Synthesis, characterization and antimicrobial studies of 2-(4-antipyrine azo)-4-nitroaniline with some metal ions.

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Abstract

Transition metal complexes of Co(II), Ni(II), Cu(II), and Zn(II) with 2-(4-antipyrine azo)-4-nitroaniline derived from 4-aminoantipyrine and 4-nitroaniline 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 studied following the mole ratio and continuous variation methods, Beer's law obeyed over a concentration range (1×10^{-4} - 3×10^{-4} M). High molar absorbtivity of the complex solutions were observed. From the analytical data, the stoichiometry of the complexes has been found to be 1:2 (metal:ligand). On the basis of physicochemical data octahedral geometries were assigned for the complexes. The ligand and metal complexes were screened for their antimicrobial activity.

Keywords: - spectral studies, complexes, antimicrobial activity

Introduction

Aso dyes are a class of compounds containing a (N=N) double bond and due to their ability to absorb visible light, and ease of synthesis have been extensively used in the textile, fiber, leather, paint and printing industries for more than a century(1,2). Metal complexes of azo compounds containing heteroaryl ring systems find various applications. These type of molecules have several advantage, for example the azo group is photocromic, redox responsive and in textile industry(3-6). Pyrazoles are an important class of compounds because of their biological and pharmacology activities(7). Also, fused pyrazole moieties have a wide range of interesting properties such as anti-hyperglycemic analgesic, anti-inflammatory, anti-pyretic, anti-bacterial and sedative-hypnotic activities(8). Recently, some pyrazoles were reported to have non-nucleoside HIV-1 reverse transcriptase inhibitory activities(9,10). Azo pyrazole derivatives and its metal complexes can be used as inkjet, they have got fastness properties for dying papers and pocess high dying power on fibers(11-14). In this work, azo dye derived from 4-aminoantipyrine as diazo component and 4-nitroaniline as coupling agent. The complex of this ligand with some metal ions has been prepared and characterized physciochemically.

Experimental

Instrumentation

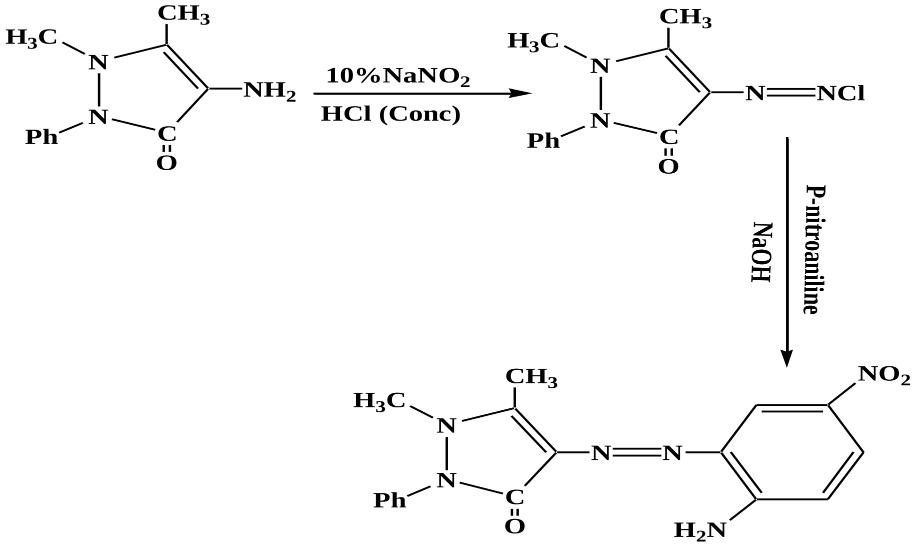
UV- Vis spectra were recorded on a Shimadzu UV- 160A Ultra Violet-Visible Spectrophotometer. IR- spectra were taken on a Shimadzu, FT-IR- 8400S Fourier Transform Infrared Spectrophotometer in the 4000- 400 cm^{-1} spectral region with samples prepared as KBr discs. Atomic absorption was obtained using 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 the 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^{o}C using Philips PW- Digital Conductimeter. Magnetic susceptibilities were performed by using Brucker Magnet B.M.6 instrument at 25C^o. In addition, melting points were obtained using Stuart Melting Point Apparatus.

Materials and Reagents

The following chemicals were used as received from suppliers; cobaltous chloride hexahydrate 98.8%, nickel chloride hexahydrate 99.9%, copper chloride dihydrate 98% and zinc chloride 98.8%,(Merck), 4-aminoantipyrine and 4-nitroaniline (B. D. H).

