Spectrophotometric Determination of Co(II) in Vitamin B12 Using 2-(biphenyl-4-yl)-3-((2-(2,4-dinitrophenyl) hydrazono)methyl) imidazo [1,2-a]pyridine as Ligand by Flow Injection–Merging Zone Analysis

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Abstract

Nitro Schiff base ligand of 2-(biphenyl-4-yl)-3-((2-(2,4-dinitrophenyl) hydrazono) methyl) imidazo [1,2-a] pyridine (BDNHMIP) was synthesized, identified and used in spectrophotometric determination of Co(II) at 530nm. The suggested method of Co(II) determination was performed at optimum analytical parameters of 3.2 ml/min flow rate, 137.4µl ligand volume, 98.1µL Co(II) volume, 1minutes residence time in reaction coil, and as a result 48 sample/hour of method throughput was accomplished. The proposed method performed linearity of 25-400 ppm, molar absorptivity coefficient of 121.5704 L.mol.−1.cm−1, and L.O.D of 2.28ppm. The suggested method was successful applied for determination of Co(II) content of vitamin B12 injection samples with high recovery of 99.5-100.3% and minimum relative error percent of -0.3 - 0.5%.

1. Introduction

Hydrazones are resulted compounds from reaction between carbonyl compounds (aldehydes or ketones) and hydrazine derivatives. Hydrazones are special class of Schiff base compounds, these categories are specified by presence of a cyclic group of \(\text{C} = \text{N} - \text{C} = \text{N}\), which make hydrazones with other donor atoms high flexible and versatile poly dentate reagents in determination various transition and inner transition metal ions [1].

Hydrazones have wide range of applications such as analytical reagents [2, 3] for purpose of metal ions determination, as biological active compounds [4], as enzyme inhibitors [5], etc.

Compound of 2-(biphenyl-4-yl)-3-((2-(2,4-dinitrophenyl) hydrazono) methyl) imidazo[1,2-a] pyridine (BDNHMIP) is hydrazone Schiff base with more than one donor atom of heterocyclic nitrogen and nitro group in addition to characterized azomethine group. In our proposed method BDNHMIP is suggested as analytical chelate reagent for Co(II) spectrophotometric determination of vitamin B12 injections by standard addition style with high precision and simple economic technique. The aim of this research is making determination process of Co(II) in its samples as routine, inexpensive, low consuming reagents, and available analysis using chelate ligand of BDNHMIP with merging zone-flow injection technique.

Nitro group of hydrazone Schiff bases is strong withdrawing group and because of it’s steric effects nitro group has played a vital role in reactivity of hydrazone Schiff bases either it is participated in coordination [6] with metal ions or not [7-9].

2. Experimental Instrumentation

1. Double beam spectrophotometer UV-1800 from Shimadzu Corporation Company.
2. FTIR-8400 (Fourier Transform Infrared) spectrophotometer from Schimadzu Company with potassium chloride disk (in region 4000-400) was used to identify the prepared compounds.
3. Single beam spectrophotometer of V-5000(320-1000 nm) from Metash company.
4. Sensitive balance of KERAN ABS.
5. Magnetic Susceptibility Balance (JM) Johnson Matthey.
6. Heater with magnetic starrier.
7. Flow System: FI system was consisted of the following parts:
   - Peristaltic Pump: four lines peristaltic pump was used (Shenchen LabM1).
   - VIS-Optima spectrophotometer: single beam spectrophotometer was used to detect analyte response in visible region.
   - Flow Cell.
Synthesis of complex
According to synthesis method in reference [12], the complex in this study was synthesized. A 1 mmole (0.478 gm) 2-(biphenyl-4-yl)-3-(2-(2,4-dinitrophenyl) hydrazono) methyl imidazo[1,2-a] pyridine was dissolved in 5ml of ethanol and the mixture was heated under reflux for few minutes until it dissolved completely. A 0.5mmole (0.119 gm) of cobalt chloride hexa hydrate CoCl₂.6H₂O was dissolved in 5ml ethanol and added slowly to ligand solution with refluxing for 4hours, and then the precipitate with reddish brown color was filtered and dried.

3. Preparation Sample of Vitamin B12 Ampoule
Solution of one ampoule of vitamin B₁₂ was heated in furnace for 1 hour at 700 °C. The solution was cooled and 1-2ml of concentrated HNO₃ was added with heating to ensure complete dissolution. The acidity of solution was

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adjusted by adding sodium hydroxide solution to reach pH=7 and completing with deionized water to required volume according to cobalt ion content in vitamin B\textsubscript{12} ampoules [13].

