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Synthesis and Characterization of 3,5-Dimethyl-2- (4-nitrophenyl azo)-Phenol Complexes with Co(II) and Ni(II) and Study Its Effect on the Activity of Ach Enzyme(invitro)

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Abstract

Transition metal complexes of Co(II) and Ni(II) with azo dye 3,5-dimethyl-2-(4-nitrophenylazo)-phenol derived from 4-nitoaniline and 3,5-dimethylphenol were synthesized. Characterization of these compounds has been done on the basis of elemental analysis, electronic data, FT-IR,UV-Vis and ^{1}HNMR, as well as magnetic susceptibility and conductivity measurements. The nature of the complexes formed were studies following the mole ratio and continuous variation methods, Beer's law obeyed over a concentration range ($1x10^{-4}$- $3x10^{-4}$ M). High molar absorbtivity of the complex solutions were observed. From the analytical data, the stoichiomerty of the complexes has been found to be 1:2 (Metal:ligand). On the basis of physicochemical data tetrahedral geometries were assigned for the complexes. The inhibitory effect of prepared compounds was used to study the type of inhibition. The results from line weaver- Burk plot indicated that the inhibitor type was non competitive with a range (31.76-83-21%).

Keywords: - spectral studies, complexes, azo dyes, inhibitors.

1. Introduction

The azo compounds with the general structure R-N=N-R$^-$; where R and R$^-$ are alkyl, aryl or heterocyclic radicals (Vadher & Zala2011), have found many applications, as dyes for textile fibres, coloring of different materials (Faghihi & Hajieygi 2003, Ebenso *et al.* 2008, Nahapatra & Panda 2010, Anitha *et al.* 2011, Turcas & Sebe 2012).Several bidentate azo dyes in which the phenolic-OH group and azo nitrogen are present in such a way that they form six membered ring with metal ions were also found wide application as textile dyes (Hrdina *et al.* 2004, Modhavadiya 2011). These azo compounds found other applications as optical data storage devices (Lemr *et al.* 2000, Samieh *et al.* 2008, Oututu 2013) recently; azo metal chelates have also found great attention due to their important electronic and geometrical structures in connection with their application for molecular memory storage, nonlinear optical elements and printing systems (Wang *et al.* 2000, Kirkan & Gup 2008, Kumar & Chaudhary 2010) . In this work, a ligand of azo functional group derived from 4-nitroaniline as diazo component and 3,5-dimethylphenol as coupling agent, were prepared. The complex of this ligand with some metal ions has been prepared and characterized physciochemically. Hundards of compounds have been synthesized and tested as cholinesterase inhibitors. They belong to different types of organic and organometalic classes such as alkaloids, physostigmine, organophosphorus (Ballantyne & Marrs 1992).

Acetylcholinesterase is present in all vertebrates, particularly in the muscle and nervous tissue (Silver 1974, ligand *et al.* 2008). Structurally and functionally acetylcholinesterase is aserine hydrolase (AChE, Acetylcholine hydrolase EC.3.1.1.7). The enzyme is found in both synaptic and non-synaptic tissues (Quinn 1987). In synaptic tissue the main role of acetylcholinesterase is the hydrolysis of neurotransmitter acetylcholine (Ach). The catalytic activity of this enzyme is essential for normal cholinergic transmission and neuromuscular function(Rosenberry 1975, Silman & Sussman2005) . In a non-synaptic context, AChE and BuchE are expressed in early embryonic development some time before the formation of cholinergic synopsis, where its function is the formation and regulation of the acetylcholine gradient which guides the growth of nerve cells (Layer & Gilgert 1985).

A noteworthy amount of acetylcholinesterase is also present in blood cells, particularly in erythrocytes and lymphocytes (Kawashima & Fuji 2008).

2. Experimental

2.1 Instruments

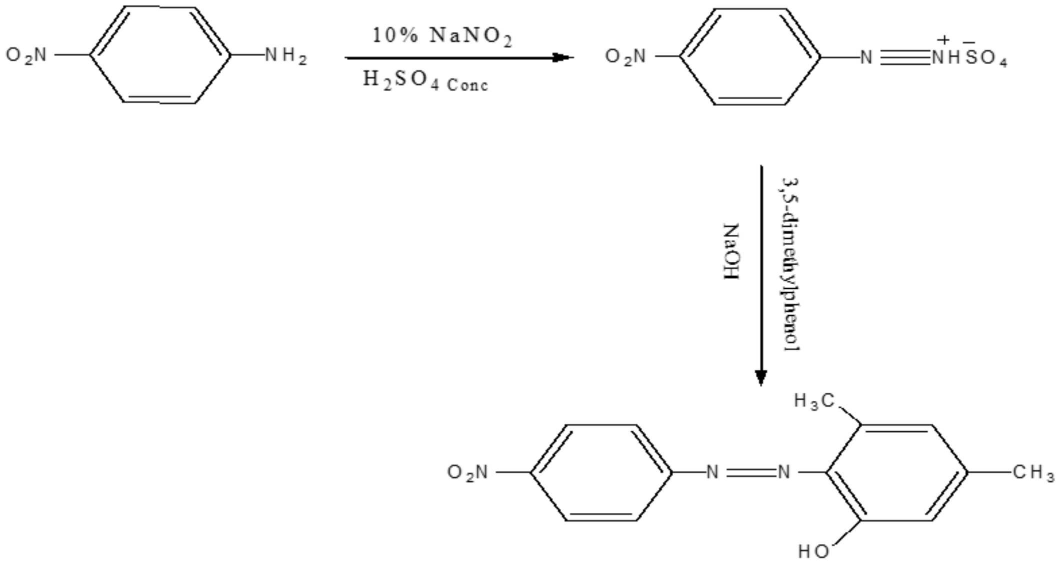
UV- Vis spectra were recorded on a Shimadzu UV- 160A Ultra Violet-Visible Spectrophotometer. IR- spectra were taken on a Shimadzu, FT-IR- 8400S Fourier Transform Infrared Spectrophotometer in the 4000- 400 cm^{-1} spectral regions with samples prepared as KBr discs. Atomic absorption was obtained using a Shimadzu A.A-160A Atomic Absorption/Flame Emission Spectrophotometer. The ^{1}H-NMR spectra were recorded on a Brucker-300 MHz Ultra Shield spectrometer at University of Al- al- Bayt using DMSO as the solvent and TMS as the reference. Microelemental analysis (C.H.N) were performed at the Al- al- Bayt University, Jordan, using Euro vector EA 3000A Elemental Analyser. Conductivities were measured for 10^{-3} M solutions of complexes in ethanol at 25°C using Philips PW- Digital Conductimeter. Magnetic susceptibilities were performed by using Brucker Magnet B.M.6 instrument at 25C°. In addition, melting points were obtained using Stuart Melting Point Apparatus.

2.2 Materials and Reagents

The following chemicals were used as received from suppliers; cobalt chloride hexahydrate 98.8% and nickel chloride hexahydrate 99.9% (Merck), 4-nitroaniline and 3,5-dimethylphenol (B.D.H).