Preparation of the Ligand(15)

A solution of 4-aminoantipyrine (0.50 g,1mmole) (10ml) of EtOH solution contain (2ml) conc.HCl was diluted with 10 ml H_2O, and diazotized at 5^oC with $NaNO_2$ solution. The diazo solution was added drop wise with stirring to a cooled ethanolic solution of (0.34 g, 1mmole) of 4-nitoaniline. 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 and elemental analysis.



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

Buffer Solution

Buffer solution, covering the pH range of 4-8, were prepared as 0.01 M solutions of ammonium acetate in distilled water. The required pH was obtained by the addition of either ammonia solution or glacial acetic acid.

Metal Salt Solutions

Solutions of (1.10^{-5}- 1.10^{-3} M) of the metal salt were prepared by dissolving appropriate weight of the metal salt in the buffer solutions.

Ligand Solution

Ligand solutions of (1.10^{-5}- 1.10^{-3} M) were prepared by dissolving a required weight of the ligand in ethanol.

Study of Biological Activity

Two selected types of bacteria were used in this study Escherichia Coli (E.Coli) as Gram Negative Bacteria and Staphylococcus Aureus (Staph. Aurous) as Gram Positive Bacteria in Neutrient Agar medium, using (DMSO) as a solvent and as a control, the concentration of the compounds in this solvent was 10^{-3}M, using disc sensitivity test. This method involves the exposure of the zone of inhibition toward the diffusion of micro- organism on agar plate. The plates were incubated for 24hrs at $37C^{o}$.

Preparation of Metal Complexes (general procedure)

An ethanolic solution of the ligand (0.352g,1mmole) was added gradually with stirring to the 0.118g, 0.118g, 0.085g and 0.068g (1mmole) of $CoCl_2.6H_2O$, $NiCl_2.6H_2O$, $CuCl_2.2H_2O$ and $ZnCl_2$ respectively dissolved in the buffer solution of the required pH. The mixture was cooled until dark color precipitate was formed, filtered and washed several times with (1:1) water: ethanol then with acetone.

Results and Discussion

The ligand (L) was prepared by coupling 4-nitroaniline with the 4-aminoantipyrine in alkaline solution. The ligand (L) was sparingly soluble in water but soluble in organic solvents such as DMSO and DMF, 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. The ^{1}HNMR spectrum of the ligand in DMSO (Fig 1) shows multiplet signal at δ=7.426-7.967 ppm, which refers to aromatic protons(16). On the other hand, the signals at δ=2.368 ppm and δ=1.176 ppm due to $\delta(N\text{-}CH_3)$ and $\delta(CH_3)$ of pyrazole respectively(17). The signal at δ= 3.441 ppm is assigned to $\delta(NH_2)$ and the signal at δ=2.50 ppm referred to DMSO-d_6(18) .

Interaction of the metal ions Co(II),Ni(II),Cu(II) and Zn(II) with the prepared ligand (L) has been studied in solution; an aqueous-ethanolic solution was always performed over wide molar concentration and acidity range. Colours of these mixed solutions were varied from brown or green.

Different range of molar concentrations (10^{-5}-10^{-3} M) of the mixed solutions, only concentrations in the range of 10^{-4} M obey the

Lambert- Beer's law, and only these solutions showed intense colour. A calibration curve was fitted to data points in the range $3.10^{-4} – 5.10^{-4}$ M, which shows absorbance against molar concentration (Figure 2). Best fit straight lines were obtained with correlation factor of R> 0.998.

The optimal concentration was chosen for complex solutions, and it was observed that the absorption maximum (λ_{max}) remained the same at different pH values. The influence of pH was also studied in the pH range of 4-8, and the absorbance- pH curves for each metal ion measured at λ_{max} are plotted in Figure 3. Figure 3 shows selective pH-absorbance curves. The plateaus of the curves represent the completion of the reaction and consequently represent the optimum pH value.

The composition of complexes formed in solutions has been established by mole ratio and job methods. In both cases the results reveal a 1:2 metal to ligand (L) ratio. A selected plot is presented in Figure 4.

Table 2 summarizes the results obtained, as well as conditions for the preparation of the complexes.

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 DMSO solutions of these complexes indicated their non- electrolytic nature;(19) 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.

