Article

a special issue for the scientific conference held by the Department of Chemistry- College of Education for Girls/University of Kufa and in cooperation with Hilla University College, under the title (**5'th Postgraduate Students Annual Conference**) **(PSAC2024)**, which held for Wednesday, **24/4/2024.**

Study of complexation behavior for Cobalt(II) with a new ligand derived from pA benzoic acid to estimate Co^{2+}content in vitB12

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Abstract:

The spectrophotometric method was suggested for the determination of trace amounts of cobalt. The method is based on the reaction of cobalt (selected between some ions) with a novel prepared reagent 4-((1-ethoxy-1,3-dioxobutan-2-yl) diazenyl)benzoic acid at pH 9 in the presence of sodium hydroxide as a basic medium to form a distinct color complex that shows maximum absorption at λ_{max} = 421nm. Under specific conditions, Beer's law is obeyed to control the concentration mode of 10-60 ppm. The molar absorptivity was 106.0695 $L.mol^{-1}cm^{-1}$, Sandell′s sensitivity was 2.6×10^{-5}, and the detection coefficient was 0.9984. The method was applied with good results to estimate cobalt in multiple doses of vitamin B12 (tablets and injection), then compared with FAAS instrumental, for which the results were close.

1. Introduction

Azo compounds are recognized as valuable reagents and serve as a foundation for coordination chemistry due to their uniformity and ability to form complexes with various transition metal ions [1,2]. Their preparation methods are generally similar, with minor adjustments, except for those tailored for producing heterocyclic azo compounds, including those with five- and six-membered rings [3-4].

Azo dye compounds demonstrate favorable reaction rates and high stability, along with ease of purification and intense colors that exhibit wavelength shifts upon interaction with transition metal ions [5,6]. Among these compounds, chelate complexes with five- or six-membered chelate rings [7] are particularly stable and hold substantial importance, playing crucial roles in industrial technologies and biological processes [8-9].

They have long captivated and motivated scientists all over the globe because of their potential uses in a variety of domains. Its significance is shown by the breadth, volume, and current research articles' abundance on the topic as well as the variety of applications. The Azo group is characterized by a nitrogen atom, which may chelate if it is attached to an aromatic ring that has more donor sites.

Azo compounds are primarily synthesized by coupling diazotized heterocyclic amines with aromatic hydroxyl and amino compounds. These molecules offer numerous advantages. Among them, the azo group exhibits photochromism, responds to redox reactions, shows sensitivity to pH changes, stabilizes low-valent metal oxidation states owing to a low-lying azo-centered molecular orbital, and serves as a metal ion indicator in complexometric titrations, as well as in dyes and pigments within the textile industry [10-11].

Azo compounds exhibit vibrant colors and exceptional thermal and optical attributes and serve crucial roles in applications like optical data storage, photoswitching, and the development of nonlinear optical materials. The bonding characteristics of azo compounds are notable, primarily due to the N-N group present within their structure. This feature enables them to create diverse metal complexes with transition metal ions, resulting in unique structural and magnetic properties. [12-13]

Scholars have delved deeply into examining these complexes through various physicochemical methods. Numerous studies have elucidated the properties of azo complexes utilizing various techniques, including analysis of electronic transitions, studies of infrared spectra, magnetic susceptibility measurements, and ESR spectra examinations. [14-15].

2. Experimental Work

2.1.Instrumentation

The following devices and instruments were utilized in the current research:

- Double-beam (UV-Vis) spectrophotometer, UV1800 Shimadzu, Japan.
- Test-scan Shimadzu FT-IR 8400 Series, Japan.
- Electrically sensitive balance A D Company, Limited, Dool, CE HR 200, Japan**.**
- OvenPhilip Harris Limited, Shenstone, England.
- E163694 Germany pH-meter, WTW, listed laboratory equipment (beakers, pipettes, and volumetric flasks of different sizes).
- Atomic Absorption Spectrophotometer, AA-7000, Shimadzu, Japan.

2.2.Preparation of standard solutions

1. Stock solution for reagent 4-((1-ethoxy-1,3-dioxobutan-2-yl) diazenyl)benzoic acid

A stock solution for reagent (1-EDBBA) at a concentration of $1x10^{-1}$ M was prepared by dissolving 0.1391 g of pure reagent in a 5 mL volumetric flask. The solution was made up to the mark with ethanol to give $1x10^{-1}$ M. Other standard solutions with a concentration of $1x10^{-4}$ M were prepared by subsequent dilution using ethanol in the stock solution.

2. Storage solution ($CoCl_2$) at 100 ppm concentration

200 milliliters of distilled water were utilized to dissolve 0.044 grams of cobalt chloride (II) (CoCl2) to produce the storage solution. The solution was made with distilled water to give 100 ppm.

