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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, 98.1 μL 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 121.5704×10^3 $L.mol.^{-1}cm^{-1}$, and L.O.D of 2.28ppm. The suggested method was successful applied for determination of Co(II) content of vitamin B_{12} injection samples with high recovery of 99.5-100.3% and minimum relative error percent of -0.3 - 0.5%.

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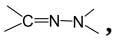
1. Introduction

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  , 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.

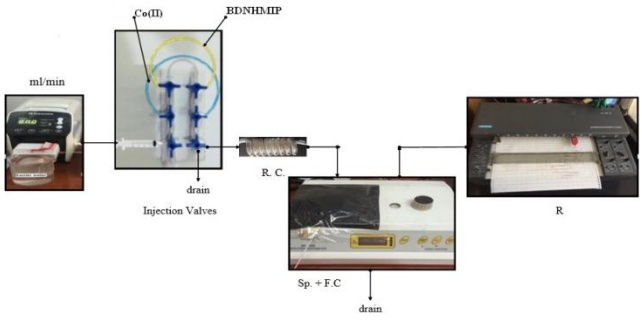
Nitro group of hydrazone Schiff bases is strong withdrawing group and because of it's steric effects nitro group has played a vital role in reactivity of hydrazone Schiff bases either it is participated in coordination [6] with metal ions or not [7-9].

2. Experimental

Instrumentation

1. Double beam spectrophotometer UV-1800 from Schimadzu 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.

- Injection Valve: homemade six – three way plastics valve was used to insert metal ion and ligand solutions (small volumes in µl) into water carrier stream flow. Injection and loading modes of valve system are illustrated in Scheme 1.
- Reaction Coil.
- Kompensograph C1032 recorder from Siemens.



Scheme 1. The setup of FIA merging zones system where P: peristaltic pump to flow carrier water, homemade injection valves (in injection status), R. C.: reaction coil, Sp.+F.C: spectrophotometer+ flow cell as detection unit, R.: recorder and drain system.

Chemicals

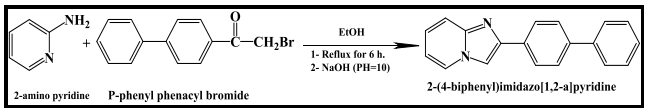
The chemicals with high purities from Fluka, BDH, Riedel de-Haen, Alpha, Alpha Aesar, and Merck were used.

Synthesis process

Synthesis process of ligand of 2-(biphenyl-4-yl)-3-((2-(2,4-dinitrophenyl) hydrazono) methyl) imidazo[1,2-a]pyridine (BDNHMIP) was included three steps which were [10,11].

Synthesis of 2-(4-biphenyl) imidazo[1,2-a]pyridine by cyclization step

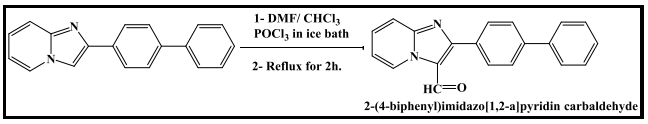
2.75 gm (1mmol) of p-phenyl phenacyl bromide (as limiting reactant) was mixed with 0.96gm (1 mmol) of 2-amino pyridine. 10-12ml of ethanol was added to the resulted mixture and refluxed with magnetic stirring for 6 hours. The resulted solution was cooled and basified to pH = 10 by adding 2-3 ml of 5% sodium hydroxide (5 gm of NaOH in 100ml H_2O). Solid crystals was formed, filtered, washed with water, and dried well in oven. The product yield was 81.18% and the synthesis process was summarized in Scheme 2.



Scheme 2. Preparation of 2-(4-biphenyl) imidazo[1,2-a] pyridine.

Synthesis of (4-biphenyl) imidazo[1,2-a]pyridine-3-carbaldehyde

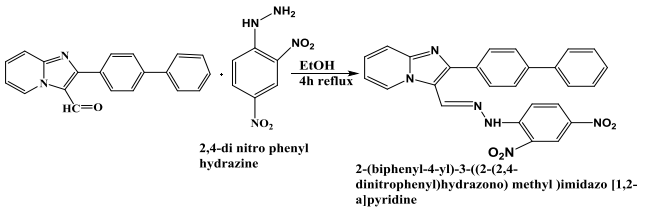
The synthetic process was summarized in Scheme 3 and it included mixing of 5ml of chloroform was with 1 ml of dimethyl formamide in ice bath. To this mixture, 2 ml of $POCl_3$ was added drop by drop to maintain reaction temperature under 10C° because reaction was exothermic. Then 2.70 gm (1 mmol) of solid 2-(4-biphenyl) imidazo [1,2-a] pyridine with 5-7 ml chloroform were added alternatively to cold mixture with stirring. The resulted mixture was reflexed with magnetic stirring for 2hours. The resulted solution was cooled with ice bath, filtered, washed with ethanol, and dried in oven. The product crystals of 2-(4-biphenyl) imidazo [1,2-a] pyridine-3-carbaldehyde yield was 80 %.



Scheme 3. preparation of 2-(4-biphenyl) imidazo [1,2-a] pyridine-3-carbaldehyde.

Synthesis ligand (BDNHMIP) of 2-(biphenyl-4-yl)-3-((2-(2,4-dinitrophenyl) hydrazono) methyl) imidazo [1,2-a] pyridine

10-15 ml of ethanol was added to mixture of 1.491gm (0.5 mmol) of 2-(4-biphenyl) imidazo [1,2-] pyridine-3-carbaldehyde and 0.99 gm (0.5 mmol) of 2,4-dinitro phenyl hydrazine, then the mixture was refluxed with stirring for 4 hours. The product was cooled, filtered, and washed with water. The product yield is 78.26 %. It was recrystallized by ethanol. The synthesis process was summarized in Scheme 4.