Two ampoules of Methylcobal vitamin B\textsubscript{12} injection were treated by above procedure and completed volume to 10ml. A 2 ml of prepared injection solution was added in each of three (5 ml) volumetric flask and mixed with 0, 1, and 2 ml of 25 ppm standard solution CoCl\textsubscript{2} respectively with completing volume to 5 ml by deionized water.

One ampoule of Pancylo vitamin B\textsubscript{12} injection was treated by above procedure and completed volume to 100ml. A 2ml of prepared injection solution was added in each of three (5ml) volumetric flasks and mixed with 0, 1.5, and 2.5 ml of 25 ppm standard solution of CoCl\textsubscript{2} respectively with completing volume to 5ml by deionized water.

**Identification of prepared ligand (BDNHMIP) and its complex**

According to many of analyses, BDNHMIP ligand and [Co(BDNHMIP)\textsubscript{2}]Cl\textsubscript{2} were identified.

**Appearance and physical properties**

The basic properties of BDNHMIP ligand and its complex of [Co(BDNHMIP)\textsubscript{2}]Cl\textsubscript{2} were listed in Table 1.

| Appearance | Melting point, °C |
|------------|------------------|
| BDNHMIP ligand | Yellowish orange | 158-160 |
| [Co(BDNHMIP)\textsubscript{2}]Cl\textsubscript{2} | Reddish brown | 198-200 |

**Table 1.** Physical properties of BDNHMIP ligand and its complex.

**HNMR of BDNHMIP**

As shown in Figure 1 HNMR spectrum included the signals which were summarized in Table 2 for ligand identification [14].

| 2-(biphenyl-4-yl)-3-((2-(2,4-dinitrophenyl) hydrazono) methyl) imidazo[1,2-\textalpha] pyridine | HNMR data, ppm |
|-----------------------------------------------|----------------|
| Multiplate (10H) of aromatic                  | 7.10-7.47      |
| Singlet (1H) of H\textsuperscript{C}=N group  | 8.71           |
| Singlet 1H of NH group                        | 8.38           |

**Table 2.** HNMR data of BDNHMIP ligand.
Figure 1. HNMR of 2-(biphenyl-4-yl)-3-((2-(2,4-dinitrophenyl) hydrazono) methyl) imidazo [1,2-a] pyridine (BDNHMIP).

FT-IR analysis of BDNHMIP and Co(BDNHMIP)₂Cl₂.

Based on FT-IR spectra of both of BDNHMIP ligand and its complex which were shown in Figures 2 and 3, the decreasing in intensities of stretching frequencies of nitrogen bonds in addition to new peaks appearing reflected complex formation [14, 15]. The results were summarized in Table 3.

Table 3. The difference in IR spectrum peaks between 2-(4-biphenyl) imidazo [1,2-a] pyridine-3-(2,4-dinitrophenyl) hydrazone and its complex Co(BDNHMIP)₂Cl₂.

| Frequency of bond vibration in cm⁻¹ | ν =CH | ν aliphatic C–H | ν aromatic C–H | ν C=N of ring | ν C=N of Schiff base | ν NH | ν C=C aromatic | ν N–O | Co–N |
|-----------------------------------|-------|-----------------|---------------|---------------|---------------------|-----|----------------|-------|------|
| BDNHMIP Ligand                    | 3110  | 2958            | 3091          | 1618          | 1650                | 3294| 1514           |       |      |
|                                  |       |                 |               |               |                     |     | Symmetric 1332 | Asymmetric 1490 | –    |
| Co(BDNHMIP)₂Cl₂ complex          | 3180  | 2952            | 3056          | 1560          | 1645                | 3276| 1515           | 1311  | 441  |
|                                  |       |                 |               |               |                     |     | 1456           | 582   |      |
Figure 2. IR spectrum of 2-(biphenyl-4-yl)-3-((2-(2,4-dinitrophenyl) hydrazono) methyl) imidazo [1,2-a] pyridine.

Figure 3. IR spectrum of Co(BDNHMIP)$_2$Cl$_2$ complex.

**XRD of BDNHMIP ligand**

As shown in Figure 4 the results were treated and summarized in Table 4.
Figure 4. XRD analysis of BDNHMIP ligand.