2.3 Preparation of the Ligand (Nair & Sheela 2008)

A solution of 4-nitroaniline (0.34g, 1mmole) (10ml) of EtOH solution contain (2ml) conc.H_2SO_4 was diluted with 10 ml H_2O, and diazotized at 5°C with $NaNO_2$ solution. The diazo solution was added drop wise with stirring to a cooled ethanolic solution of (0.305g, 1mmole) of 3,5-dimethylphenol. 25 ml of 1M sodium hydroxide solution was added to the dark colored mixture. The precipitate was filtered, washed several times with (1:1) ethanol: water, mixture then left to dry. The reaction is shown in (scheme 1), while Table 1 describes the physical properties.



Scheme 1. Synthesis of the azo dye Ligand (L).

2.4 Buffer Solution

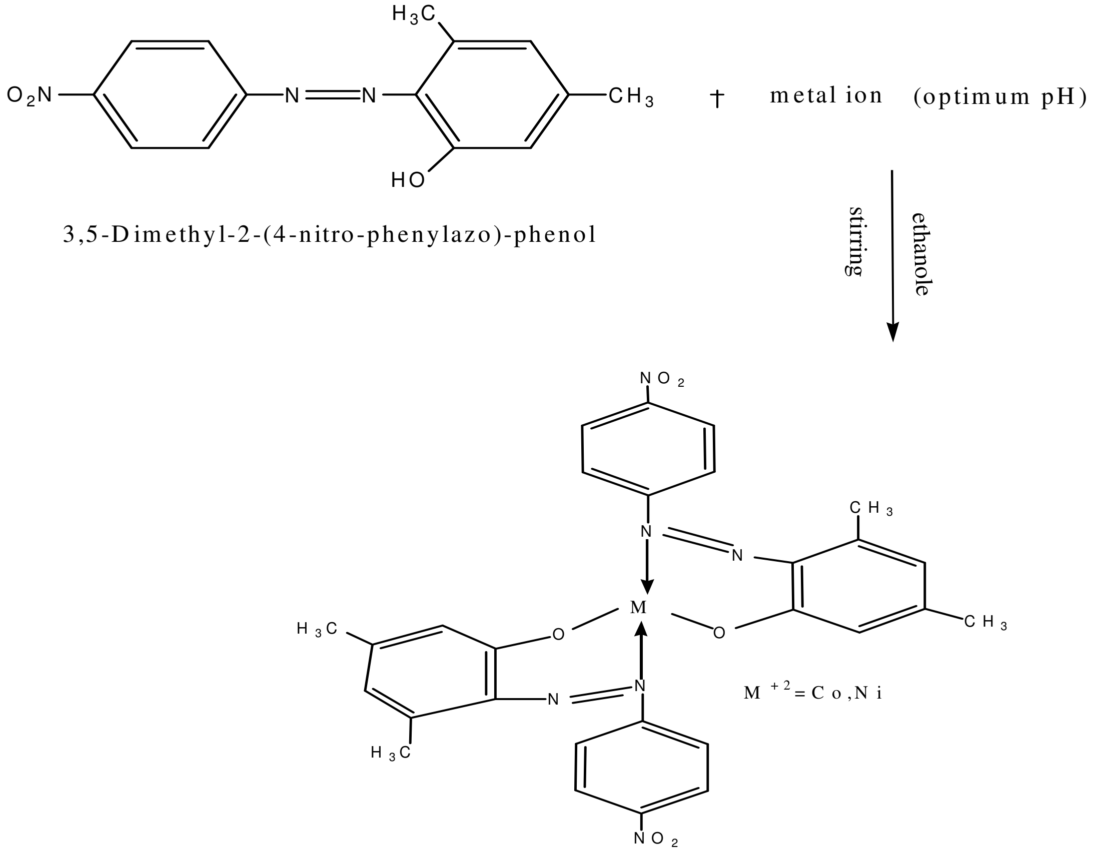
(0.01M, 0.771 gm) of ammonium acetate was dissolved in one liter of deionized water. For adjusted pH range (5-8) was used glacial acetic acid or ammonia solution.

2.5 Standard Solution

A series of standard solutions of metal chlorides of [Co(II) and Ni(II)] were prepared in different concentration (10^{-3}-10^{-5} M) at pH range (5-8). At the same time a series of ethanolic solutions of ligand within the range of concentrations (10^{-3}-10^{-5} M) was also prepared.

2.6 Preparation of Metal complexes (general procedure)

An ethanolic solution of the ligand (0.271g, 2mmole) was added gradually with stirring to the 0.118g (1mmole) of $CoCl_2.6H_2O$ and $NiCl_2.6H_2O$ respectively dissolved in the buffer solution of the required pH (scheme 2). The mixture was cooled until dark color precipitate was formed, filtered and washed several times with (1:1) water: ethanol then with acetone.



Scheme 2. Synthesis of the complexes.

2.7 Enzyme assay

This compounds were dissolved in dimethyl sulphoxide (DMSO) and stock solutions were made for each compound-different volumes from these stock were added to the assay mixture and the enzyme activity was determined according to aslightly modified WHO procedure (Vandekar 1978). Volume of (2.250 mL) of phosphate buffer (pH=7.3, 0.2M), 50 µL of DTNB solution and 10 µL serum was served as the assay mixture. In a 3 mL curette, 2 mL of this mixture was taken and 34 µL of substrate (Acetylthiocholine iodide) was added and the absorbance was measured at 430 nm. DMSO was used as a vehicle solution (control) and showed no inhibitory effect on the activity of the enzyme (Jaffer et al.1988).

3. Results and Discussion

For the preparation of the ligand (L) a coupling of 3,5-dimethylphenol with the appropriate diazotized in alkaline solution was carried out. The solubility of the ligand was investigated and was found to be soluble in organic solvents and was stable toward air and moisture. Synthesized ligand (L) was characterized by ^{1}H-NMR, FT-IR, Elem.Anal. (C.H.N) and UV-Vis spectroscopic technique. An aqueous-ethanolic solutions were always performed to study the interaction of the metal ions Co (II) and Ni (II) with the prepared ligand (L).The Colors of these mixed solutions over the molar concentration and acidity range performed were varied from brown to green.

3.1 ^{1}HNMR Spectrum

The ^{1}HNMR spectrum of the ligand in DMSO Figure 1. shows multiplet signal at δ=6.86-7.86 ppm, which refers to aromatic protons (Carballo 2001) .A signal at δ=5.22 ppm appear due to hydrogen atom of the phenolic group (SI et al. 2005) .The signal at δ=3.13 ppm is assigned to δ(CH3) and the signal at δ=2.50 ppm referred to DMSO-d_6 (Pal & Sinho 2001) .

3.2 Calibration Curve

Several molar concentration (10^{-3}–10^{-5} M) of mixed aqueous-ethanolic of ligand and metal ions, only the concentration in the range ($1\text{-}3\times10^{-4}$M) obeyed Beer's law and appeared perspicuous intense color. Best fit straight lines were obtained with correlation factor R>0.9980 as shown in Figure 2.

3.3 Optimum Conditions

To investigate the interaction between the prepared ligand and metal ions under study for the preparation of the complexes, the spectra of blending solutions for the ligand and metal ions to reach to optimum pH and concentration, as well as fixed wave length (λ_{max}) were studies first .Then mole ratio metal to ligand (M:L) was appointed to prepare the complexes. Optimum concentration was chosen for complex solution based on which solution gives the highest absorbance at constant (λ_{max}) at different pH, and results are described in Table 2.