Scheme 4. Synthesis of 2-(biphenyl-4-yl)-3-((2-(2,4-dinitrophenyl) hydrazono) methyl) imidazo[1,2-a] pyridine (BDNHMIP).

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 $CoCl_2.6H_2O$ 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 B_{12} was heated in furnace for 1 hour at 700 °C. The solution was cooled and 1-2ml of concentrated HNO_3 was added with heating to ensure complete dissolution. The acidity of solution was

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_{12} ampoules [13].

Two ampoules of Methylcobal vitamin B_{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 $CoCl_2$ respectively with completing volume to 5 ml by deionized water.

One ampoule of Panclo vitamin B_{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 $CoCl_2$ respectively with completing volume to 5ml by deionized water.

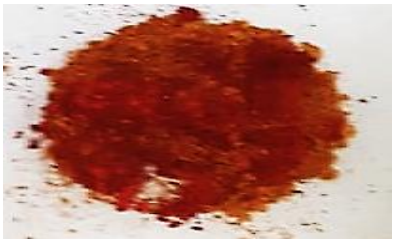
Identification of prepared ligand (BDNHMIP) and its complex

According to many of analyses, BDNHMIP ligand and $[Co(BDNHMIP)_2]Cl_2$ were identified.

Appearance and physical properties

The basic properties of BDNHMIP ligand and its complex of $[Co(BDNHMIP)_2]Cl_2$ were listed in Table 1.

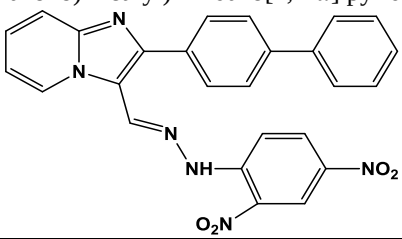
Table 1. Physical properties of BDNHMIP ligand and its complex.

	Appearance	**Melting point, °C**
BDNHMIP ligand	Yellowish orange  **Image 1:BDNHMIP crystals.**	158-160
$[Co(BDNHMIP)_2]Cl_2$	Reddish brown  **Image 2: complex crystals.**	198-200

HNMR of BDNHMIP

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

Table 2. HNMR data of BDNHMIP ligand.

2-(biphenyl-4-yl)-3-((2-(2,4-dinitrophenyl) hydrazono) methyl) imidazo[1,2-*a*] pyridine 		HNMR data, ppm
	Multiplate (10H) of aromatic	7.10-7.47
	Singlet (1H) of HC=N group	8.71
	Singlet 1H of NH group	8.38

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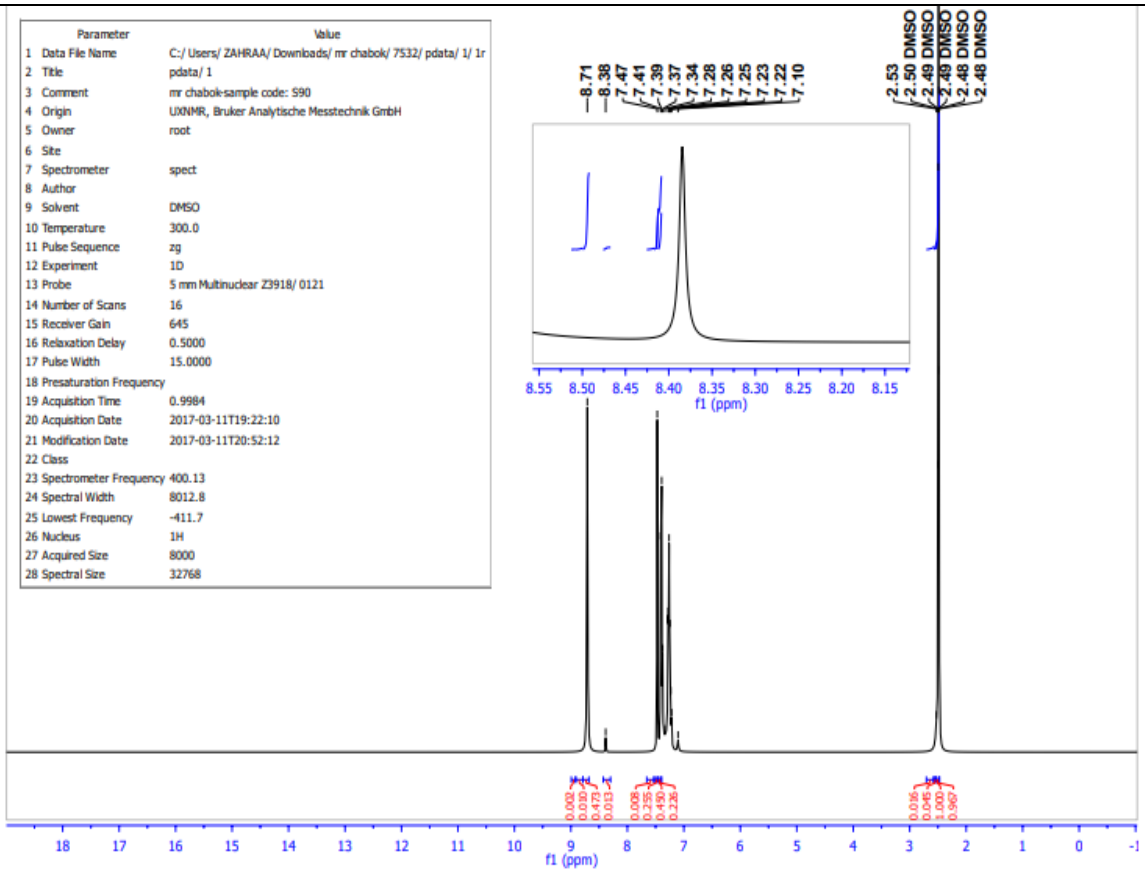

Figure 1. HNMR of 2-(biphenyl-4-yl)-3-((2-(2,4-dinitrophenyl) hydrazono) methyl) imidazo [1,2-*a*] pyridine (BDNHMIP).