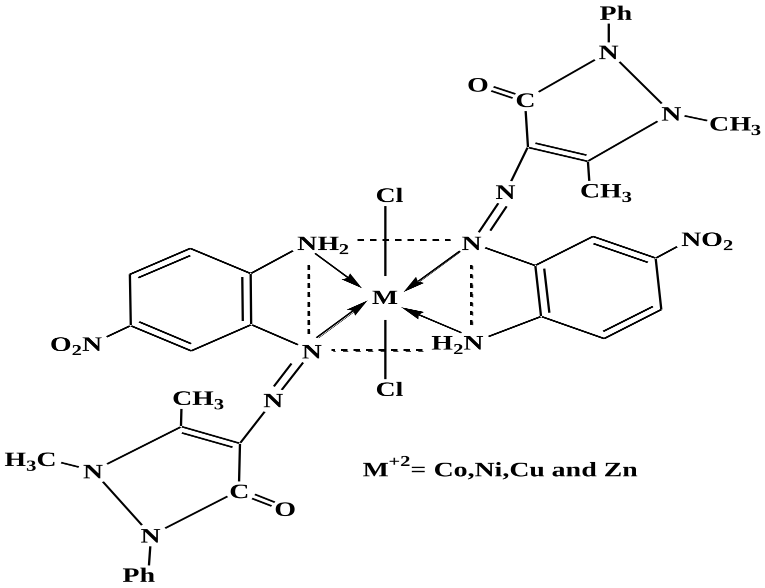
The effective magnetic moments (Table 2) of the complexes lie in the range (1.68-4.71) B.M. This value refers to a paramagnetic (high spin) which has been reported for most octahedral geometry. In case of Zn^{II} complex because of filled-d orbital, therefore the magnetic moment ($\mu=0$) is diamagnetic (20).

The UV- Vis spectrum of the ligand 10^{-3} M in DMSO (Fig 5) displayed mainly three peaks, the first and second peaks were observed at 259 nm and 295 nm were assigned to the moderate energy

π- π^* transition of the aromatic rings. The third peak (λ_{max}) was observed at the 425 nm was referred to the π- π^* transition of intermolecular charge- transfer taken place from benzene through the azo group (-N=N)(21). The spectrum of Co(II) complex (fig 6) showed peaks at 337 nm and 370 nm due to charge transfer. Other two peaks at 482 nm and 620 nm were found to be caused by (d-d) electronic transition type $^4T_{1g(f)} \rightarrow {}^4T_{1g(p)}$ and $^4T_{1g(f)} \rightarrow {}^4A_{2g(f)}$ respectively(22). The spectrum of Ni(II) complex appeared absorption peaks at 296 nm and 335 nm were related to charge transfer, then other two peaks at 462 nm and 640 nm were assigned to electronic transition type $^3A_{2g(f)} \rightarrow {}^3T_{1g(P)}$ and $^3A_{2g(f)} \rightarrow {}^3T_{1g(f)}$ respectively(23). The spectrum of Cu(II) complex gave absorption peak at 340 nm due to charge transfer. The peak at 455 nm was caused by electronic transition(24) $^2E_g \rightarrow {}^2T_{2g}$ .The spectrum of Zn(II) complex showed absorption peaks at 310 nm and 341 nm due to charge transfer. The peak at 456 nm in the visible region was detected in the complex solutions spectra with respect to that of the free ligand. The high shift in the (λ_{max}) gave a good indication for complex formation, this is a good result for octahedral complex.(25)

In order to study the bonding mode of the ligand (L) with the metal ions, the FT-IR spectra of the free ligand (L) and the prepared complexes have been compared, and the data was tabulated in Table 4. The IR spectrum of the ligand (L) (fig 7) exhibited bands at 3388 cm^{-1} and 3377cm^{-1} in the ligand spectrum ascribed to the $\upsilon(NH_2)$ stretching vibration(26,27), on complexation (fig 8) a shiften with change in shape were observed from these bands, while increasing in intensity were noticed. The band at 1716 cm^{-1} appear due to the stretching mode of $\upsilon(C=O)$. Since no change in this band was noticed, the possibility that coordination occurs via the coordination of oxygen atom of this group was excluded(28,29). Band characteristic of the azo bridge vibration at 1567 cm^{-1} shifted to lower frequency with change in shape in spectra of complexes, which is an indication of the engagement of this group in the coordination with the metal ion(30,31). The appearance of new bands in the region of 424-497 cm^{-1} are tentatively assigned to υ(M-N) (Metal-Ligand) stretching bands(32,33).

According to the results obtained, anoctahedral structure has been tentatively suggested to these complexes (Scheme 1).



Scheme 1

Finally, the biological activities of the ligand (L) and their complexes have also been examid against selected type of bacteria; Table 4 shows the deactivation capacity against the bacteria specimen of the prepared compounds under study. The results show that the Co-complex has a relatively strong deactivating capacity.

Table 1. Physical Properties and Elemental Analysis of the Complexes.