Table 4. Data of XRD analysis of BDNHMIP ligand.

|                | a length in Å | b length in Å | c length in Å | α, β, and γ angles | Suggested Crystal structure | Unit cell volume in Å³ |
|----------------|---------------|---------------|---------------|-------------------|-----------------------------|------------------------|
| BDNHMIP        | 17.05         | 4.09          | 7.318         | 90° for each angle.| Each of three lattice dimensions of a, b, and c are unequaled. All three angles of α, β, and γ are equaled to 90°. As a result the suggested configuration of ligand is orthorhombic. | 510.5 |

**Evaluation effective magnetic moment.**
The calculations were done on experimental results of magnetic properties evaluation [16] which were listed in Table 5. According to calculation of angular momentum constant the complex is low spin complex with three unpaired electrons.

Table 5. evaluation parameters of magnetic properties of BDNHMIP and Co(BDNHMIP)₂Cl₂.

|                | R₀          | R  | Wt. of tube + Sample gm | Wt. of empty tube in gm | X₂ x10⁻⁶ |
|----------------|------------|----|------------------------|-------------------------|----------|
| BDNHMIP        | Zero       | 10 | 1.7756                 | 1.7591                  | 0.909    |
| Co(BDNHMIP)₂Cl₂| Zero       | 70 | 1.7157                 | 1.6854                  | 3.465    |

**Absorption spectrum of ligand and its complex**
The difference between the distinguished wave lengths of complex and its ligand (530 nm of reddish brown colored complex and 226 nm in addition to 264 nm and 318 nm of yellow ligand) makes spectrophotometric analysis of complex to determine Co(II) content possible and dependable analysis. The difference in colors of BDNHMIP ligand, and Co(BDNHMIP)₂Cl₂ complex and distinguished difference in maximum wave lengths of both of them were recorded in Figure 5.

Image 3. The difference in colors of BDNHMIP and Co(BDNHMIP)₂Cl₂ (from left to right).
Figure 5. absorption spectrum of [Co(BDNHMIP)2]Cl2 complex against ligand as blank (in blue line) and BDNHMIP ligand spectrum against DMF solvent (in black line).

Shelf life of ligand
During storage period, the ligand of BDNHMIP was stable for one month in solvents of ethanol, DMF, and DMSO under dark storage condition. The spectrum of ligand was changed completely after storage period of one month and the analytical results of ligand in cobalt ion quantification cannot depend on. As shown in Figure 6 and related Table 6, the peaks of freshly prepared ligand and old prepared one were different and when ligand dissolved in DMSO and exposed to light, solution color changed from yellow to green rapidly.

Table 6. Changes in peaks positions of ligand with storing period.

| Absorbance at peaks and valleys in spectrum of fresh ligand | Absorbance at peaks and valleys in spectrum of old prepared ligand |
|------------------------------------------------------------|---------------------------------------------------------------|
| "Peak",364, 2.632,""                                      | "Peak",680, 0.466,""                                        |
| "Peak",271, 2.541,""                                      | "Peak",667, 0.377,""                                        |
| "Peak",237, 2.925,""                                      | "Peak",495, 2.777,""                                        |
| "Valley",290.00,1.643,""                                  |                                                               |

Influence of ligand solvent
Despite of that the absorbance of complex in DMSO solvent was greater than it’s absorbance in other solvents as listed in Table 7, but it was necessary to keep ligand solution (in DMSO solvent) in dark container to avoid ligand color change as shown in Image 4.

Table 7. Influence of ligand solvent on complex color intensities.

| Solvent of ligand | Absorbance |
|-------------------|------------|
| DMF               | 0.206      |
| DMSO              | 0.2        |
| EtOH              | 0.166      |

Image 4. BDNHMIP ligand in different solvents.

Quantification of Co(II) ion using flow injection - merging zone analysis
Based on the considerable difference in maximum wavelength of BDNHMIP ligand and its complex with Co(II), it was possible to spectrophotometric determine of Co(II) by flow injection analysis with spectrophotometric detection unit.

Optimizing conditions of flow injection analysis
By injection 200 ppm Co(II) solution and 400 ppm of BDNHMIP ligand solution with or without buffer addition, the optimum parameters for Co(II) determination reaction were studied.