The empirical results proved that the absorbance of all prepared complexes are maximum and constant in a buffer solution of ammonium acetate in the pH range(5-8).It was found that all prepared complexes had optimum performance at (pH = 7) as is shown in Figure 3.

3.4 Stoichiometry of Complexes

The composition of the prepared complexes performed in solution by mole ratio method at exact pH and concentration at certain wavelength of maximum absorption (λ_{max}). The ratio was (1:2) metal ion to ligand at pH 7, Figure 4. The conditions for the preparation of complexes are presented in Table 2.

3.5 Physical properties

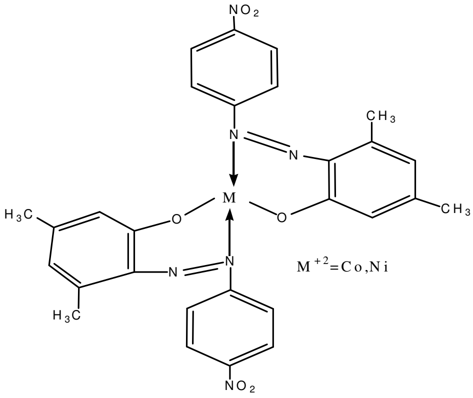
The solid complexes have been prepared by direct reaction of alcoholic solution of the ligand (L) with the aqueous solution of the metal ions at the optimum pH and in a M:L ratio of 1:2. The result of C.H.N analysis and the metal content of these complexes were in good agreements with the calculated values. The molar conductance of 10^{-3} M in ethanolic solutions of these complexes indicated their non- electrolytic nature (Geary 1971), data are presented in Table 2. The UV-Vis spectra of the prepared complexes dissolved in ethanol (10^{-3} M) have been measured and the data obtained are included in Table 2.The effective magnetic moments Table 2 of the complexes at 4.74 and 3.19 B.M. This value refers to a paramagnetic (high spin) which has been reported for most tetrahedral geometry (El-Ghar et al.2007).

3.6 Electronic spectra

The UV- Vis spectrum of an ethanolic solution of the ligand 10^{-3} M displayed mainly three peaks, the first and second peaks were observed at 218 nm and 263 nm were assigned to the moderate energy π- π^* transition of the aromatic rings. The third peak (λ_{max}) was observed at the 350 nm was referred to the π- π^* transition of intermolecular charge- transfer taken place from benzene through the azo group (-N=N) (Hamil et al. 2009). The spectrum of Co (II) complex showed peaks at 243 nm and 282 nm due to ligand felid. Other three peaks at 386 nm, 504 nm and 968 nm were found to be caused by charge transfer and (d-d) electronic transition type ${}^4A_{2(F)} \rightarrow {}^4T_{1(P)}$ and ${}^4A_{2(F)} \rightarrow {}^4T_{1(F)}$ respectively(Jarad et al. 2013). The spectrum of Ni(II) complex appeared absorption peaks at 256 nm and 288 nm were related to ligand felid, then other three peaks at 394 nm , 520 nm and 984 nm were assigned to charge transfer and electronic transition type ${}^3T_{1(F)} \rightarrow {}^3T_{1(P)}$ and ${}^3T_{1(F)} \rightarrow {}^3A_{2(F)}$ respectively(Raman et al.2007). Figure 5. Shows spectra of the ligand and their complexes.

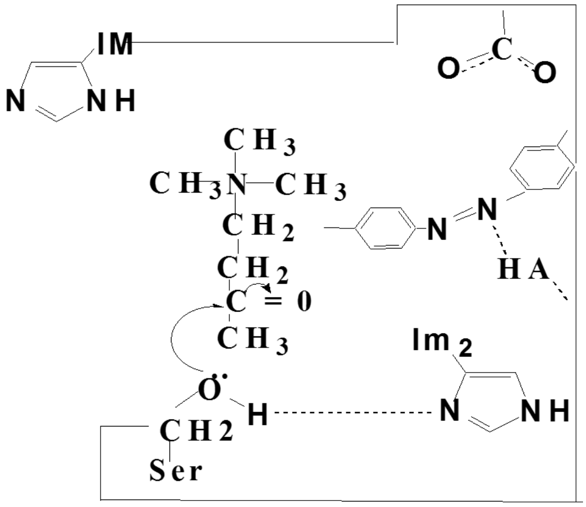
3.7 Fourier transforms infrared spectra

The FT-IR spectra of the free ligand (L) and the prepared complexes have been compared, and the data was tabulated in Table 3. The IR spectrum of the ligand (L) (Figure 6) exhibited broad band at 3421 cm^{-1}, which was assigned to the stretching vibration of υ(OH) group. This band was absent in the spectra of all prepared complexes Figure 7., which indicated deprotonation and involvement of the enol oxygen in chelation (Jarad 2013).Band characteristic of the azo bridge vibration at 1521 cm^{-1} shifted to lower frequency with change in shape in spectra of complexes, Figure 8. Which is an indication of the engagement of this group in the coordination with the metal ion (Al-Mousway *et al.* 2013). The appearance of new bands in the region of 443-489 cm^{-1} are tentatively assigned to υ(M-N) and υ(M-O) (Metal-Ligand) stretching bands(Hakim et al.2008, Rajavel et al. 2008). According to the results obtained, a tetrahedral structure has been tentatively suggested to these complexes.



3.8 Enzymic activity

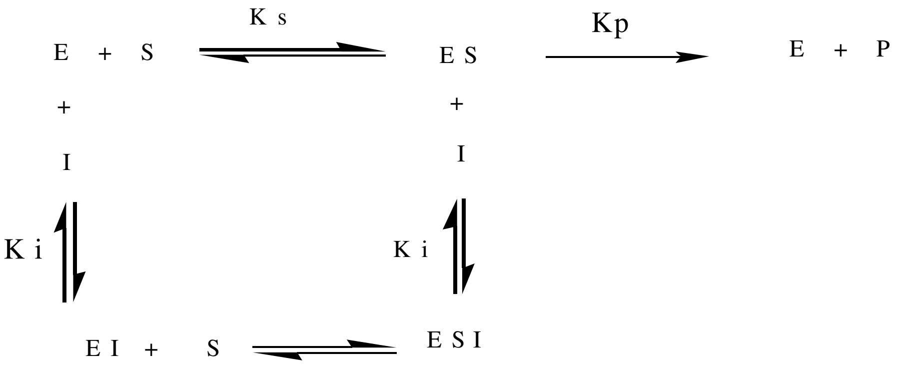
The effects of azo compound on serum cholinesterase activity invitro were carried out. Some of this compound showed encouraging inhibitors as dibucaine and Naf (Stepankova & Komer 2008). The percentages of inhibition of these compounds are shown in table (4, 5, 6). The variation in inhibition percentage of intend compounds may be attributed to the nucleophility of nitrogen atoms in these compounds this mean that these compound have pair of electron and behave as base to accept H from active side in the mechanism of hydrolysis of enzyme (CHE), OH^- group of serine as strong nucleophile to attack the (C=O) group of ester choline, and at the same time these compounds accept hydrogen H-A for Tyrosine to connect with, therefore prevent its coordination with oxygen of (C=O)(CHE), This make its electrophility C of (C=O) weak towards OH^- of serine, according this inhibited the enzyme activity in 3,5-dimethyl-2-(4-nitrophenyl azo)-phenol (Al-Ahdami 2001), but in compound Co, Ni the inhibition effect decrease which cause the nitrogen atom connect with Co,Ni complexes instead of attact of hydrogen H-A for tyrosine as shown in the suggested chart.