3. 0.1 M Sodium hydroxide solution (NaOH)

A sodium hydroxide solution was prepared by dissolving 0.1 g of it in a small beaker and dissolving it with distilled water. Transferring the solution to a volumetric vial of 25 mL capacity and completing the volume to the mark with distilled water.

4. 0.1 M solution of hydrochloric acid (HCl)

0.2 mL of hydrochloric acid was transferred to a volumetric vial of 25 mL capacity, and the volume was completed with distilled water.

5. Standard solutions of the detected metal ions

The standard solutions of the detected metal ions were prepared at a concentration of 100 ppm.

6. Buffer solution with 0.1M and pH 9

The buffer solution was prepared by dissolving 6.20 g of boric acid in 500 ml of distilled water using a 1000-ml volumetric flask. After that, a solution was made up to the mark using distilled water after adding 41.5 ml of NaOH at a 4 M concentration.

7. 0.1 M Sodium carbonate solution (Na_2CO_3)

A 0.265 g sodium carbonate solution was prepared in 25 mL of distilled water using a 25 mL volumetric vial flask.

8. Diluted ammonia solution (NH_4OH)

A diluted ammonia solution was prepared at a ratio of 1:2 by adding 10 ml of concentrated ammonia hydroxide to 10 ml of distilled water in a 20-ml volumetric vial flask.

2.2.1 Preliminary tests of the reagent with ions

A preliminary test was conducted for 1-EDBBA with 20 ions when the reaction conditions of concentration, temperature, and acid function were fixed.

From these results, the Co^{+2} ion was chosen to be complex with the prepared reagent 1-EDBBA (Ge^{+4}, Mg^{+2}, Ni^{+2}, Cu^{+2}, Al^{+3}, Au^{+1}, Cu^{+2}, Hg_2^{+2}, Co^{+2}, Na^{+1}, Ce^{+2}, Pt^{+2}, Cd^{+2}, Sn^{+2}, Mo^{+6}, $NH4^{+1}$, Li^{+1}, Co^{+2}, Cr^{+2}, Ba^{+2}).

2.3.1.Determination λ_{max}

Measuring λ_{max} of the Reagent

To estimate the wavelength at maximum absorbance for the reagent (1-EDBBA), a UV-Vis device was utilized in an 800-200 nm range against an absolute ethanol solution. A double beam spectrometer (Cavetti) of 1 cm thickness (bath length) was utilized as a blank solution.

Measuring λ_{max} of the complex

To estimate the wavelength at maximum absorbance for (complex) by using UV-Vis has been recorded in the range (800-200 nm) against reagent solution as a blank solution by using a double beam spectrometer, (Cavetti) of 1cm thickness (bath length).

2.3.2. Limitation of the optimum conditions

As shown in the following steps, many tests were performed to indicate the optimum conditions for metal complexes to get high sensitivity and selectivity using spectroscopic methods.

1- The effect of (pH)

To achieve optimal pH values, the absorbance of metal complex solutions was studied in a pH range of 5–12 using NaOH (0.1 M) for basic solutions and HCl (0.1 M) for acidic solutions of complexes.

2- Effect of base

The complex was prepared to study the best base by taking 2 ml of the reagent (1-EDBBA) at a concentration of $1x10^{-4}$ M with 2 ml of cobalt chloride ion at a concentration of 100 ppm in a 5 ml volumetric vial and completing the volume to the mark with absolute ethanol while holding the acid function constant with the following rules: NaOH, NH_4OH, Na_2CO_3, and Buffer at calculated concentrations.

3- addition effect

A 2 mL reagent was prepared at a concentration of 1×10^{-4} M. A solution of the metal ion $CoCl_2$ was prepared at 100 ppm, from which 2 ml was taken, and 0.15 ml of NaOH was added at the optimum pH in a 5 ml volumetric vial, completing the volume to the mark with absolute ethanol. The absorbance was measured for each addition.

4-The effect of time

2 ml of the reagent (1-EDBBA) at a concentration of $1x10^{-4}$ M with 2 ml of the ion ($CoCl_2$) at a concentration of 100 ppm was added to 0.15 ml of sodium hydroxide to stabilize the acid function in a 5 ml volumetric vial, complete the volume to the mark with absolute ethanol, and measure the highest absorbance of the complex at different times against the reagent as a blank solution at λ_{max} = 421 nm.