Influence of flow rate
At flow rate 1.95 ml/min, peak of detection response peak was splatted and this may be due to the fact that slow flow rate led to decrease analyte response peak, peak broadening, and peak distortion [17] as shown in Chart 1, related Figure 7 and Table 8 the optimum flow rate with best detection response was 3.2 ml/min.
Table 8. Influence of flow rate on analytical detection response.

| Flow rate, ml/min | Av. peak height ($\bar{X}$)of $n = 3$, mv | S.D. ($\sigma_{n-1}$) | R.S.D. % | Confidence limits C.L. = $\bar{X} \pm t_{0.05} \frac{\sigma_{n-1}}{\sqrt{n}}$ |
|------------------|--------------------------------------|---------------------|--------|----------------------------------|
| 1.95             | 78                                   | 0                   | 0      | 78±0.0                            |
| 2.4              | 80                                   | 2                   | 2.5    | 80±3.4                            |
| 2.8              | 100                                  | 0                   | 0      | 100±0.0                           |
| 3.2              | 136                                  | 0                   | 0      | 136±0.0                           |
| 3.55             | 124                                  | 2                   | 1.6    | 124±3.4                           |

Figure 7. Effect of flow rate on analytical detection response.

Chart 1. Flow rate influence on analytical detection response.

Influence of BDNHMIP injected volume

As shown in Chart 2 and related Figure 9 the results were summarized in Table 9 and 137μL (70 cm of loop length with constant diameter of 0.5 mm) was the best choice of BDNHMIP injected volume to obtain maximum analytical response at constant length of Co(II).loop = 40 cm.

Table 9. Influence of BDNHMIP volume on analytical detection response.

| Ligand vol., μL | Av. peak Height of $n = 3$, mv | S.D | R.S.D % | C.L. = $\bar{X} \pm t_{0.05} \frac{\sigma_{n-1}}{\sqrt{n}}$ |
|-----------------|-------------------------------|-----|---------|----------------------------------|
| 68.69           | 92                            | 4   | 4.3     | 92±6.7                           |
| 98.13           | 116                           | 0   | 0       | 116±0.0                           |
| 137.38          | 136                           | 0   | 0       | 136±0.0                           |
| 166.61          | 120                           | 0   | 0       | 120±0.0                           |
Influence of Co(II) injected volume
As shown in Chart 3 and related Figure 9, the results were summarized in Table 10. 98.1 μL (50 cm loop length with constant loop diameter of 0.5 mm) was the best choice to perform the best analyte response at constant length of Co(II) loop = 40 cm.

| Co(II)vol., μL | Av. peak height of n = 3, mv | S.D | R.S.D% | C.L = \bar{X} ± t_{0.05} \frac{σ_n-1}{\sqrt{n}} |
|---------------|----------------------------|-----|--------|---------------------------------|
| 68.69         | 108                        | 1.6 | 1.48   | 108±2.7                         |
| 98.13         | 116                        | 0   | 0      | 116±0.0                         |
| 137.38        | 84                         | 2   | 2.38   | 84±3.4                          |
| 166.61        | 60                         | 0   | 0      | 60±0.0                          |

Influence of medium acidity
The influence of pH on detection response was shown in Chart 4 and related Figure 10 and summarized in Table 11. Basic medium caused precipitation of Co(II) and as a result solution turbidity, while acidic medium may be caused ligand dissociation.
Table 11. Effect of medium acidity on analytical detection response.

| Medium acidity, pH | Av. Peak height of n = 3, mv | S.D | R.S.D% | C.L = $\bar{X} \pm t_{0.05} \frac{s_{n-1}}{\sqrt{n}}$ |
|-------------------|-----------------------------|-----|--------|----------------------------------|
| 0                 | 136                         | 0   | 1      | 136±0.0                          |
| 1                 | 124                         | 2   | 1.613  | 124±3.4                          |
| 2                 | 140                         | 4   | 2.587  | 140±6.7                          |
| 3                 | 128                         | 0   | 0      | 128±0.0                          |
| 4                 | 142                         | 2   | 1.408  | 142±3.4                          |
| 5                 | 116                         | 4   | 3.448  | 116±6.7                          |
| 6                 | 104                         | 0   | 0      | 104±0.0                          |
| 7                 | 180                         | 1.2 | 0.667  | 180±2.0                          |
| 8                 | 200                         | 0   | 0      | 200±0.0                          |

Figure 10. pH influence on analytical response.

