

3.3 Effect of pH on the absorption spectra of the complexes

Fig. 4 shows the spectral curves obtained from the study of the effect of pH on Zn(II) complexes with murexide for the three investigated ratios. At ratios of 8:2 and 6:4, the complexes exhibited weak absorption under strongly acidic conditions (pH less than 3), where the color of the solution disappeared. This difference is attributed to the rapid decomposition of murexide under strongly acidic conditions [28]. Additionally, the complex's absorption under strongly basic conditions (pH higher than 7) at a ratio of 8:2 nearly disappears due to the precipitation of Zn(II) ions as hydroxide in this medium, while the complex showed the highest absorption at this ratio in a neutral medium. However, at a ratio of 3:7, the complex exhibited high absorption under all pH conditions. The ideal pH values for complex formation are presented in Table 2.

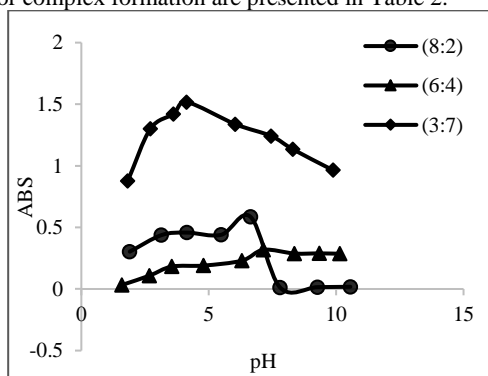


Fig. 4. Effect of pH on complex absorption for three solvent/solution ratios

Table 2. Various parameters of Zn-murexide complexes at three different solvent ratios

H ₂ O:OH	λ _{max}	pH	L:M	ε	pK _f	R ²
8:2	455	6 -6.70	1:2	2109.1	10.86	0.9917
6:4	460	7 - 7.50	2:1	21250	11.04	0.9826
3:7	455	3 - 4	1:1	13795	5.6	0.9845

3.4 Effect of time on the absorption spectra of the complexes

Fig. 5 shows the obtained spectra of the Zn-murexide complexes, which represent the time effect for the three investigated solvent ratios. The effect of time was studied at the previously obtained optimal pH values. These curves show that these complexes form rapidly (color changes immediately upon addition) and exhibit stability and resistance to decomposition during the studied time period. This supports their use for estimating Zn(II) in solution. Additionally, a slight decrease (10%) in the absorption value was observed after 3 hours at a ratio of 6:4.

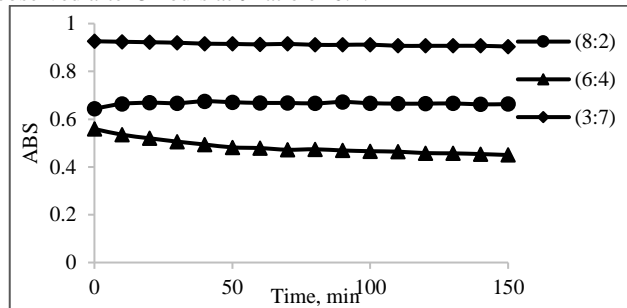


Fig. 5. Effect of time on complex absorption for three solvent at different ratios

3.5 Determination of complex stoichiometry by the Job method [27]

Fig. 6 shows the Job plots of the absorbance versus the mole fraction of murexide for Zn(II) complexes in different solvent mixtures. The plots indicate that the stoichiometry of the complexes is 1:1 (ML) when the water-to-ethanol ratio is 3:7 and 1:2 (ML₂) when the ratio is 8:2 or 6:4. The higher water content in the latter case facilitates the formation of ML₂ complexes due to the reduced steric hindrance around the Zn(II) ion [29].