FT-IR analysis of BDNHMIP and $Co(BDNHMIP)_2Cl_2$.

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-] pyridine-3-(2,4-dinitrophenyl) hydrazone and its complex $Co(BDNHMIP)_2Cl_2$.

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	–
$Co(BDNHMIP)_2Cl_2$ complex	3180	2952	3056	1560	1645	3276	1515	1311 1456	441 582

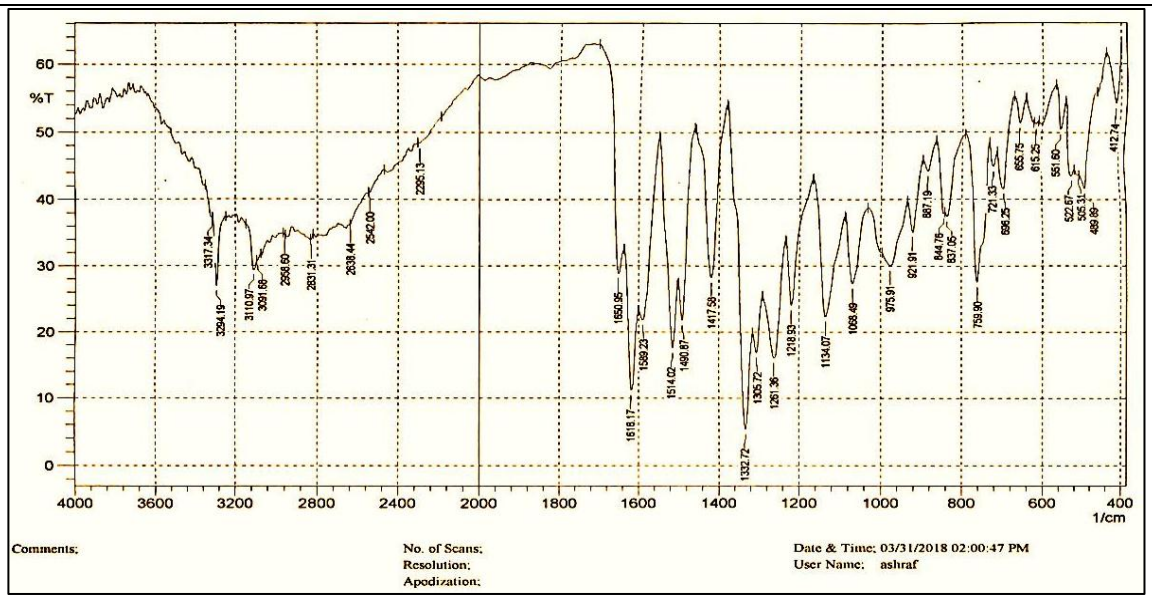

Figure 2. IR spectrum of 2-(biphenyl-4-yl)-3-((2-(2,4-dinitrophenyl) hydrazono) methyl) imidazo [1,2-a] pyridine.

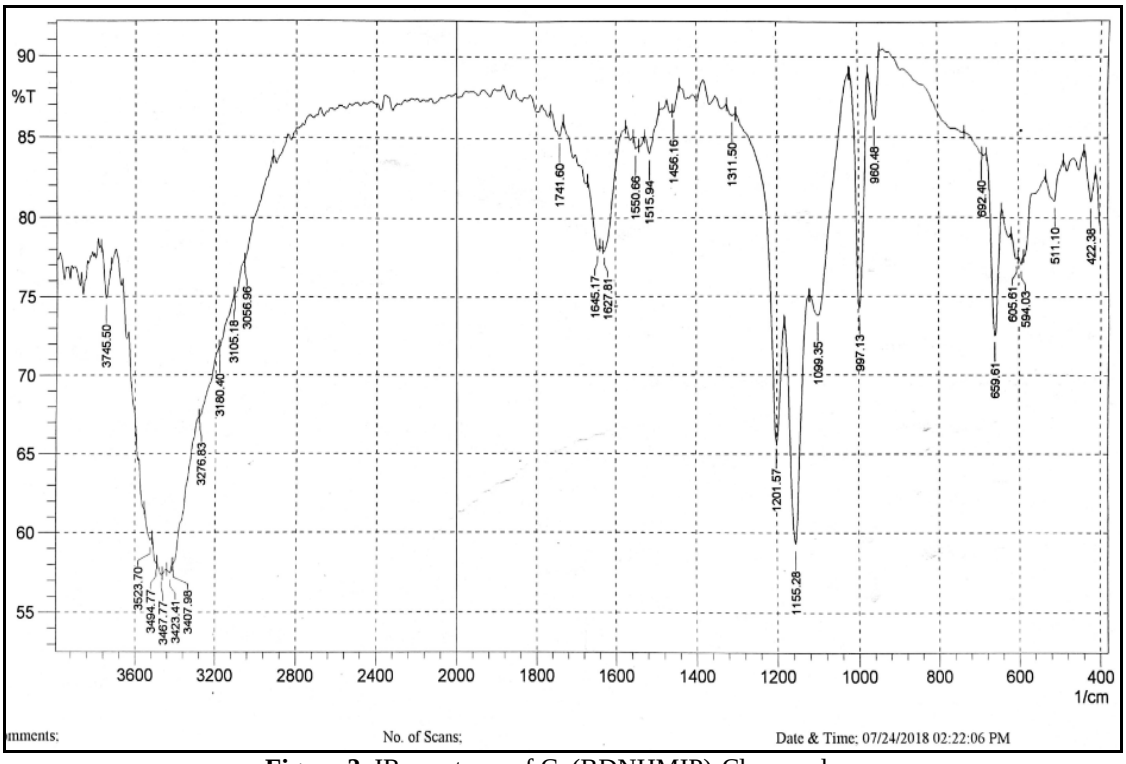

Figure 3. IR spectrum of $Co(BDNHMIP)_2Cl_2$ complex.