Chart 4. Effect of pH on analytical response.

Influence of reagents injection sequence
As shown in Table 12 the result of best sequence of reagents addition was BDNHMIP (loop 1) then Co(II) (loop 2).

Table 12. Influence of reagents addition sequence on analytical response.

| Sequence of reagents addition | Av. of peak height of n = 3, mv | S.D  | R.S.D% | C.L = $\bar{X} \pm t_{0.05} \frac{s_{n-1}}{\sqrt{n}}$ |
|-------------------------------|---------------------------------|------|--------|--------------------------------------------------|
| BDNHMIP.(Loop₁)+ Co(II)(Loop₂)| 136                             | 0.4  | 0.294  | 136±0.7                                         |
| Co(II) (L₁) + BDNHMIP.(L₂)     | 116                             | 4    | 3.448  | 116±6.7                                         |

Influence of ligand concentration
As shown in Figure 11 and related Table 13, double fold of BDNHMIP in comparison with Co(II) was the best choice of BDNHMIP concentration to obtain the best analytical response.

Table 13. Influence of BDNHMIP ligand concentration on analytical response.

| Ligand conc.,ppm | Av. of peak height n = 3, mv | S.D  | R.S.D% | C.L = $\bar{X} \pm t_{0.05} \frac{s_{n-1}}{\sqrt{n}}$ |
|-------------------|-------------------------------|------|--------|--------------------------------------------------|
| 100               | 52                            | 2    | 3.846  | 52±3.4                                          |
| 150               | 78                            | 0    | 0      | 78±0.0                                          |
| 250               | 102                           | 1    | 0.98   | 102±1.69                                        |
| 400               | 135                           | 0    | 0      | 135±0.0                                         |
| 500               | 114                           | 1    | 0.877  | 114±1.69                                        |
Influence of residence time.
The best residence time of reactants was tested and listed in Table 14.

Table 14. Effect of residence time of reactants in reaction coil on analytical response.

| Holding time of reactants in mixing coil, min | Av. of Peak height, mv | S.D | R.S.D% | C.L = $\bar{X} \pm t_{0.05} \frac{S.D}{\sqrt{n}}$ |
|---------------------------------------------|------------------------|------|--------|----------------------------------|
| 1                                          | 130                    | 0    | 0      | 130±0.0                          |
| 3                                          | 130                    | 4    | 3.077  | 130±0.0                          |
| 5                                          | 120                    | 1    | 0.833  | 120±0.0                          |

The optimum conditions for reaction formation of [Co(BDNHMIP)₂]Cl₂ were summarized in Table 15.

Table 15. The analytical conditions of proposed method in flow injection technique.

| Parameter                  | Optimum value | Parameter                  | Optimum value |
|----------------------------|---------------|----------------------------|---------------|
| Wave length                | 530nm         | pH                         | Without buffer addition |
| Flow rate, ml/min          | 3.2           | BDNHMIP volume, µ          | 137.38 L      |
| Reagents injection sequence| BDNHMIP in 1ˢᵗ loop | Co(II) in 2ⁿᵈ loop | Co(II) volume, µ | 98.12 L   |
| BDNHMIP conc., ppm         | 400           | Residence time             | 1 minutes     |

Repeatability
By taking in consideration that repeatability is another meaning of method precision [17], the repeatability calculations was done according to Chart 5 and related Table 16 using.

Table 16. Repeatability calculations.

| Injection number | Peak height, mv | Av. of peak = 154.8 height, mv | S.D = 1.789 | R.S.D% = 1.156 |
|------------------|-----------------|--------------------------------|--------------|----------------|
| 1                | 156             |                                |              |                |
| 2                | 152             |                                |              |                |
| 3                | 154             |                                |              |                |
| 4                | 156             |                                |              |                |
| 5                | 156             |                                |              |                |

Diffusion factor
By depending on Chart 6 using 275 ppm of Co(II) concentration, reagent dilution determined by diffusion factor calculation [18] according to following relation $D = \frac{C}{c}$ and the result was $D = \frac{6.5}{5} = 1.3$. 

Figure 11. Influence of BDNHMIP concentration on analytical response.
Calculation of method throughput
75 seconds was the required time for Co(II) determination to complete analyte determination from injection point to analyte peak appearance, therefore method throughput was 48 sample/hour.