Suggested chart

3.9 Kinetic of inhibition

Type of inhibition was determined by line weaver–Burk plot, Figures (9, 10) which indicate that the inhibition proceeds as non competitive inhibition due to change in V_{max} values while K_m remained constant. The non competitive inhibition could be represented as follows (Al-Mudaffer 1985, Cook, & Celand2007).



Vmapp was calculated from

$$\frac{1}{V}=\frac{Km}{Vmax}\left[1+\frac{I}{Ki}\right]\frac{1}{[S]}+\frac{1}{Vmax}\left[1+\frac{I}{Ki}\right]$$

$$\frac{1}{Vmapp}=\frac{1}{Vmax.Ki}[I]+\frac{1}{Vmax}$$

$$\frac{1}{Vmapp}=\frac{1+\frac{[I]}{Ki}}{Vmax}$$

4. Conclusion

The synthesis azo dye derived from 4-nitroaniline and 3,5-dimethylphenol. Azo dye and its metal complexes were characterized by different modern analytical techniques. The analytical data of azo dye and its metal complexes gave satisfactory results corresponding to metal:ligand ratio 1:2 for complexes. IR spectra of complexes indicate that azo dye coordinate to metal ions through the hydroxyl group and the azo nitrogen. According to the physicochemical data tetrahedral geometries were assigned for the complexes. The prepared compounds have been tested as cholinesterase inhibitors.

5. Acknowledgment

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Table 1. Physical properties of the ligand and its complexes.

Compounds	**Color**	**M.P $^{\circ}$C**	**Yield %**	**Analysis Calc (Found)**			
				M%	**C%**	**H%**	**N%**
Ligand(L)	Brown	250	82	-	61.99 (60.55)	4.79 (4.31)	15.49 (14.73)
[Co(L)$_2$]	Deep brown	›300	74	9.85 (9.26)	56.41 (55.88)	4.00 (3.93)	14.02 (13.75)
[Ni(L)$_2$]	Green	›300	78	9.69 (9.13)	56.18 (55.92)	4.01 (3.89)	14.04 (13.96)

Table 2. Conditions for the preparation of the complexes and UV-Vis, magnetic susceptibility and conductance measurements Data.

Compounds	**Optimum pH**	**Optimum Molar Conc. x 10^{-4}**	**M:L Ratio**	**(λ nm)**	**ABS**	**ϵ_{max} (L.mol^{-1}.cm^{-1})**	**Λ_m (S.cm^2.mol^{-1}) In Absolute ethanol**	**μ_{eff} (B.M)**
Ligand(L)	-	-	-	218 263 350	1.522 0.532 1.951	1522 532 1951	-	-
[Co(L)$_2$]	7	2	1:2	243 282 386 504 968	1.786 1.823 1.288 1.079 0.527	1786 1823 1288 1079 527	10.56	3.74
[Ni(L)$_2$]	7	1.5	1:2	256 288 394 520 984	1.552 1.611 0.682 0.883 0.264	1552 1611 682 883 264	8.33	2.79

Table 3. The main frequencies of the ligand and their complexes (cm^{-1}).

Compounds	**ν (OH)**	**ν(-N=N-)**	**ν (M-N)**	**ν (M-O)**
Ligand(L)	3421 br.	1521 sh.	-	-
$[Co(L)_2]$	-	1511 sh.	489 w.	443 w.
$[Ni(L)_2]$	-	1517 s.	487 w.	443 w.

br =broad, sh = sharp, s = strong, w = weak

Table 4. The effect of different concentrations of 2-(4- phenyl azo)-3,5- dimethyl phenol on AchE activity in serum .

inhibitor Conc.(M)	**Enzyme activity μmol / ml / 3min**	**Inhibition %**	**Recovery %**
Nil	6.55 ± 0.18	0.0	100
4.5×10^{-3}	1.10 ± 0.21	83.27	16.79
4.5×10^{-4}	1.24 ± 0.19	86.07	18.93
4.5×10^{-5}	1.54 ± 0.21	76.49	23.51
4.5×10^{-6}	1.83 ± 0.34	72.07	27.93
4.5×10^{-7}	1.96 ± 0.31	70.08	29.92
4.5×10^{-8}	2.14 ± 0.33	67.33	32.67

Table 5. The effect of different concentrations of cobalt complex on AchE activity in serum.

inhibitor Conc.(M)	**Enzyme activity µmol / ml / 3min**	**Inhibition %**	**Recovery %**
Nil	4.85 ± 0.41	0.0	100
4.5×10^{-3}	3.31 ± 0.18	31.76	68.24
4.5×10^{-4}	3.38 ± 0.35	30.31	69.69
4.5×10^{-5}	3.45 ± 0.17	28.87	71.13
4.5×10^{-6}	3.67 ± 0.18	24.20	75.67
4.5×10^{-7}	4.21 ± 0.24	13.20	86.80
4.5×10^{-8}	4.40 ± 0.23	9.08	90.92

Table 6. The effect of different concentrations of nickel complex on AchE activity in serum.

inhibitor Conc.(M)	**Enzyme activity µmol / ml / 3min**	**Inhibition %**	**Recovery %**
Nil	5.09 ± 0.21	0.0	100
4.5×10^{-3}	3.44 ± 0.31	32.42	67.58
4.5×10^{-4}	3.49 ± 0.17	31.44	68.56
4.5×10^{-5}	3.56 ± 0.19	30.06	69.94
4.5×10^{-6}	3.69 ± 0.24	27.51	72.49
4.5×10^{-7}	3.91 ± 0.17	23.19	76.81
4.5×10^{-8}	3.21 ± 0.18	17.28	82.71

Table 7. kinetic properties of AchE using line weaver – Burk polt.

No. of comp.	Conc. Of Inh. (M)	Vmapp µmol / ml / 3min	Ki (M)
Ligand(L)	4.5×10^{-8}	10.31	3.4×10^{-8}
$[Co(L)_2]$, $[Ni(L)_2]$	4.5×10^{-8}	7.40	6.11×10^{-8}