Compounds	Color	M.P°C	Yield %	Analysis Calc (Found)			
				M%	C%	H%	N%
Ligand(L)	Reddish Brown	180	76	-	57.95 (57.26)	4.54 (3.97)	23.86 (22.75)
[Co(L)$_2$Cl$_2$]	Red	210	71	7.07 ()	48.92 (48.53)	3.83 (3.15)	20.14 (19.84)
[Ni(L)$_2$Cl$_2$]	Pale Brown	225	87	6.96 ()	48.97 (48.63)	3.84 (3.61)	20.16 (19.69)
[Cu(L)$_2$Cl$_2$]	Dark Green	220	74	7.62 ()	48.63 (48.17)	3.81 (3.42)	20.02 (19.75)
[Zn(L)$_2$Cl$_2$]	Dark Brown	234	72	7.73 ()	48.57 (48.08)	3.80 (3.17)	20.00 (19.68)

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 DMSO	μ_{eff} (B.M)
Ligand(L)	-	-	-	259 295 425	0.923 0.870 0.879	923 870 879	-	-
[Co (L)$_2$ Cl$_2$]	7	4.5	1:2	337 370 482 620	1.454 1.507 1.382 0.167	1454 1507 1382 167	27.3	4.71
[Ni (L)$_2$ Cl$_2$]	6	4.0	1:2	296 335 462 640	1.435 1.529 1.408 0.267	1435 1529 1408 267	16.8	3.06
[Cu (L)$_2$ Cl$_2$]	6	4.5	1:2	340 455	1.788 1.043	1788 1043	25.4	1.68
[Zn (L)$_2$ Cl$_2$]	6	4.0	1:2	310 341 456	0.681 0.719 0.649	681 719 649	25.6	Dia

Table 3. The Main Frequencies of the Ligand and Their Complexes (cm^{-1}).

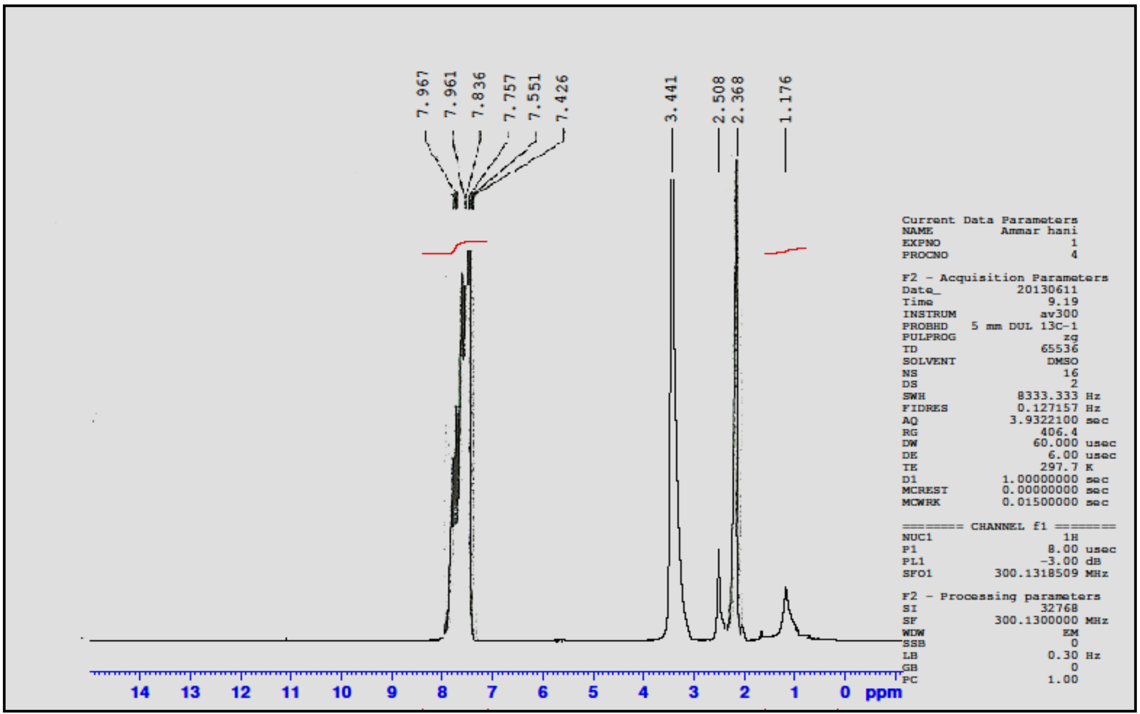
Compounds	ν(NH_2)	ν(C=O)	ν(-N=N-)	ν (M-N)azo,NH_2
Ligand(L)	3388 br. 3377 br.	1716 sh.	1567 s.	-
[Co $(L)_2 Cl_2$]	3448 br. 3414 br.	1716 sh.	1521 s.	468 w. 424 w.
[Ni $(L)_2 Cl_2$]	3448 br. 3425 br.	1716 sh.	1521 s. 1510 s.	462 w. 433 w.
[Cu $(L)_2 Cl_2$]	3437 br. 3425 br.	1716 sh.	1537 sh.	498 w. 435 w.
[Zn $(L)_2 Cl_2$]	3489 br. 3415 br.	1716 sh.	1519 s. 1504 s.	470 w. 449 w.