XRD of BDNHMIP ligand

As shown in Figure 4 the results were treated and summarized in Table 4.

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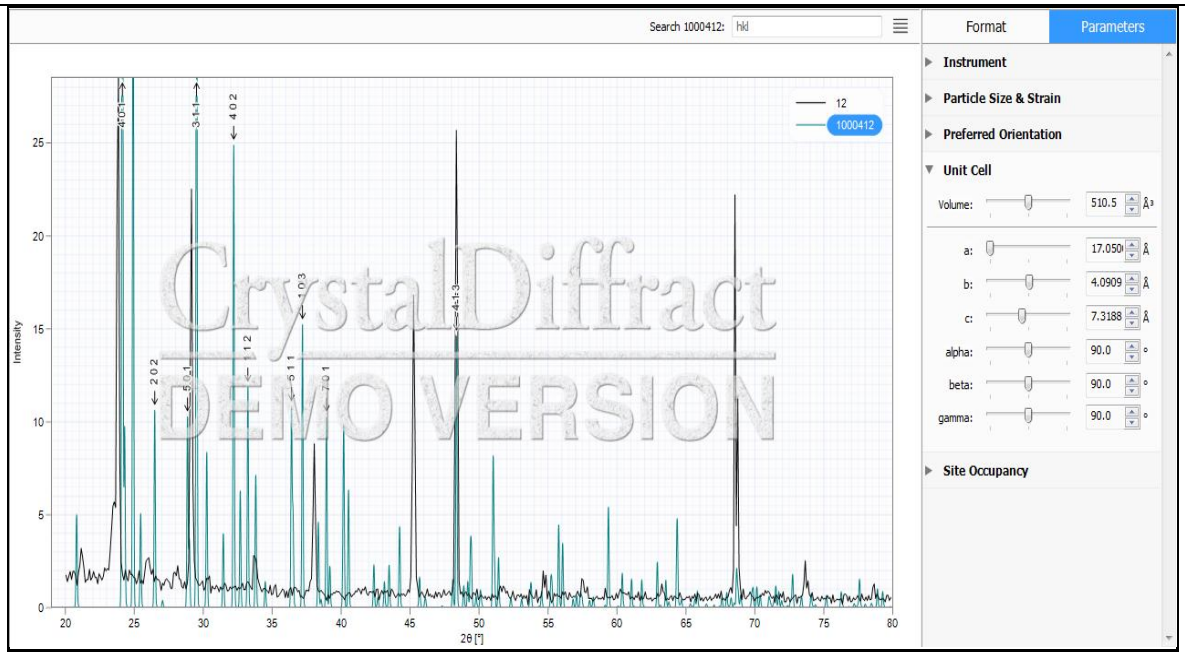

Figure 4. XRD analysis of BDNHMIP ligand.

Table 4. Data of XRD analysis of BDNHMIP ligand.

	a length in A°	**b length in A°**	**c length in A°**	**$\alpha, \beta, and\ \gamma$ angles**	**Suggested Crystal structure**	**Unit cell volume in A^3**
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 $\alpha, \beta, and\ \gamma$ 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 $Co(BDNHMIP)_2Cl_2$.

	R_o	**R**	**Wt. of tube + Sample, gm**	**Wt. of empty tube in gm**	**$X_g \times 10^{-6}$**
BDNHMIP	Zero	10	1.7756	1.7591	0.909
$Co(BDNHMIP)_2Cl_2$	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 $Co(BDNHMIP)_2Cl_2$ 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 $Co(BDNHMIP)_2Cl_2$ (from left to right).

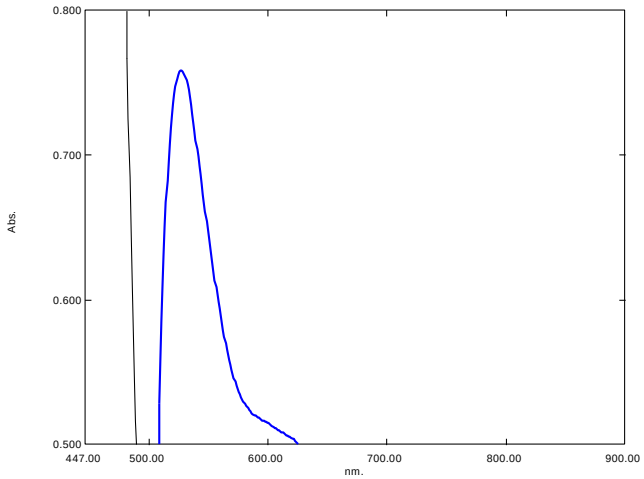

Figure 5. absorption spectrum of $[Co(BDNHMIP)_2]Cl_2$ complex against ligand as blank (in blue line) and BDNHMIP ligand spectrum against DMF solvent (in black line).

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.