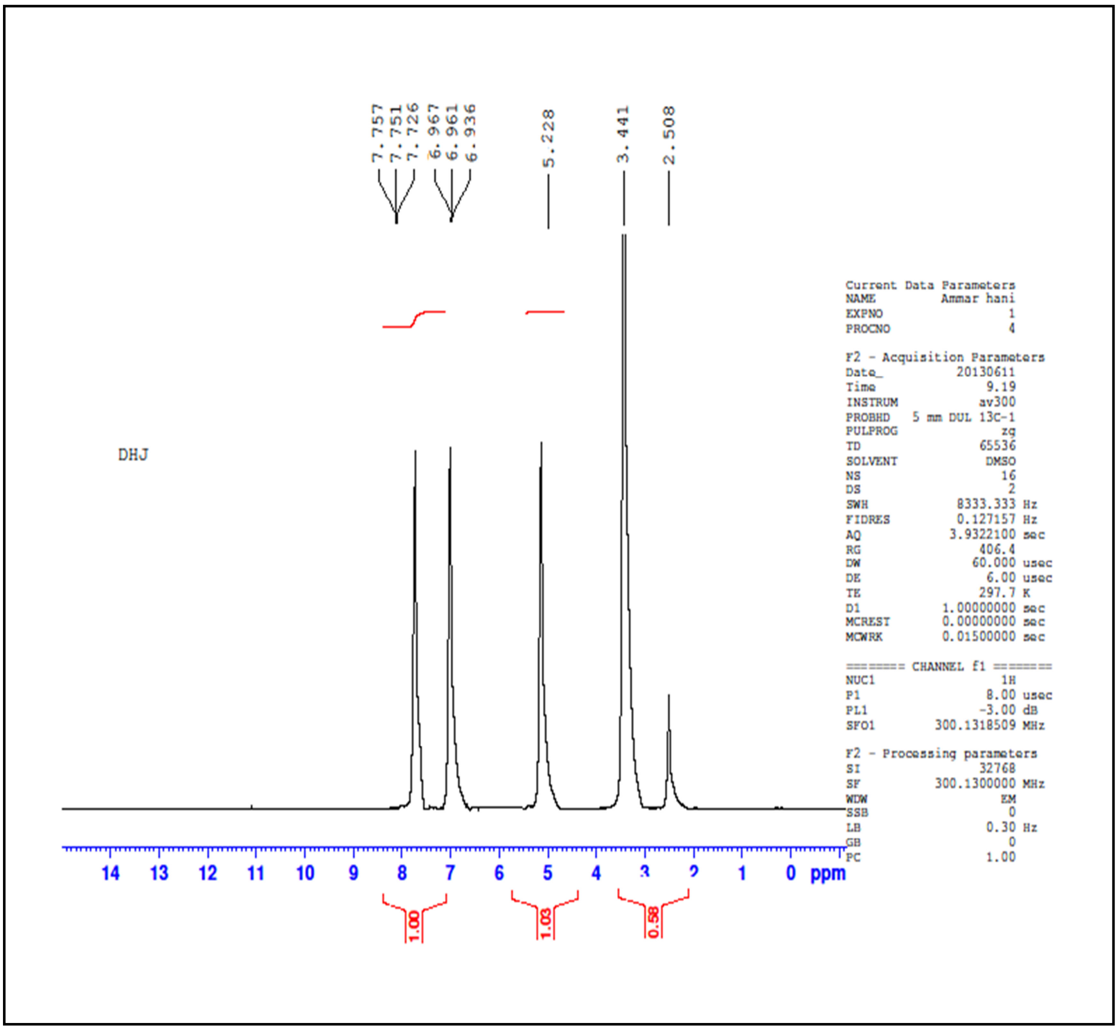

Figure 1. 1HNMR spectrum of the ligand.

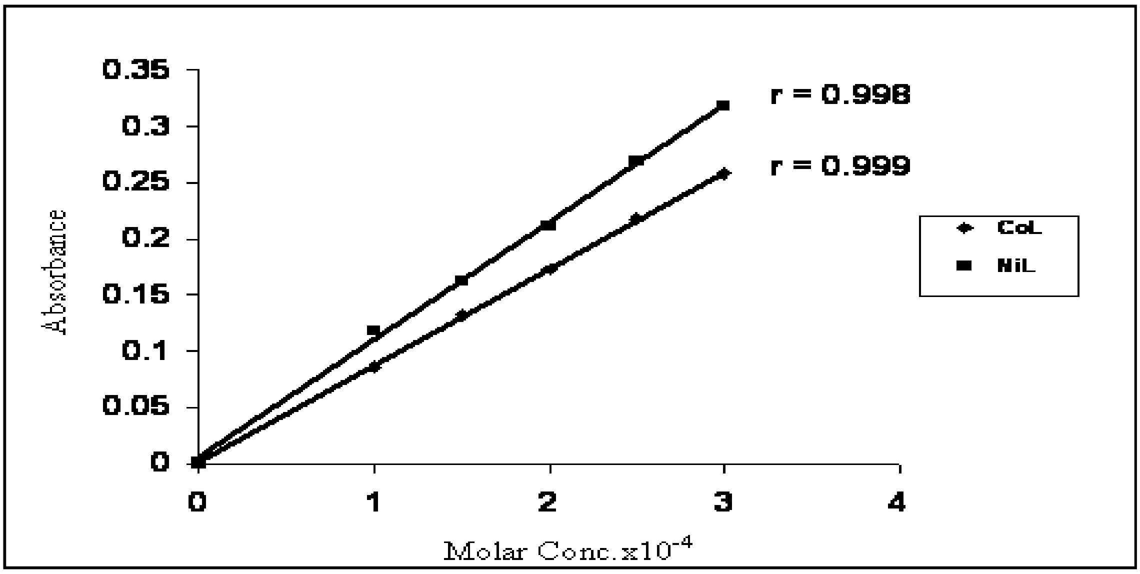

Figure 2. Linear correlation between molar concentration and absorbance.

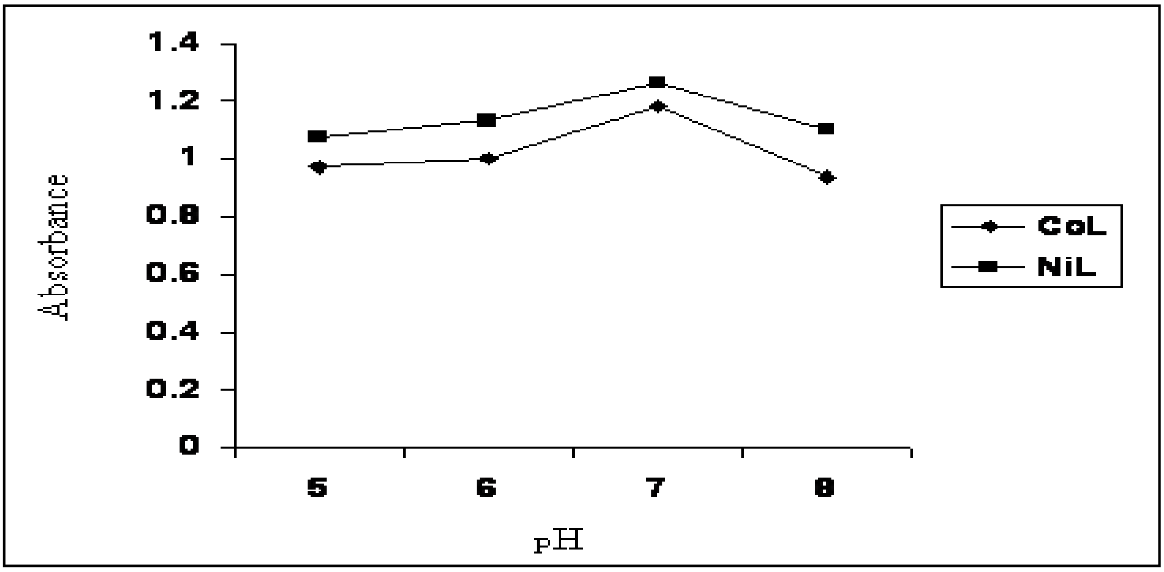

Figure 3. Effect of pH on absorbance (λ_{max}) for complexes.