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

Table 4. Diameters(mm) of Deactivation of Bacteria for the Ligand and its Complexes.

Compounds	Staphylococcus Aurous	Escherichia Coli
Ligand (L)	+	-
[Co $(L)_2 Cl_2$]	+++	-
[Ni $(L)_2 Cl_2$]	++	-
[Cu $(L)_2 Cl_2$]	-	-
[Zn $(L)_2 Cl_2$]	++	-

(-) = No inhibition
(+) =Inhibition diameter(6-8) mm.
(++) =Inhibition diameter(8-10) mm.



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EXPNO                  1
PROCNO                 4

F2 - Acquisition Parameters
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PROBHD   5 mm DUL 13C-1
PULPROG               zg
TD                 65536
SOLVENT             DMSO
NS                    16
DS                     2
SWH             8333.333 Hz
FIDRES          0.127157 Hz
AQ             3.9322100 sec
RG                 406.4
DW                60.000 usec
DE                  6.00 usec
TE                 297.7 K
D1            1.00000000 sec
MCREST        0.00000000 sec
MCWRK         0.01500000 sec

======== CHANNEL f1 ========
NUC1                  1H
P1                  8.00 usec
PL1                -3.00 dB
SFO1         300.1318509 MHz

F2 - Processing parameters
SI                 32768
SF           300.1300000 MHz
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PC                  1.00
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Figure 1. ^{1}H-NMR spectrum of the azo dye ligand (L).

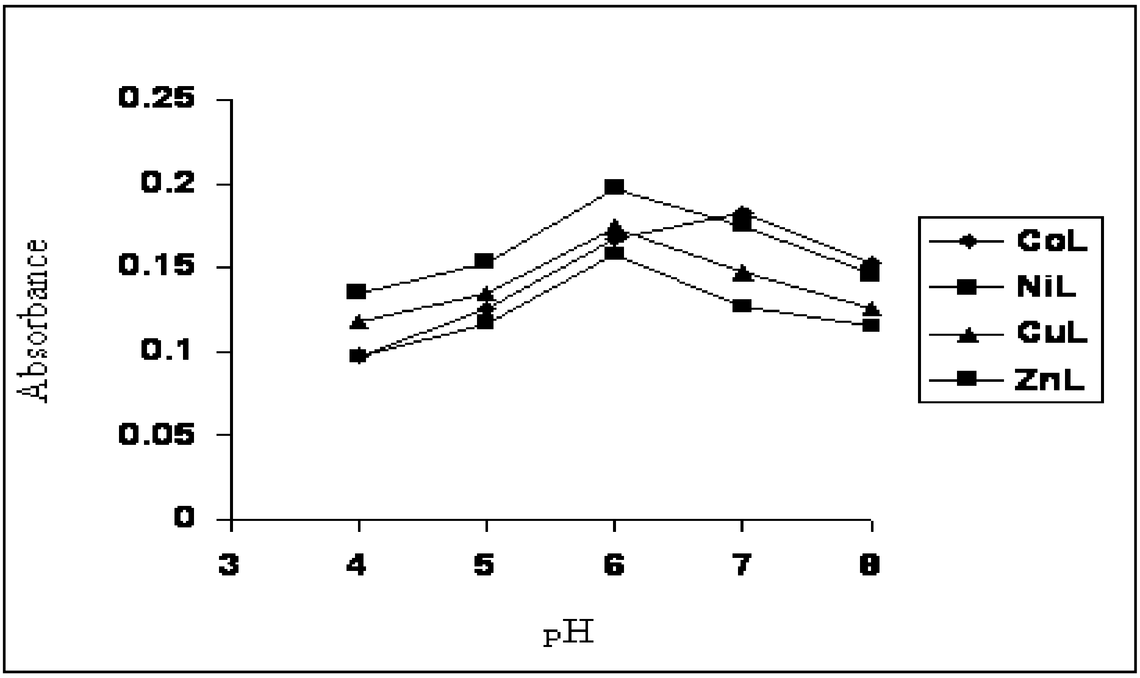

Figure 2. Linear correlation between molar concentration and absorbance.