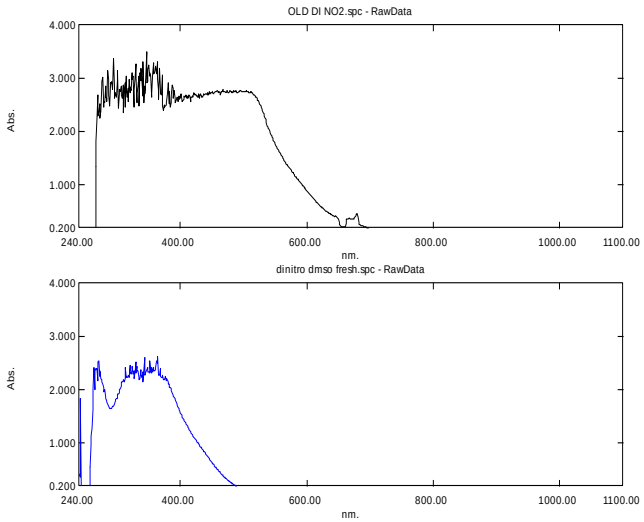

Figure 6. the difference between spectra of old prepared (before more than one month) in black and freshly prepared of BDNHMIP (in blue).

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",271, 2.541,"" "Peak",237, 2.925, "" "Valley",290.00,1.643,""	"Peak",680, 0.466,"" "Peak",667, 0.377,"" "Peak",495, 2.777,""

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

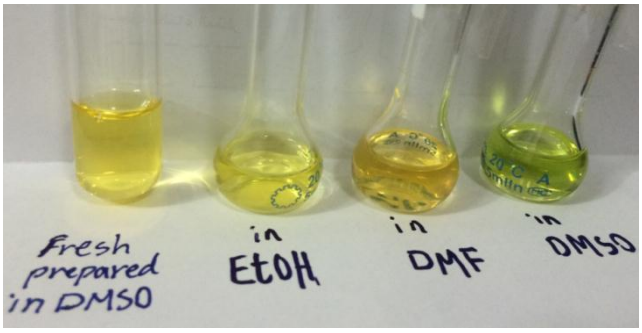

Image 4. BDNHMIP ligand in different solvents.

Quantification of Co(II) ion using flow injection-merging zone analysis

Based on the considerable difference in maximum wave length 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 broading, 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. (σ_{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

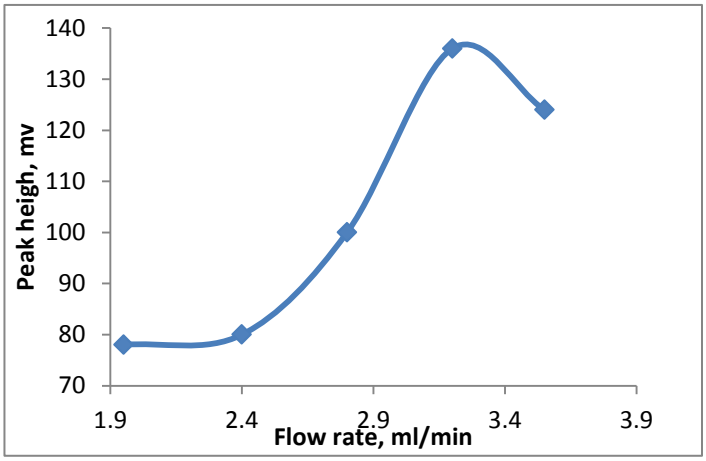

Figure 7. Effect of flow rate on analytical detection response.

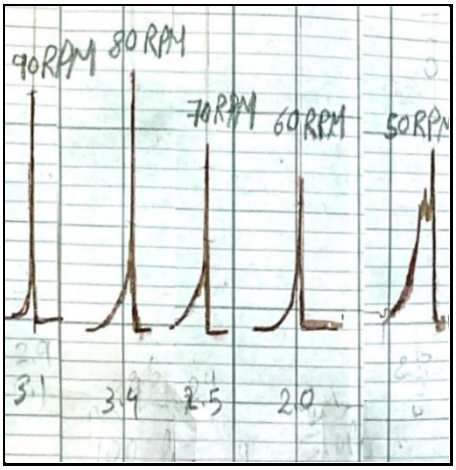

Chart 1. Flow rate influence on analytical detection response.

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

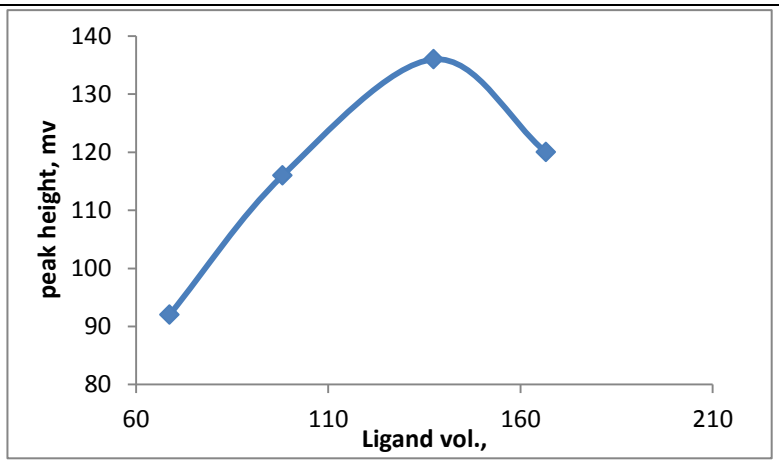

Figure 8. Effect of BDNHMIP injected volume on analytical detection response.