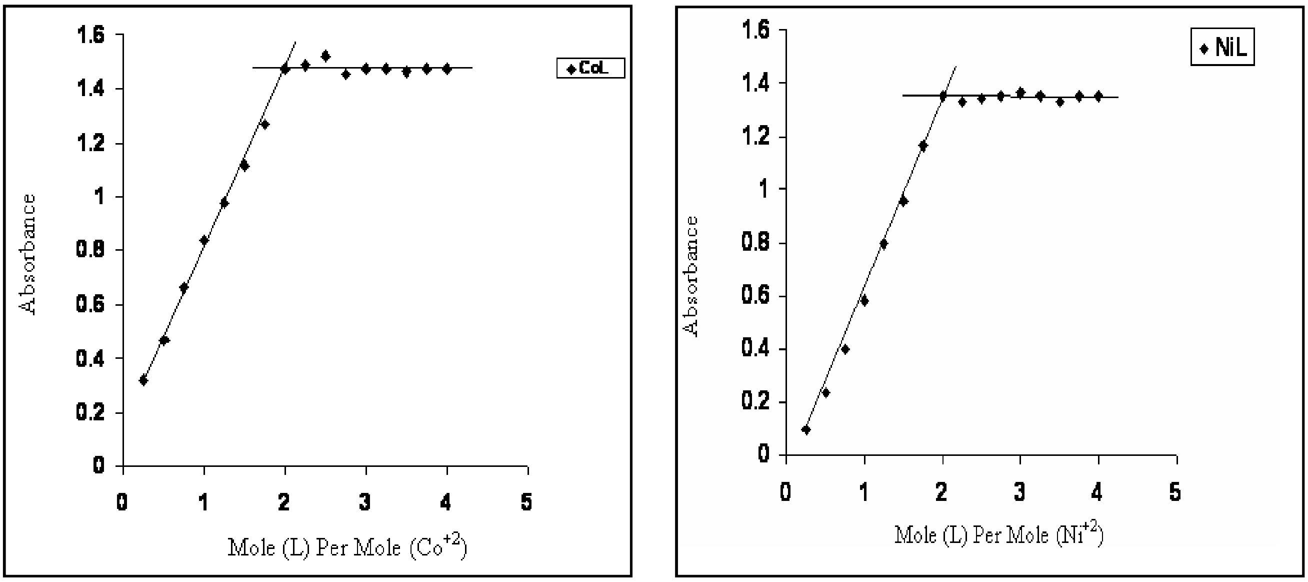

Figure 4. Mole ratio and Job methods for complexes solutions.

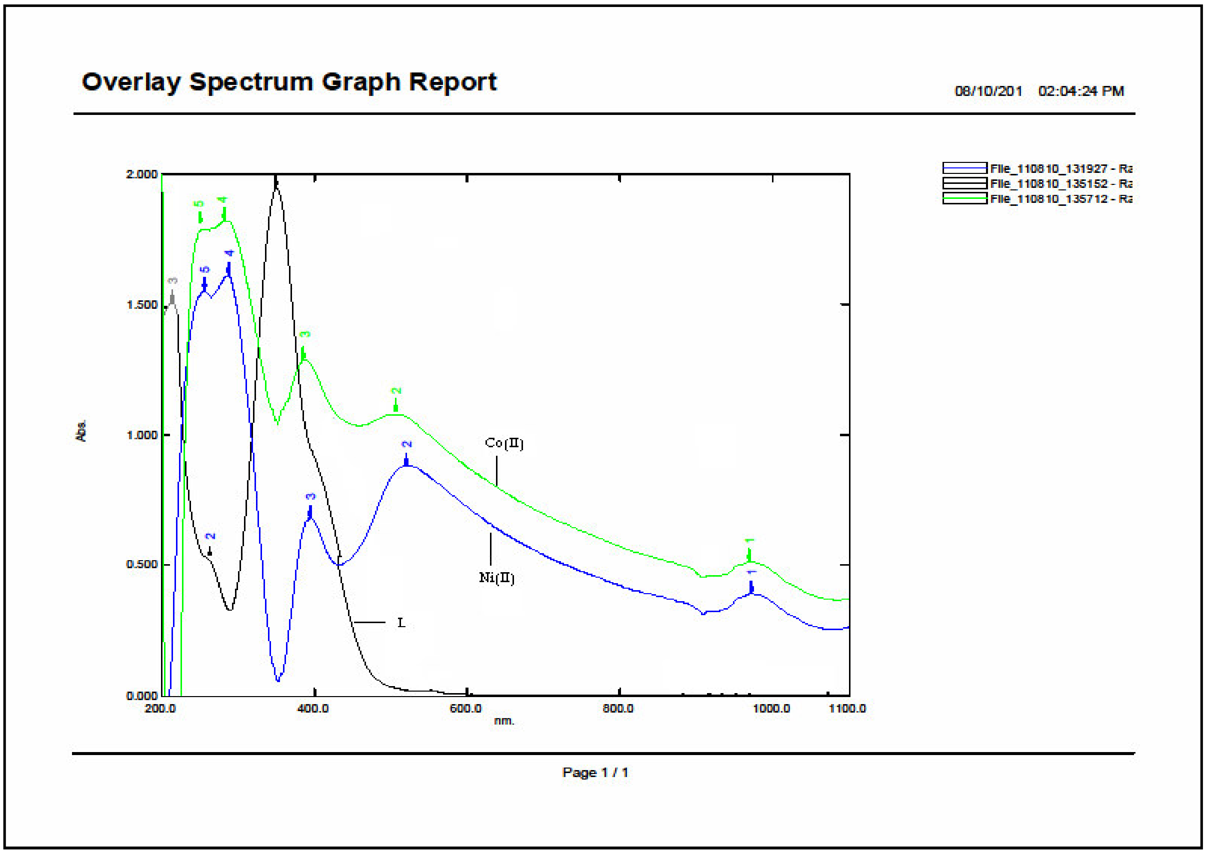

Figure 5. UV-Vis spectra of the ligand and their complexes.