5- Influence of temperature

The temperature influence on the products 1-EDBBA $CoCl_2$ color intensity was studied within the temperature range from 5 to 40°C by taking 2 ml of the reagent (1-EDBBA) at a concentration of $1x10^{-4}$ M and adding to it 2 ml of the ion ($CoCl_2$) at a concentration of 100 ppm and sodium hydroxide in a volume of 0.15 ml at the optimum pH value in a 5 ml volumetric vial, completing the volume to the mark with absolute ethanol, and measuring the absorbances of the complex at λ_{max} = 421 nm.

6- Effect of volume of ($CoCl_2$)

The maximum absorption of the cobalt ion (Co^{+2}) was studied at 421 nm at a concentration of 100 ppm and increasing volumes of 0.5, 1, 1.5, 2, and 2.5 ml with a constant concentration and volume of the reagent (1-EDBBA) $1x10^{-4}$ M, 2 ml, by adding sodium hydroxide in a 5 ml volumetric vial and completing the volume to the mark with absolute ethanol. The absorbance is measured against the reagent as a blank solution.

2.3.3. Stoichiometry of the formed products - Composition of the complex

The determination of transition metal complex composition was conducted using Job's method of continuous variations and the molar ratio method.

a. Molar ratio method

The composition of chalet complexes was investigated by the mole ratio method at a fixed volume and a fixed concentration of metal ions (2 ml, 100 ppm) and an increasing concentration of reagents ($0.5x10^{-5}$, $1x10^{-5}$, $1.5x10^{-5}$, $2x10^{-5}$, $2.5x10^{-5}$, $3x10^{-5}$, $3.5x10^{-5}$, and 4×10^{-5} M) with a fixed volume of 2 ml at optimum pH and λ_{max}.

b. The continuous variations method

The method involves preparing two solutions, the organic reagent solution and the metal ion solution, and mixing them at different volumes with the same concentrations (M), keeping the total volume of the mixture constant at 5 ml.

c. Calibration curve of metal ion

The optimal concentration for transition ions was chosen by mixing 2 ml at concentrations (10, 20, 30, 40, 50, 55, and 60 ppm) of metal ions with 2 ml ($1x10^{-4}$ M) of reagent 1-EDBBA at pH 9. The absorbance was then plotted against the concentrations.

3. Results and discussion

3.1. Determination of λ_{max}

The reddish-brown-colored solution of 1-EDBBA (dissolved in ethanol) had a λ_{max} of 358 nm, labeled the N=N moiety. Figure (1) assures that the compound was synthesized, so this band does not appear in any of the starting materials.

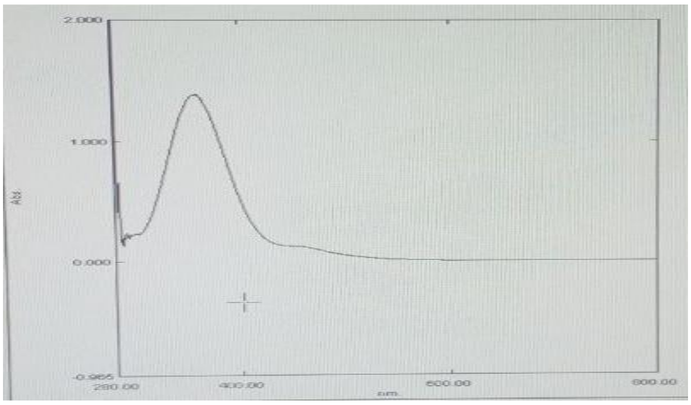

Figure (1)- UV-VIS spectra for 1-EDBBA

The UV-VIS spectra of the complex ($CoCl_2$) showed a significant absorbance at λ_{max} of 421nm, as shown in Figure (2). The complex and reagent spectrums were also plotted.

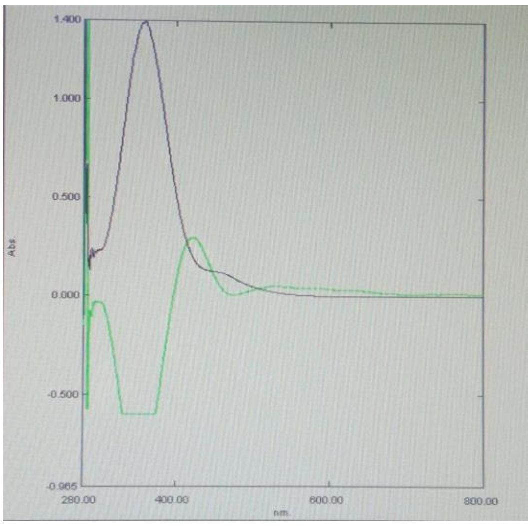

Figure (2): UV-VIS spectra for 1-EDBBA and complex ($CoCl_2$)

3.2. Limiting of Optimum Conditions

The optimum conditions are limited depending on the concentrations that obey the Lambert-Beer law. These conditions for reacting metal ions with organic reagents comprise the best acidity function and concentrations.