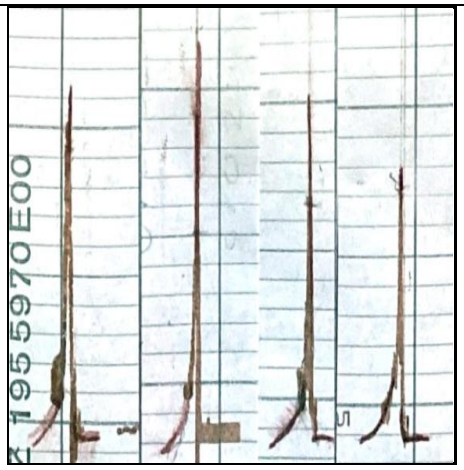

Chart 2. Effect of BDNHMIP volume on analytical detection response.

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.

Table 9. Influence of Co(II) injected volume on analytical detection response.

Co(II)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	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

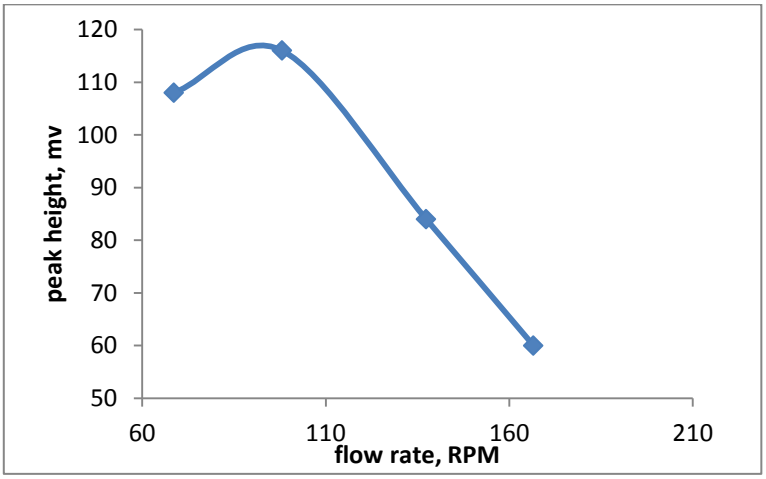

Figure 9. Effect of Co(II) injected volume on analytical detection response.

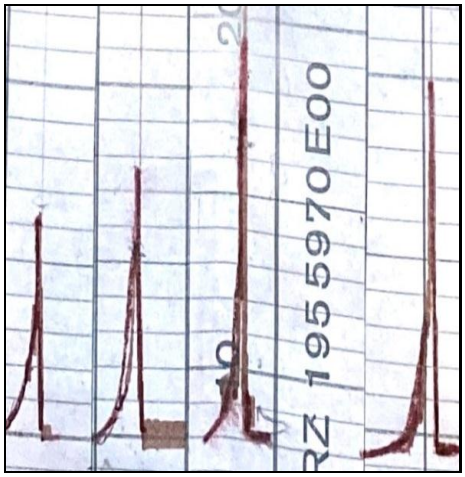

Chart 3. Effect of Co(II) injected volume on analytical detection response.

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 = $\bar{X} \pm t_{0.05} \frac{\sigma_{n-1}}{\sqrt{n}}$
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**

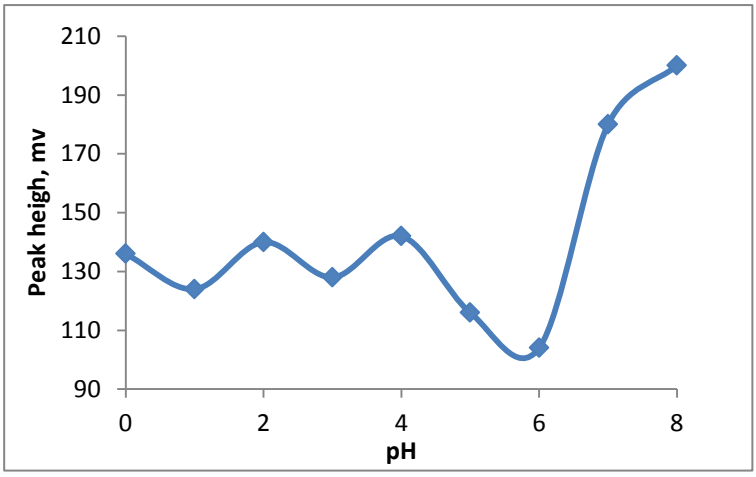

Figure 10. pH influence on analytical response.

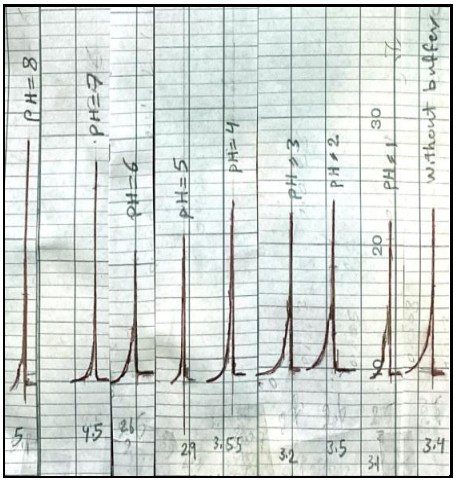

Chart 4. Effect of pH on analytical response.