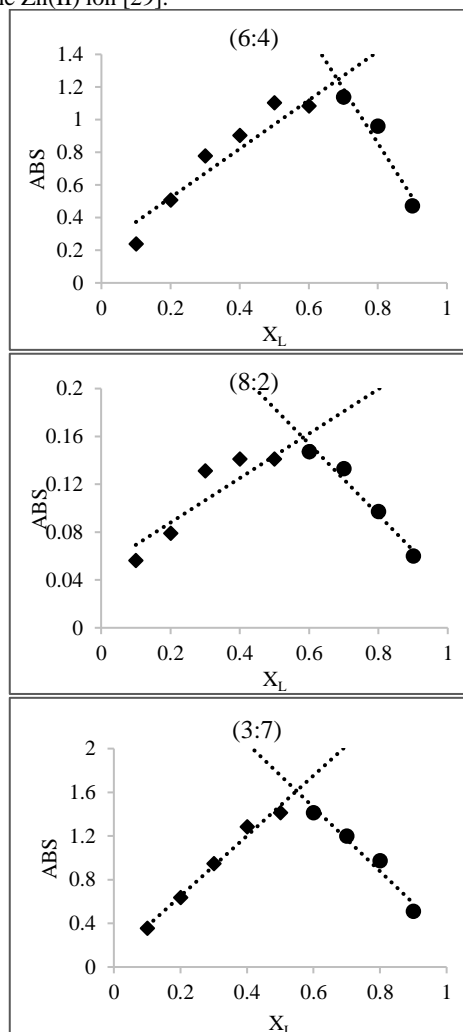


Fig. 6. Job plots for Zn-murexide complexes formed with three solvent at different ratios

3.6 Determination of the formation constant

The stability constant K_f for the 1:1 (ML) complex can be calculated using the following equation [31]:

$$K_f = \frac{1-\alpha}{a^2C} \quad (1)$$

where α represents the fraction of the dissociated complex and C denotes the concentration of the formed complex. However, for the 1:2 (ML₂) complexes, their stability constants can be calculated using the following equation:

$$K_f = \frac{1-\alpha}{4a^3C^2} \quad (2)$$

Table 2 lists the stability constants of the Zn(II) complexes with murexide at different solvent ratios. The stability constants showed that the 1:1 (ML) complex at a 3:7 ratio was the least stable. This is because the chelating effect increases the stability of the complexes in the 8:2 and 6:4 mixtures. At a 3:7 ratio, murexide forms two pentagonal rings with Zn(II) at a 1:1 binding ratio. In the 8:2 and 6:4 mixtures, murexide forms four pentagonal rings with Zn(II) in a 1:2 binding ratio. The more rings there are, the more stable the complex [23,32]. The stability constants also decrease as the water content in the solvent increases [29].

Fig. 7 shows the calibration curves for the murexide complexes with Zn(II). It is observed from the plot that all the curves were linear, indicating their compliance with the Beer-Lambert law.

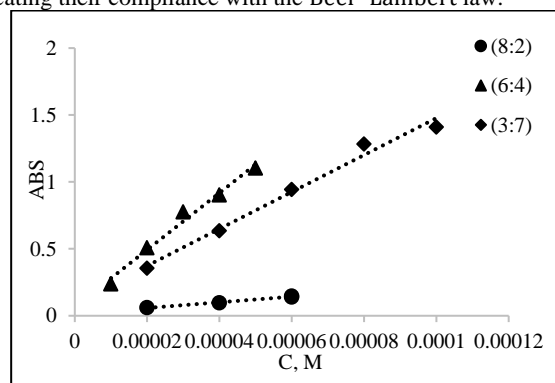


Fig. 7. Calibration curves for Zn-murexide complexes at three solvent ratios

3.7 Effect of interfering ions

Fig. 8 shows the graphs of the absorbance versus the concentration of different interfering ions: CH_3COO^- , Cl^- , NO_3^- , and SO_4^{2-} . The graphs reveal that the absorbance of the complexes is not significantly affected by Cl^- or NO_3^- ions, as it only decreases slightly. This validates the proposed method. However, the absorbance of the complexes drops sharply in the presence of SO_4^{2-} ions, especially at high concentrations (0.2-0.5 M), where the solution forms a precipitate that blocks the absorbance measurement.