3.2.1. The best Acidity Function (pH)

Acidity function involves the existence of removed protons on the donor at different pH values of the reagent molecule. According to the acidity function study, the atom in organic reagent structure also exhibits resonance state formation at a wide range of pH 5 to 12, indicating that the best pH value is between 5 and 12 because of the presence of a proton on the nitrogen atom. As the results appear to reflect in Table (1) and Figure (3), the high absorbance was at pH 9.

Table (1): Absorbance of target metal ion complexes with (4-HNPBS) in pH range

Ph	5	5.5	6.5	7	9	10.5	11	12
Abs.	0.051	0.065	0.150	0.212	0.268	0.218	0.160	0.063

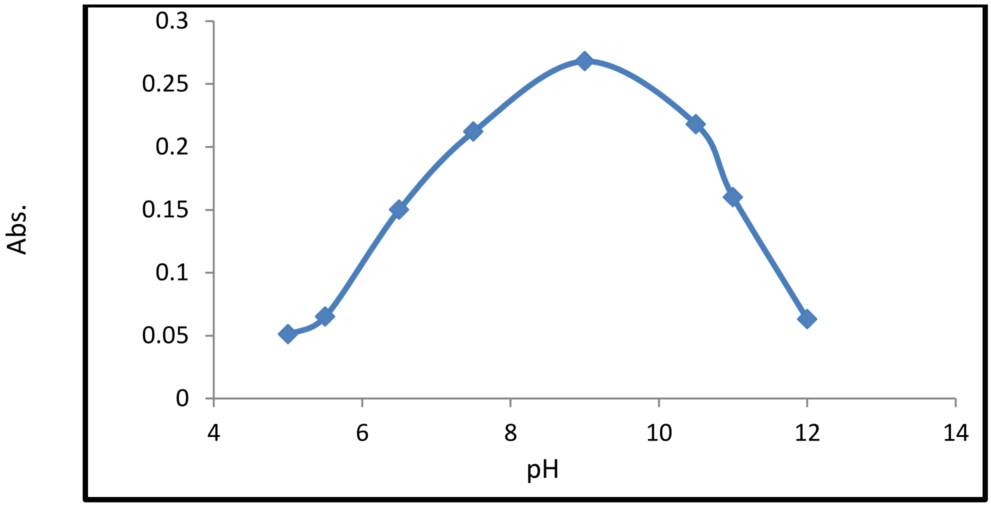

Figure (3): Effect of acidity function on Co(II) complex at optimum concentration.

3.2.2. Base effect

More than one rule was tested, and a specific absorbance was determined for each addition, as shown in Table (2). The best rule is shown in Figure (4) NaOH.

Table (2): Effect of basic solution on complex Co(ll) absorbance at optimum Conditions.

base	NaOH	Na_2CO_3	NH_4OH	Buffer
Abs.	0.268	0.200	0.194	0.126

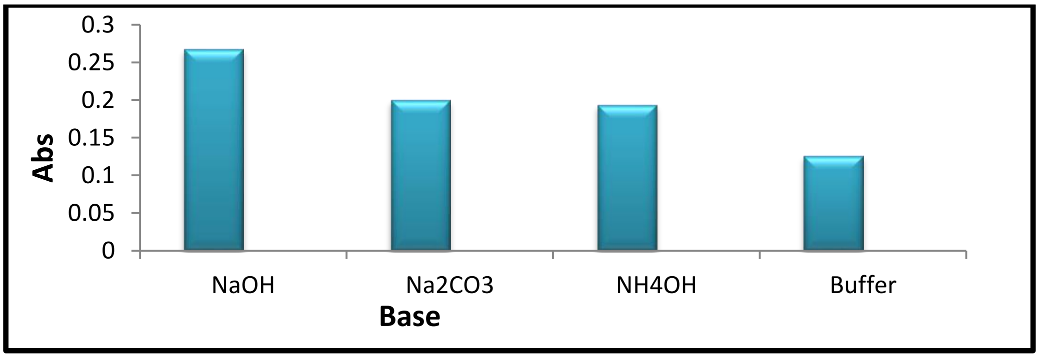

Figure (4): Effect of basic solution on complex Co(ll) absorbance at optimum concentration.

3.2.3. Addition effect

The best addition was chosen through the sequence of additions to the reagent-ion-base, where each addition gave an absorbance, as shown in Table (3).