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 = $\bar{X} \pm t_{0.05} \frac{\sigma_{n-1}}{\sqrt{n}}$
BDNHMIP.($Loop_1$)+ Co(II)($Loop_2$)	136	0.4	0.294	136±0.7
Co(II) (L_1) + BDNHMIP.(L_2)	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 = $\bar{X} \pm t_{0.05} \frac{\sigma_{n-1}}{\sqrt{n}}$
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

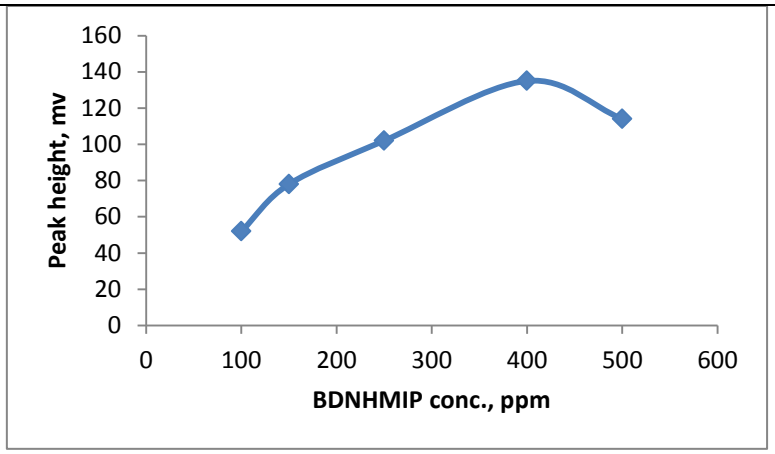

Figure 11. Influence of BDNHMIP concentration on analytical response.

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 = $\bar{X} \pm t_{0.05} \frac{\sigma_{n-1}}{\sqrt{n}}$
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 $[Co(BDNHMIP)_2]Cl_2$ 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	
1	156	**Av. of peak = 154.8 height, mv S.D = 1.789 R.S.D% = 1.156**
2	152	
3	154	
4	156	
5	156	

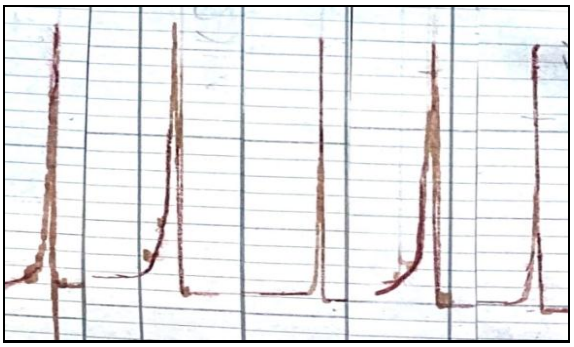

Chart 5. Repeatability test.

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 = $\frac{C_\circ}{C}$ and the result was D = $\frac{6.5}{5}$ = 1.3.

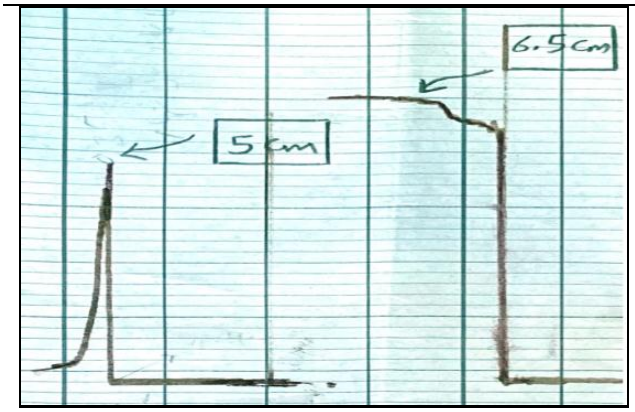

Chart 6. Test of estimation diffusion factor.

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 = $\bar{X} \pm t_{0.05} \frac{\sigma_{n-1}}{\sqrt{n}}$
25	64	2	3.125	64±3.4
50	88	2	2.273	88±3.4
75	108	4	3.704	108±6.7
100	124	2	1.613	124±3.4
150	144	0	0	144±0.0
250	188	3.2	1.702	188±5.39
300	228	0	0	228±0.0
400	260	4	1.538	260±6.7

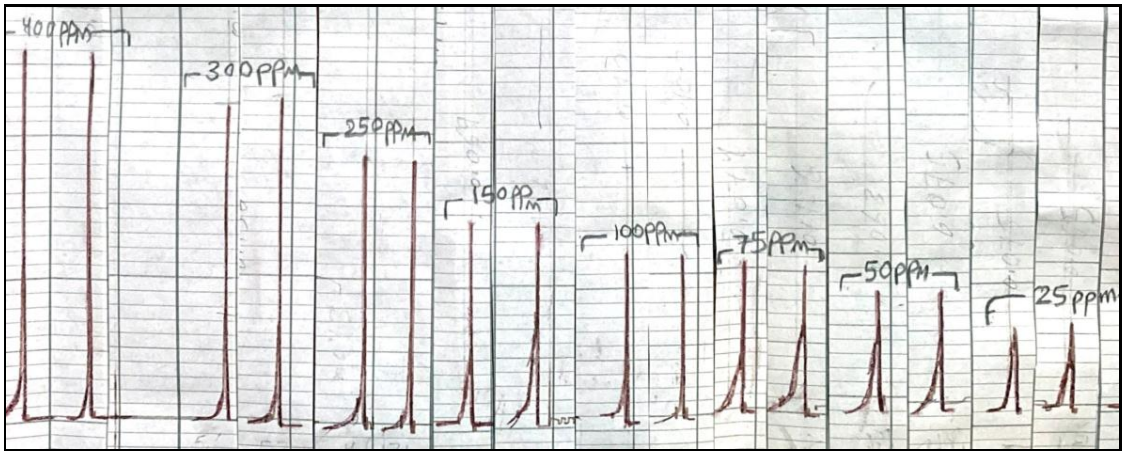

Chart 7. Evaluation linearity of proposed method by injection different concentrations of Co(II).