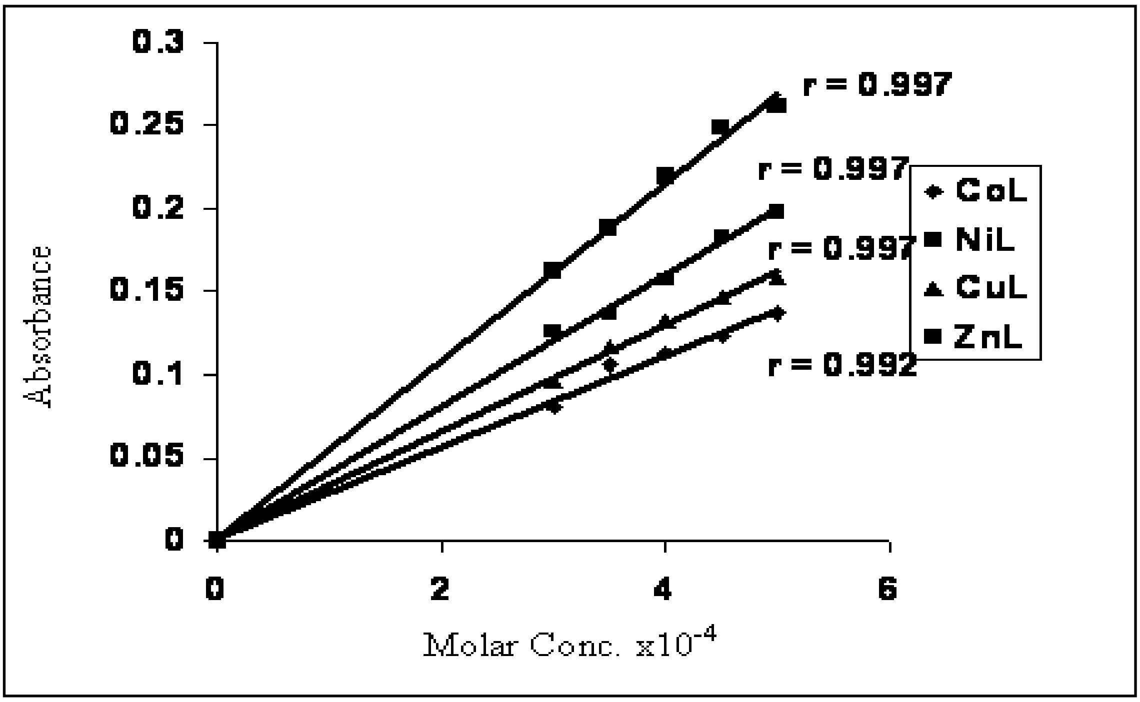

Figure 3. Effect of pH on absorbance (λ_{max}) for complexes.

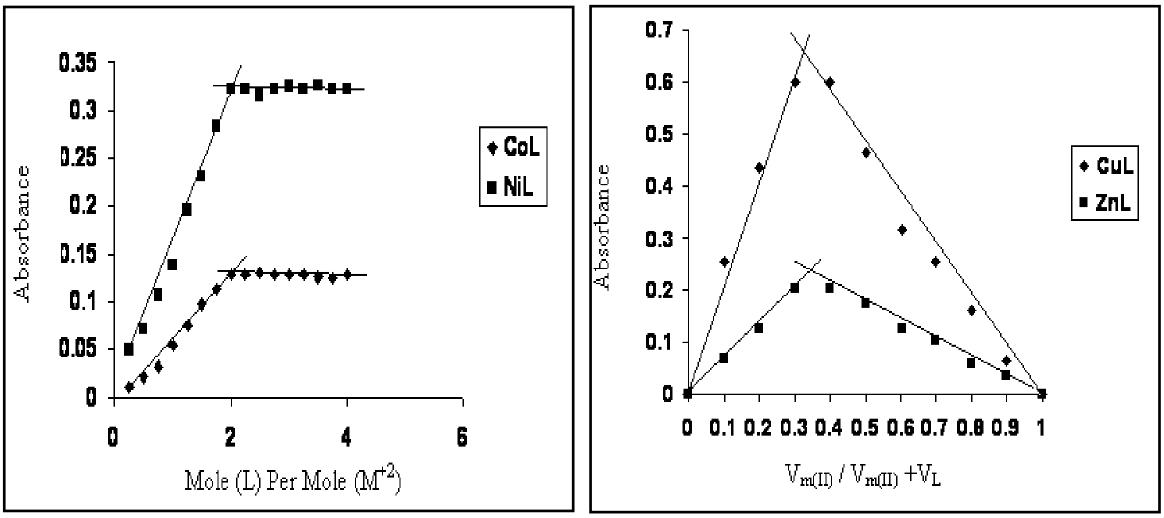

Figure 4. Mole ratio and Job methods for complexes' solutions.