Table (3): Influence of addition on absorbance

Addition	Absorbance
$CoCl_2$ + Reagent + NaOH	0.154
Reagent + $CoCl_2$ + NaOH	0.208
NaOH + Reagent + $CoCl_2$	Precipitated
NaOH + $CoCl_2$ + Reagent	Precipitated

3.2.4. Influence of time

The stability of a chelating complex is essential to the performance of optimum conditions to get a chelating complex in its solution. Therefore, the chemists deal with it in the analytical field to demonstrate this factor, recording the absorbance of the metal complex at optimum conditions for a range of 0 to 5 min. It was found that the absorption of the mineral complex is optimal when prepared immediately, considering that the complex is one of the rapid reactions. The results are shown in Figure (5).

Table (4): The effect of time on absorbance of complex at optimum Conditions.

Time (min)	0	1	3	5
Abs.	0.376	0.266	0.213	0.206

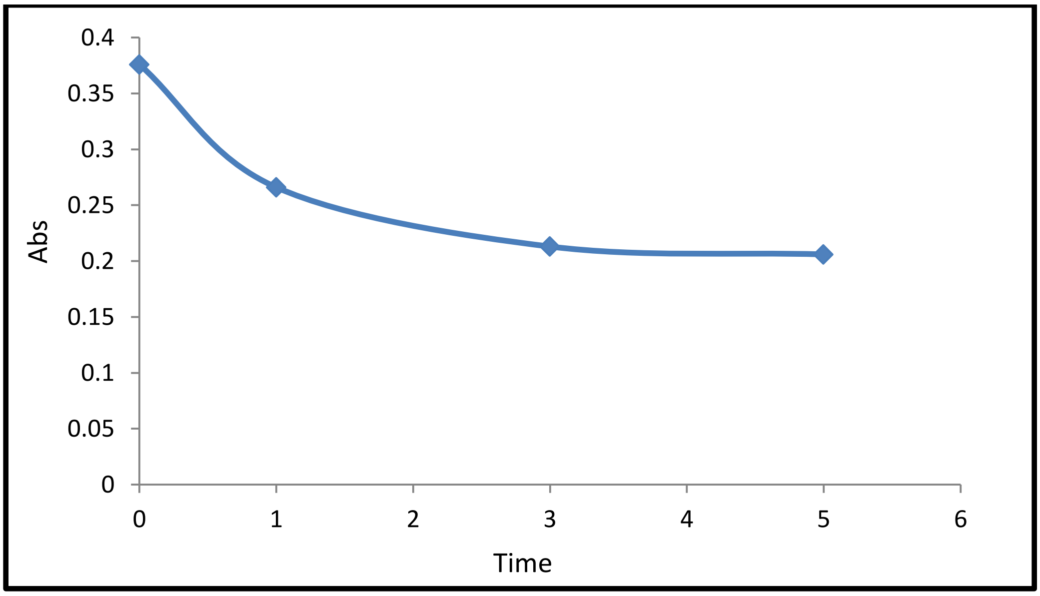

Figure (5): Effect of time on Co(II) complex at optimum concentration

3.2.5. Influence of temperature

An investigation into the impact of temperature was conducted within the range of 5 to 40°C. Observations revealed that the color intensity exhibited an increase with rising temperature up to 15 °C, followed by a gradual decrease. Consequently, the temperature of 15°C was chosen for the determination of 1-EDBBA. Figure (6) shows the effect of temperature.

Table (5): Effect of temperature on the absorbance of complex Co(ll) at optimum Conditions