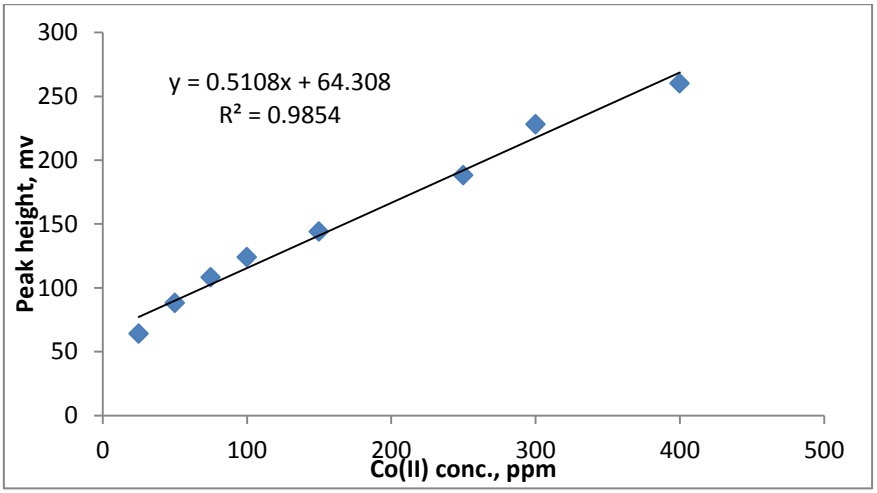

Figure 12. Standard curve of proposed flow injection method.

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, a	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 $L/mol.cm$ = slope of line $(molar)^{-1}$/path length(cm)	121.5704×10^3	LoD = $3S_b/b$, mg/L	2.284
Method throughput,sample/min	48	LoQ = $10S_b/b$, mg/L	6.853

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 in exact results which cannot be depended on.

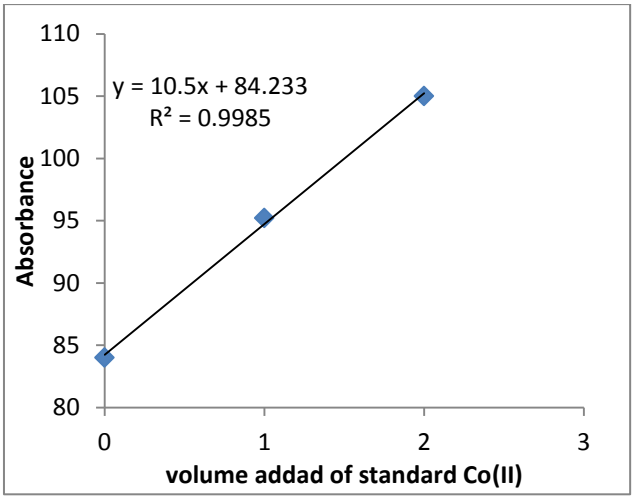

Figure 13. Standard addition method of Co(II) determination in Methylcobal B_{12} injection.

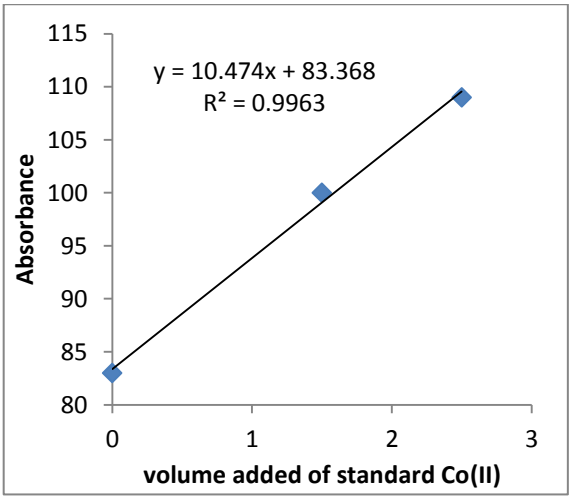

Figure 14. Standard addition method of Co(II) determination in Panclo B_{12} injection.

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} injection.	500 μg in 1 ml ampoule	100	100.277	100.3	0.277
Panclo B_{12} injection.	10 mg in 3 ml ampoule	100	99.494	99.5	–0.5

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.

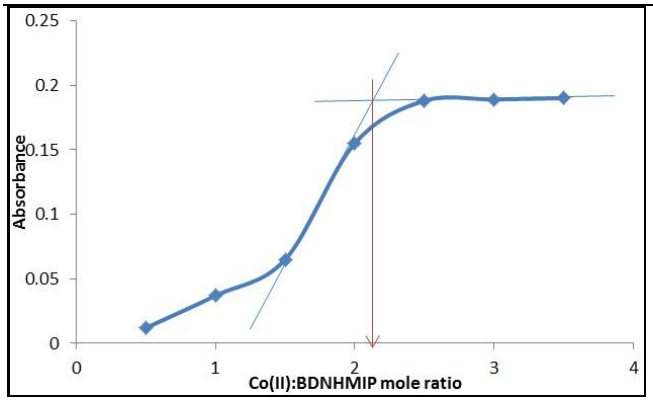

Figure 15. Mole ratio method for determination complex composition of BDNHMIP: Co(II).

Estimation stability constant of $[(BDNHMIP)_2Co]^{2+}$ complex:

$Co^{2+} + 2BDNHMIP \leftrightarrow [(BDNHMIP)_2Co]^{2+}$

Stability constant of complex is expressed as equation below:

$$K = \frac{1-\propto}{4\propto^3 C^2}$$

where C is initial concentration of ligand or metal ion and C = 0.00125M in determination reaction.

$\Delta \mathbf{G} = -RTlnK$

Table 19. The parameters for evaluation complex stability constant.

$\mathbf{A_m}$	$\mathbf{A_S}$	$\propto$	**K**	**log K**	**ΔG in joule at 300k°**
0.19	0.155	0.1842	0.519×10^7	6.715	–38566

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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