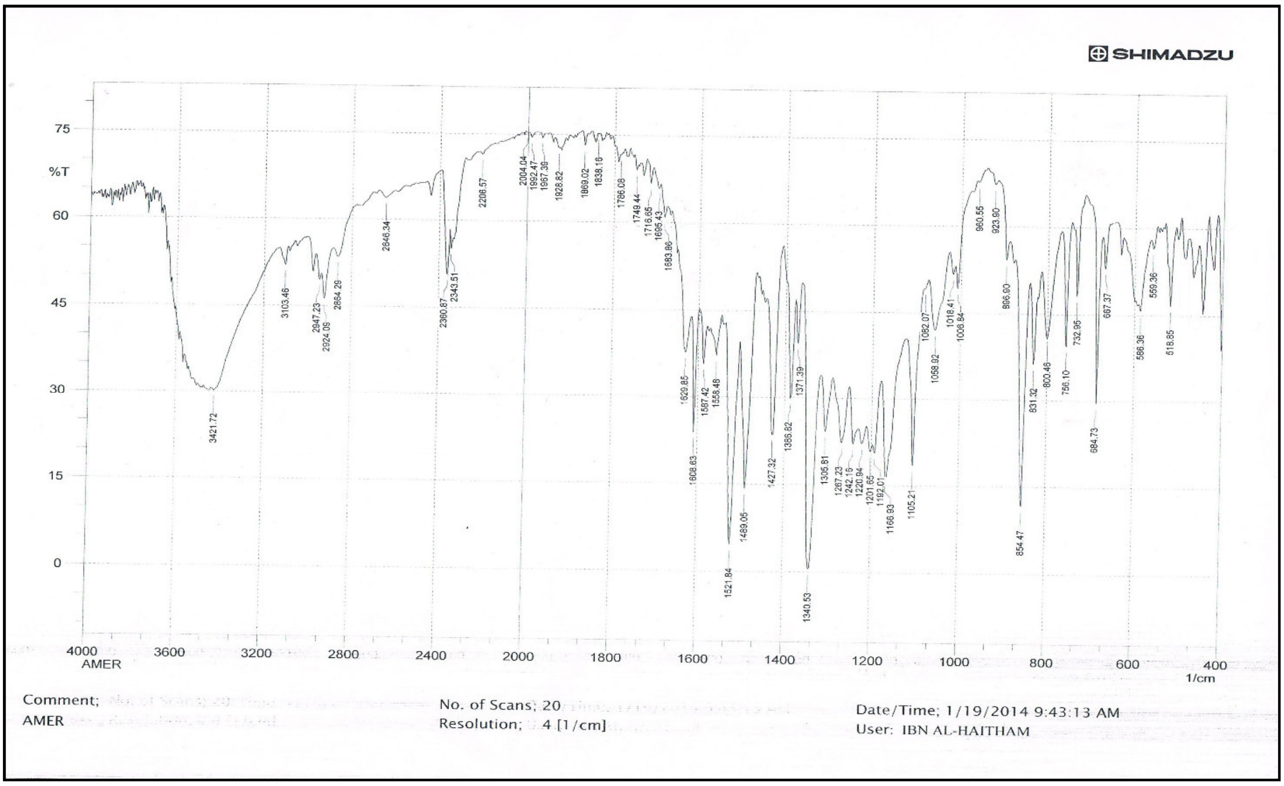

Figure 6 . FT-IR spectrum of the ligand .

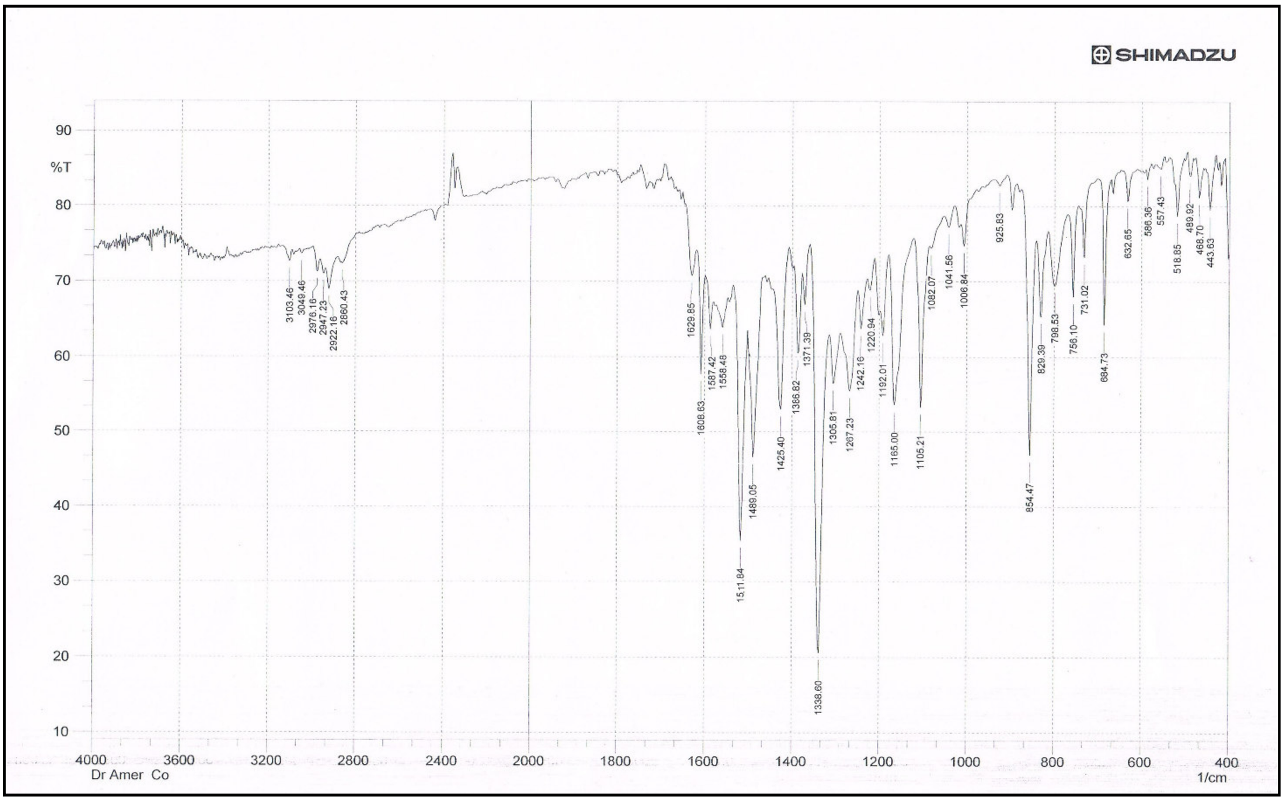

Figure 7. FT-IR spectrum of $[Co(L)_2]$ complex.