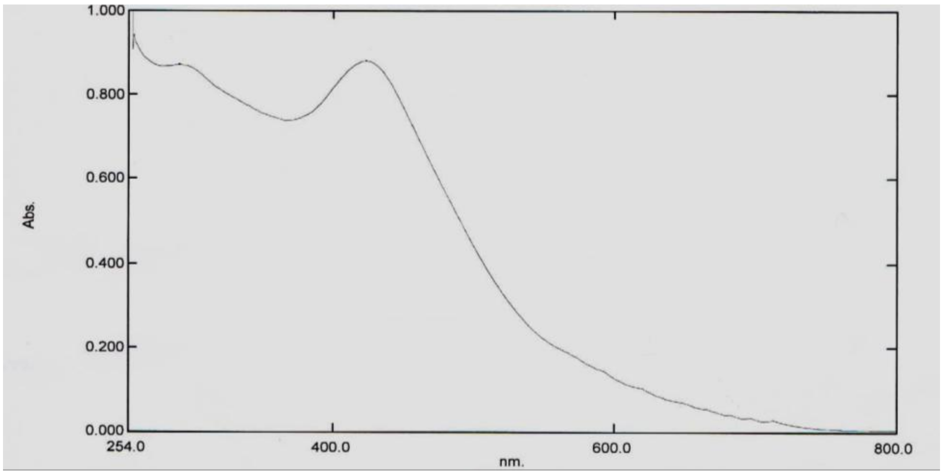

Figure 5. UV-Vis spectrum of the ligand.

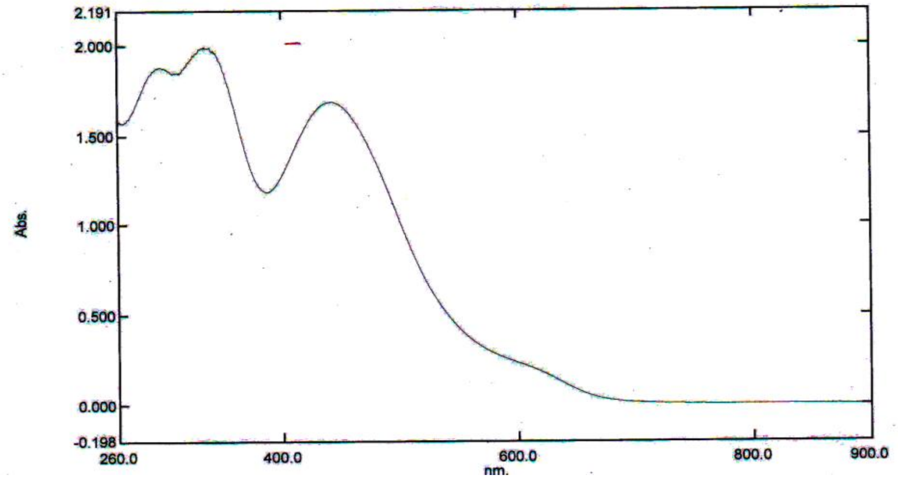

Figure 6. UV-Vis spectrum of the $[Co(L)_2Cl_2]$ complex.

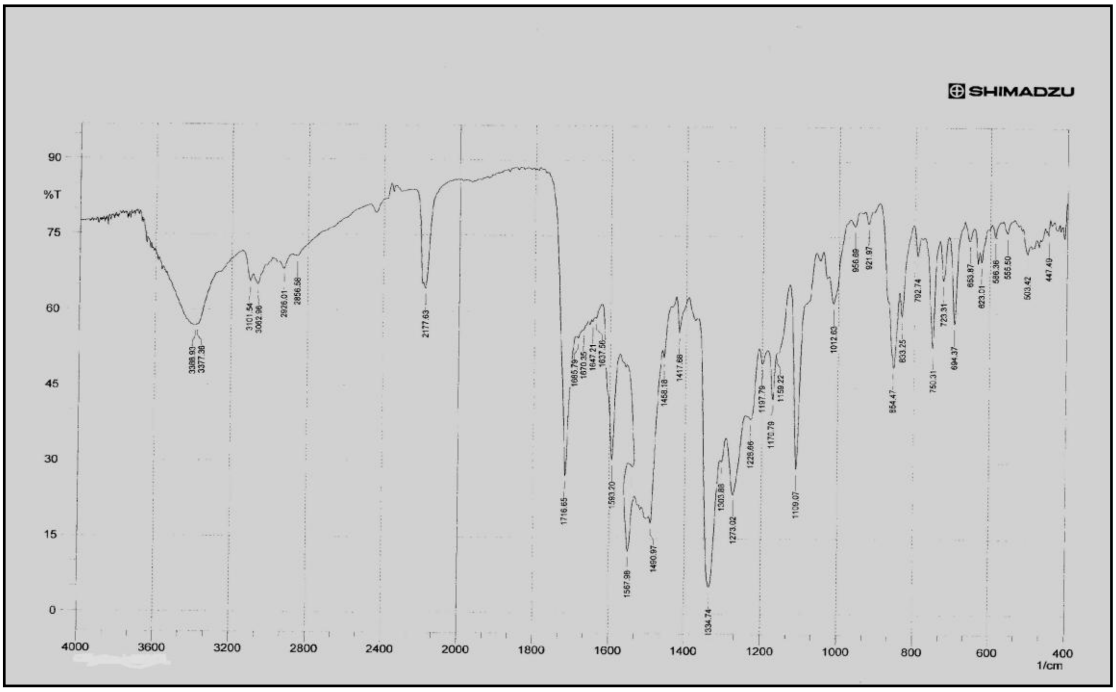

Figure 7. FT-IR spectrum of the ligand.