Method validation
The linearity of proposed method was estimated in Table 18 depending on using optimum conditions which were listed in previous Table 15 and results of Chart 7 and related Table 17.

| Co conc., ppm | Av. peak Height of n = 3, mv | S.D | R.S.D% | C.L = $\bar{X} \pm t_{0.05} \frac{s_{n-1}}{\sqrt{n}}$ |
|---------------|-----------------------------|-----|--------|-----------------------------------------------|
| 25            | 64                          | 2   | 3.125  | $64\pm3.4$                                   |
| 50            | 88                          | 2   | 2.273  | $88\pm3.4$                                   |
| 75            | 108                         | 4   | 3.704  | $108\pm6.7$                                  |
| 100           | 124                         | 2   | 1.613  | $124\pm3.4$                                  |
| 150           | 144                         | 0   | 0      | $144\pm0.0$                                  |
| 250           | 188                         | 3.2 | 1.702  | $188\pm5.39$                                 |
| 300           | 228                         | 0   | 0      | $228\pm0.0$                                  |
| 400           | 260                         | 4   | 1.538  | $260\pm6.7$                                  |

Chart 6. Test of estimation diffusion factor.

Chart 7. Evaluation linearity of proposed method by injection different concentrations of Co(II).

Figure 12. Standard curve of proposed flow injection method.
Table 18. Estimation the parameters which are related to proposed method validity.

| Statistical parameters                  | Equation               | Slope, b | 0.5108 |
|-----------------------------------------|------------------------|----------|--------|
| Regression equation                     | $y = 0.5108x + 64.308$ | Intercept | 64.308 |
| Correlation coefficient, $r^2$          | 0.9854                 | Standard deviation of residual, $S_{y/x}$ | 9.007 |
| Linearity percentage, $r^2\%$           | 98.54%                 | Standard deviation of slope, $S_b$     | 0.0254 |
| Linearity range, mg/L (ppm)             | 25-400                 | Standard deviation of intercept, $S_a$ | 5.337 |
| Molar absorptivity constant $L/mol.cm$  | $121.5704 \times 10^3$| LoD      | 2.284  |
| = slope of line (molar)$^1$/path length(cm) |                        | LoQ      | 6.853  |
| Method throughput, sample/min           | 48                     |          |        |

Determination of Co(II) in vitamin B$_{12}$

Standard addition method (SAM) is used to omit effect of sample matrix in instrumental analysis. SAM was proposed by Saxberg and Kowalski [19] and performed in spectrophotometric analyses [20-22]. It is essential to understand that SAM cannot be applied when more than sample matrix interference is achieved such as spectral, instrumental, and methodic interferences because of inexact results which cannot be depended on.

**Figure 13.** Standard addition method of Co(II) determination in Methylcobal B$_{12}$ injection.

**Figure 14.** Standard addition method of Co(II) determination in Panclo B$_{12}$ injection.

Table 19. Determination of Co(II) content in vitamin B$_{12}$ injection samples.

| Sample name            | Description              | Co(II) concn. in sample (ppm) | Co(II) founded in ppm | Recovery % | Relative error R.E% |
|------------------------|--------------------------|-------------------------------|-----------------------|------------|--------------------|
| Methylcobal B$_{12}$   | 500 $\mu g$ in 1 ml ampoule | 100                           | 100.277               | 100.3      | 0.277              |
| injection.             |                          |                               |                       |            |                    |
| Panclo B$_{12}$        | 10 mg in 3 ml ampoule    | 100                           | 99.494                | 99.5       | 0.5                |
| injection.             |                          |                               |                       |            |                    |

Mole ratio method

Mole ratio is one of two methods which are used to determine composition of complex (L:M mole ratio). This method is done by mixing different volumes of ligand with the same volume of metal ion at condition of equality concentration of both metal and ligand. As shown in Figure 15, approximately 2 mole of BDNHMIP coordinated with 1mole of Co(II) in composition of 1 mole complex.

![Mole ratio graph](attachment.png)
4. Conclusion

A new Schiff base compound was used as chelated ligand in spectrophotometric determination of Co(II) by merging zone-flow injection technique which was performed analysis with lowest reagents consuming, simple procedure, high recovery, and lowest error. Because of the suggested method did not need to any pretreatment steps, availability of ligand in high purity at any scale, simple technique and easy to work with, in addition to high method throughput, all these make proposed technique suitable and inexpensive technique to use in routine Co(II) determination works.

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