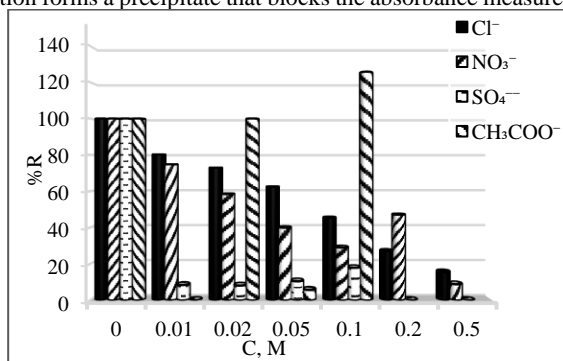


Fig. 8. Effect of various interfering ions on the stability of the Zn-murexide complex

3.8 Determination of sensitivity and detection limits

A solvent ratio of 3:7 was chosen for the spectrophotometric measurements of Zn(II) ions because this ratio had the highest stability. The sensitivity, detection limit, and quantification limit of the proposed spectrophotometric method were determined. The sensitivity was estimated by plotting the relationship between the

absorption and the concentration of the metal ion in parts per million (ppm), as shown in Fig. 9. The sensitivity represents the slope of the linear relationship between the absorption of the metal ion and its concentration. The Figure shows the linear concentrations that follow Beer's law for the metal ions and their correlation coefficients. The obtained results are presented in Table 4.

The detection limit (LOD) and quantification limit (LOQ) were estimated using the following equations [33,34]:

$$\text{LOD} = 3\text{SD} \quad (3)$$

$$\text{LOQ} = 10\text{SD} \quad (4)$$

SD stands for the standard deviation of ten blank measurements (or measurements with very low metal ion concentrations). Table 3 shows the results.

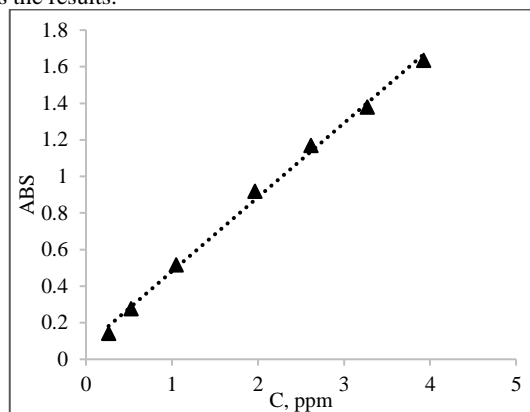


Fig. 9. Calibration curve for metal ions

Table 3. Sensitivity (S), linear range, detection limit (LOD), and quantification limit (LOQ) of the utilized spectrophotometric methods

S (ppm ⁻¹)	LOD (ppm)	LOQ (ppm)	Linear ppm	R ²
0.406	0.016	0.054	0.2 - 3.9	0.9965

3. Conclusion

In this research, a spectroscopic study was conducted to elucidate the stability of the complex formed between murexide ions and Zn(II) in mixed solvents of water and isopropanol. The stability constant, mole ratios, and molar absorptivity of the formed complexes were estimated at three different ratios of the water-isopropanol mixture. The binding ratios were 1:1 and 2:1 (L:M).

The complex formed at a ratio of 3:7 exhibited the highest stability, which was attributed to the chelation effect, which played a significant role in enhancing the stability of the complex compared to that at ratios of 8:2 and 6:4. By studying the influence of several interfering ions on the stability, it was noted that the presence of Cl^- and NO_3^- ions did not significantly affect the absorption of the complexes, as the absorption decreased only slightly. This finding supports the credibility of the proposed method. However, the absorption of the complexes noticeably decreased in the presence of SO_4^{2-} ions, especially at high concentrations where the solution precipitated, hindering the measurement of its absorption.

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