T(C˚)	5	10	15	20	25	30	40
Abs.	0.266	0.279	0.320	0.272	0.270	0.280	0.286

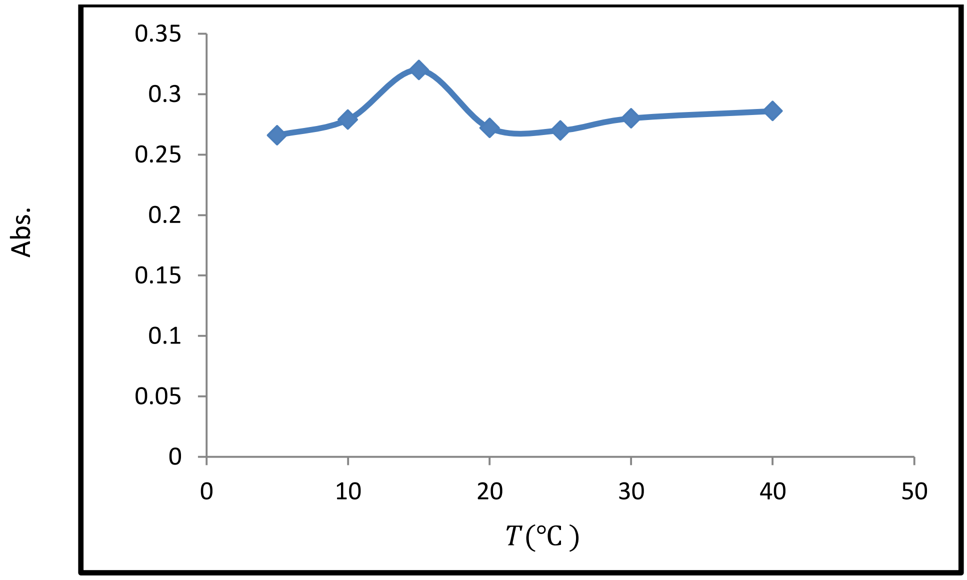

Figure (6): The Effect of temperature on Co(II) complex at optimum concentration

3.2.6. Effect of Co(ll) volume

The volumes of 0.5, 1, 1.5, 2, and 2.5 mL of metal ion for the maximum color of the product to attain at 421 nm were studied when the metal ion concentration was 100 ppm with a fixed volume and fixed concentration from the reagent (1-EDBBA). The absorbance increased with the Co(II) volume and became greatest at 2 mL, respectively, then decreased. Thus, 2 mL was selected as the optimum value for the determination process, as shown in Figure (7).

Table (6): Effect of Co(II) absorbance volume on the complex at optimum Conditions

V/Ml	0.5	1	1.5	2	2.5
Abs.	0.219	0.245	0.271	0.298	0.288

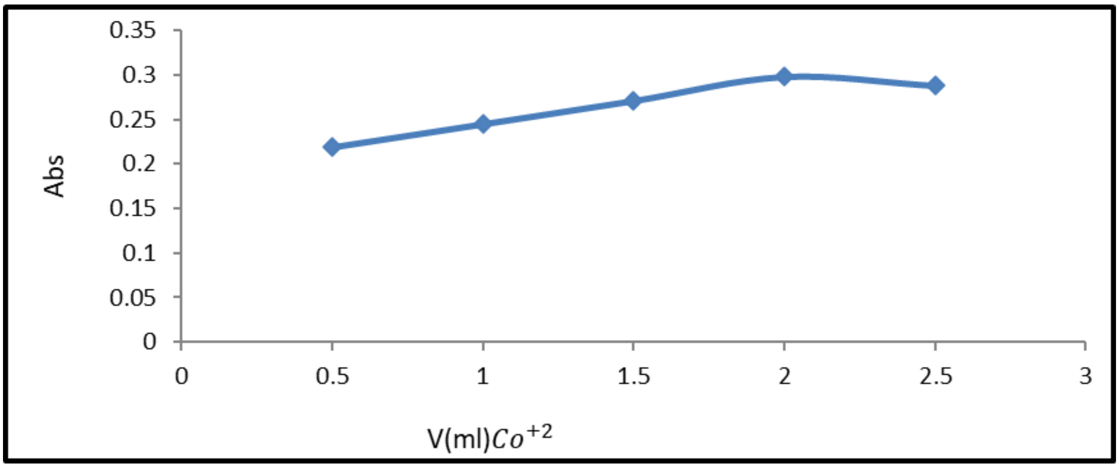

Figure (7): Effect of Co(II) absorbance volume on the complex at optimum Conditions

Stoichiometry

The continuous variation method (Job method) and the mole ratio method (YAO, Jon method [16]) were performed to determine the stoichiometry of the chelating complexes. The results in both cases reveal a 1:2 metal-to-reagent ratio that illustrates the more probable structure of Co(II) transition metal complexes.

a-Mole Ratio Method

The procedure consists of mixing a fixed volume (2 ml) and a fixed concentration (100 ppm) of metal ions with a fixed volume and different reagent concentrations (1-EDBBA). The absorbance was measured at optimum conditions. The absorbance against concentrations of the reagent was plotted. Table (7) and Figures (8) show the interaction results of the two straight lines at 2.

Table (7): Relationship between absorbance and mole ratio of metal complexes solution with reagent (1-EDBBA) at optimum complex conditions.