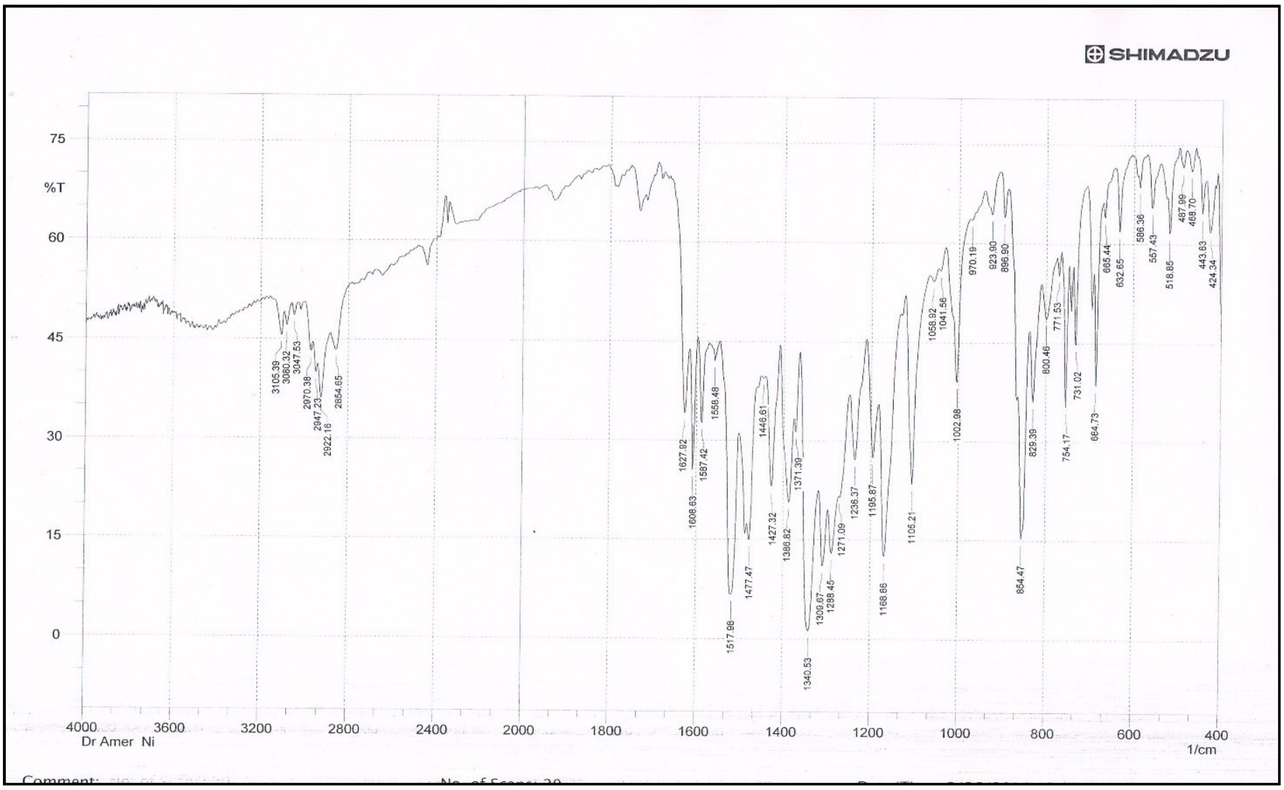

Figure 8 . FT-IR spectrum of the [Ni(L)$_2$] complex.

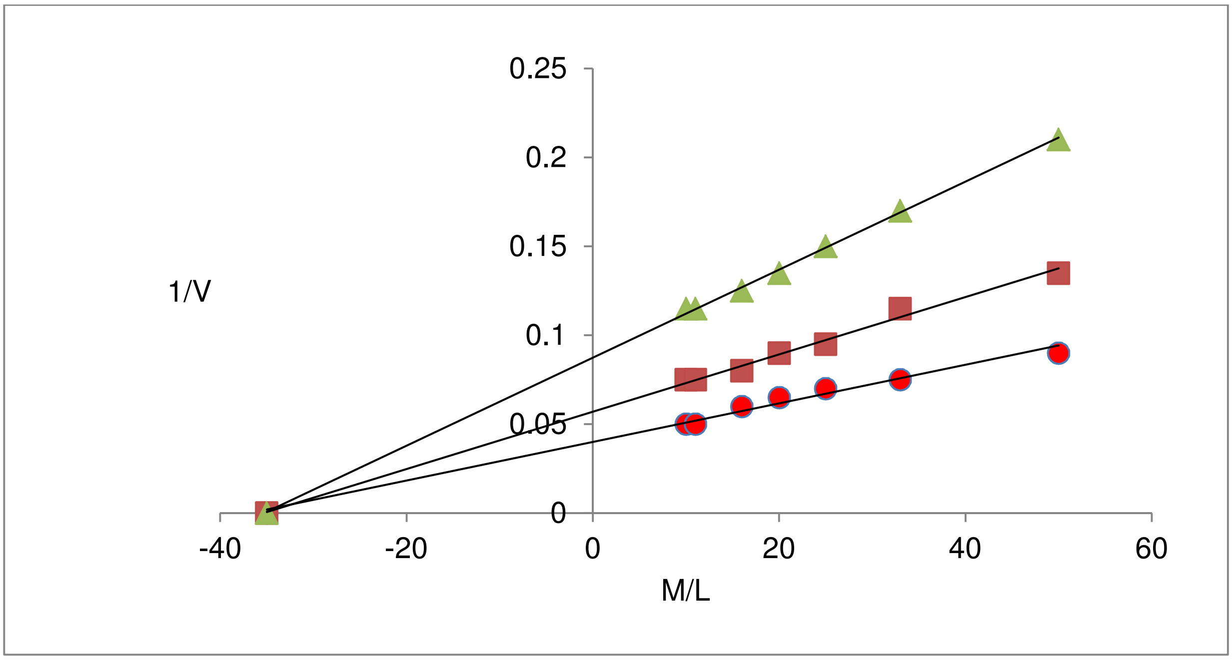

Figure 9. line weaver Burk plot determination Ki and Vampp values inhibition (AchE) (●) without (▲ , ■) with different concentration of 2-(4-nitrophenyl azo)-3,5-dimethyl phenol (4.5×10^{-3} , 4.5×10^{-8}).

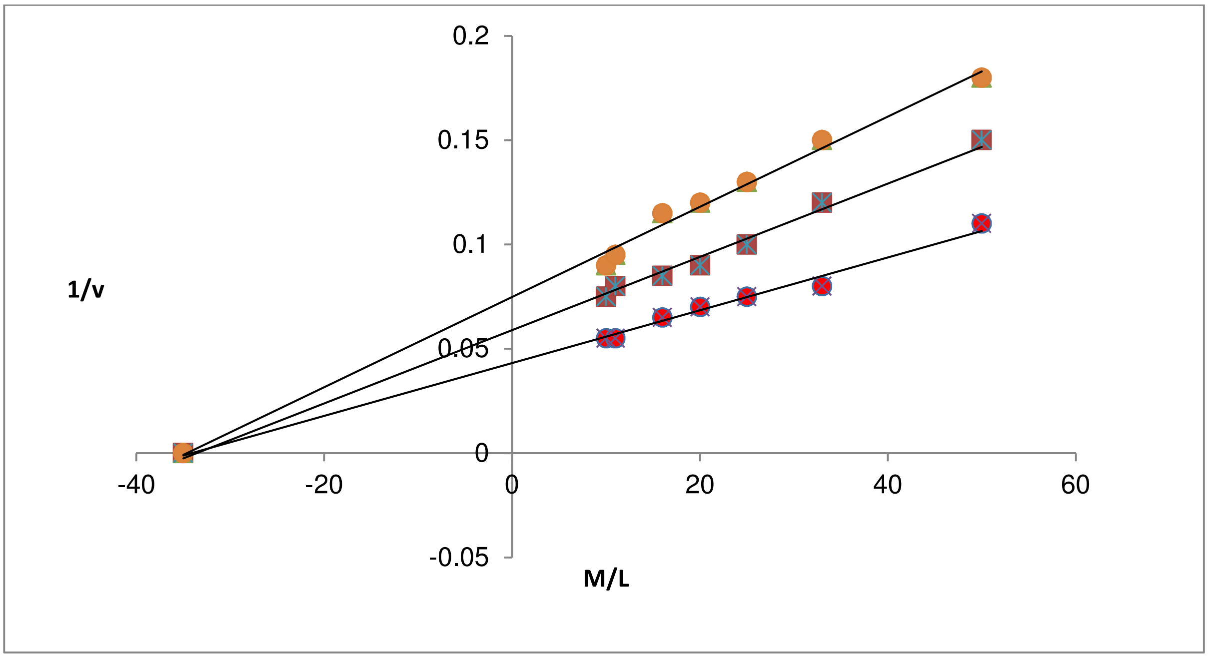

Figure 10**.** line weaver Burk plot determination Ki and Vampp values inhibition (AchE) (●) without (▲ , ■) with different concentration of cobalt and nickel complexes (4.5× 10^{-3} , 4.5×10^{-8}).

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