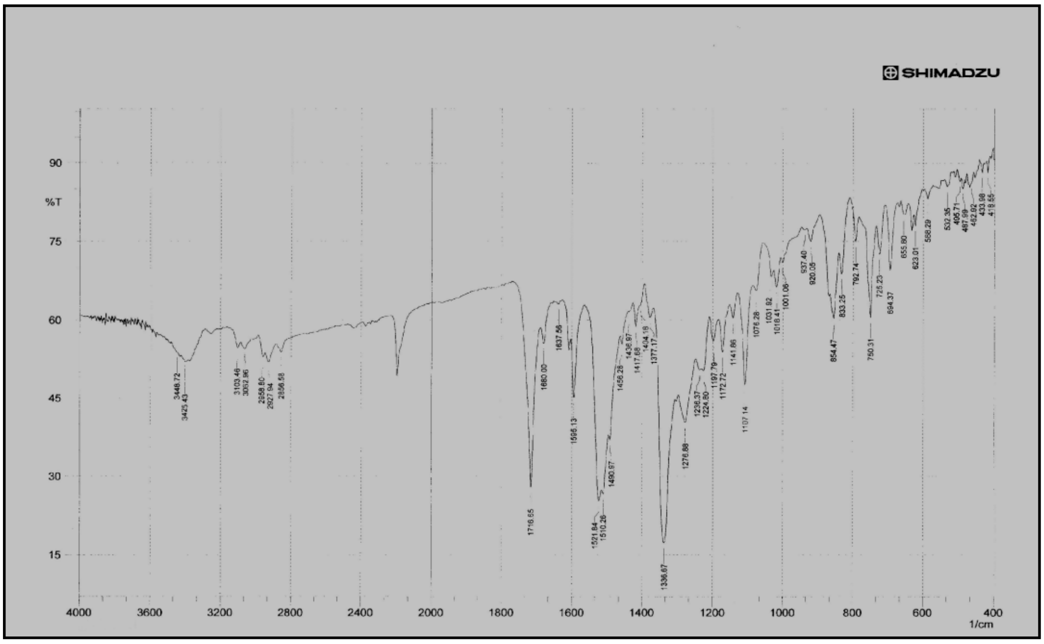

Figure 8. FT-IR spectrum of the $[Ni(L)_2Cl_2]$ complex .

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تحضير، تشخيص ودراسة الفعالية البايولوجية لمعقدات 4-(انتبيرين ازو) -4- نايترو انيلين مع بعض الأيونات الفلزية.

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الخلاصة

حضرت الليكاند 4-(4- انتبيرين ازو)- 4-نايترو انيلين من تفاعل ازدواج 4- امينو انتبيرين مع (4-نايترو انيلين). شخص الليكاند المحضر بوساطة أطياف الرنين النووي المغناطيسي والأشعة تحت الحمراء وفوق البنفسجية- المرئية والتحليل الدقيق للعناصر(C.H.N) . تمت مفاعلة الليكاند مع بعض ألايونات ألفلزية المنتخبه (Co^{II}, Ni^{II}, Cu^{II} and Zn^{II}) في وسط ايثانول – ماء وبنسبة فلز: ليكاند (2:1) وفي الدالة الحامضية المثلى، وخضعت محاليل هذه المعقدات لقانون لامبرت –بير ضمن مدى التراكيز (3×10^{-4} - 5×10^{-4} M) وتم الحصول على سلسلة من المعقدات ذات الصيغة العامة $[M(L)_2Cl_2]$.

شخصت هذه المعقدات باستخدام تقنية الامتصاص الذري اللهبي، أطياف الأشعة تحت الحمراء والأشعة فوق البنفسجية – المرئية والتحليل الدقيق للعناصر(C.H.N)، فضلا عن قياسات التوصيلية الكهربائية والحساسية المغناطيسية .درست تراكيب المعقدات باستخدام طريقتي النسب المولية والمتغيرات المستمرة ، كذلك تمت دراسة الفاعلية البايولوجية ووجد أن لهذه المعقدات قابلية متباينة على قتل الأنواع المنتخبة من البكتريا.