1-EDBBAMolar conc.	$CoCl_2$ conc.	Absorbance

0.5×10^{-5}	100 ppm	0.019
1×10^{-5}		0.03
1.5×10^{-5}		0.040
2×10^{-5}		0.049
2.5×10^{-5}		0.052
3×10^{-5}		0.055
3.5×10^{-5}		0.057
4×10^{-5}		0.059

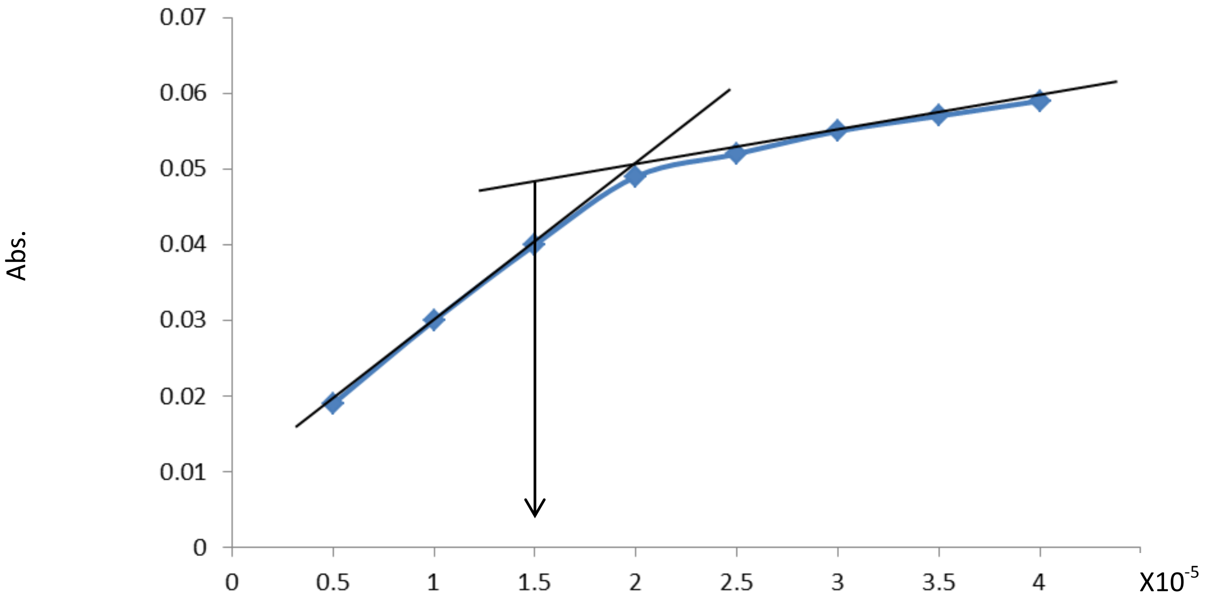

Figure (8): Mole ratio of(1-EDBBA) curve with (Absorbance)

b-Continuous Variation Method (Job′s method)

The Continuous Variation Method (Job′s method) was performed by mixing different volumes to a maximum volume of 5 ml from the metal ion solution of concentration 100 ppm with the reagent solution at optimal pH. The results inserted in Table (8) and Figure (9) gave a volumetric fraction of 0.12.

Table (8): Relationship between absorbance and Continuous Variation Method of metal complexes solution with reagent 1-EDBBA at optimum conditions of complex

1-EDBBA (V/ml)	4.5	4	3.5	3	2.5	2	1.5

$CoCl_2$ (V/ml)	0.5	1	1.5	2	2.5	3	3.5
Abs.	0.50	0.59	0.70	0.82	0.83	0.45	0.29

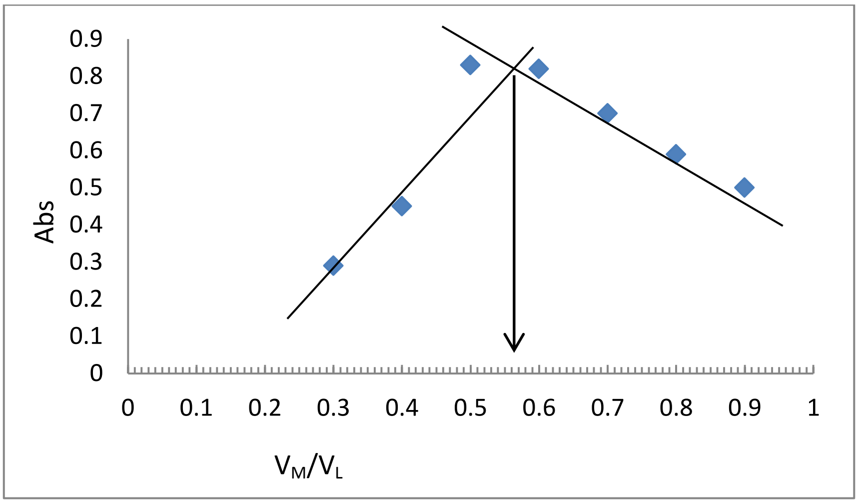

Figure (9): Continuous Variation of($CoCl_2$) curve with (Absorbance)

Figure (10) expressed the suggested structure of the [Co-2(1-EDBBA)] based on the stoichiometry study in figures (8) and (9).

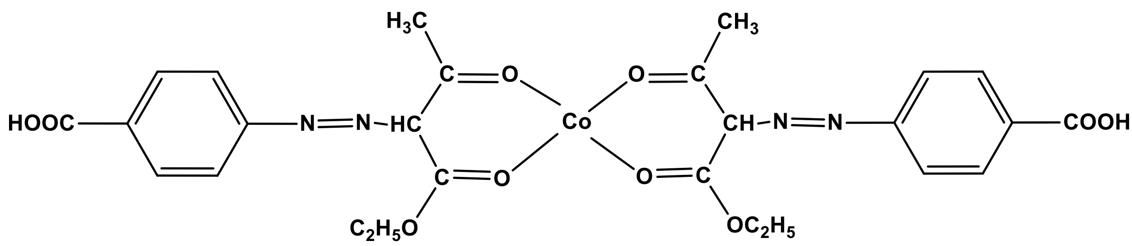

Figure (10): suggested structure of [Co- 2 (1-EDBBA)]

c- Calibration Curve of Metal Ion

The calibration curve of Co^{+2} was investigated under optimum conditions for complexation to determine the best concentration of metal ions indicated through the reaction of different concentrations (10, 20, 30, 40, 50, 55, and 60 ppm) against a fixed concentration of reagent ($1x10^{-4}$ M). Table(9) shows these results.

Table (9): Absorbance of Co (II) complexes with reagent (1-EDBBA)against a different metal ions concentration with fixed organic reagent concentration

Conc. Co^{+2}	10	20	30	40	50	55	60
Absorbance	0.018	0.047	0.075	0.102	0.128	0.140	0.150

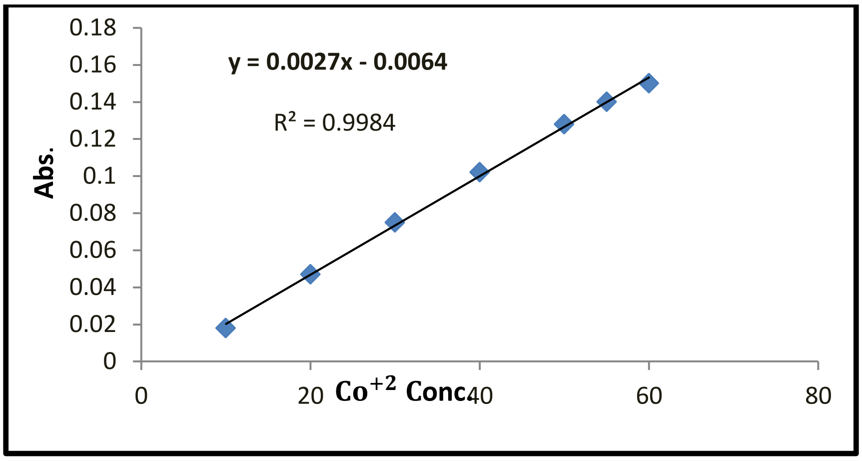

Figure (10): Calibration Curve

The curve shows its compliance with Beer's law in the range of 10 to 60 ppm of Co-ion solution at a wavelength of 421 nm. Then, some of the analytical parameters were calculated. Sandell's sensitivity (S) 2.6×10^{-5} value was found by calculating the specific absorption coefficient α=392.8501 when ε=106.0695. From Equation (1), the detection coefficient was R^2=0.9984.

$$\alpha = \frac{\varepsilon}{At.wt\times100} \qquad \text{Eq. (1)}$$

Applications

The analytical spectrophotometric method used in the research was applied to determine the cobalt (II) under specific conditions in multi-samples (tablets, ampoules) of vitamin B12. Vitamin B12 is a dietary supplement formulated as cyanocobalamin and methylcobalamin. The good results obtained in this way were compared with those of the flame atomic absorption method (FAAS), expressed in Table (10).

Table(10): Determination of Co(II) in dietary supplements vit.B12

seq	samples	Conc. $mg.L^{-1}$	Spectrophotometric method $mg.L^{-1}$	FAAS $mg.L^{-1}$
1	Vitamin B12,cyanocobalamin (tab.)	5	4.9	4.86
2	Methylcobalamin (tab.)	5	4.76	4.45
3	Vitamin B12,cyanocobalamin (Amp.)	1	0.98	0.90

Conclusions

The reagent 4-((1-ethoxy-1,3-dioxobutan-2-yl) diazenyl)benzoic acid (1-EDBBA) was prepared and identified. It can be used spectrophotometrically to determine a trace amount for a group of ions, including Co^{2+}, under specific conditions expressed previously in the research to give good results when applied to estimate the cobalt in a dietary supplement of vitamin B12 in some formulated dosage tablets and ampoules.

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