

## Full Paper

### Coordination Behavior and Biological Activity of Some Transition Metal Complexes with *p*-nitrophenyl and phenylazo 2, 6 diaminopyridine

Nadia A. AbdAlla<sup>1</sup>, Fatma Wafdi<sup>1</sup>, Marwa Fathy<sup>2</sup> and Fatma S.M. Hassan<sup>1\*</sup>

<sup>1</sup>Chemistry Department, Faculty of Science, Aswan University, Aswan 81528, Egypt.

<sup>2</sup>Clinical Pathology Department, Aswan University Hospital, Aswan, Egypt.

\* Address correspondence to Fatma S.M. Hassan, [Fatma\\_smh@yahoo.com](mailto:Fatma_smh@yahoo.com)

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#### Abstract

The objective of this study is to synthesize, characterize and evaluate the biological activity of *p*-nitrophenylazo and phenylazo 2,6-diaminopyridine and their Co(II)-, Ni(II)- and Cu(II)-chelates. The newly formed metal chelates were characterized by elemental analysis, FT-IR, mass and <sup>1</sup>HNMR spectra, thermogravimetric analysis (TGA) and biological activity. The electronic absorption spectra and magnetic susceptibility measurements refer to an octahedral geometry structure of the prepared complexes. The antibacterial and antifungal activities of the ligands and its metal complexes were examined against bacterial species (*Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*) and fungi (*Candida albicans*). Ampicillin and amphotericin were used as references for antibacterial and antifungal studies. The activity data show that the metal complexes have a conscious biological activity that can be compared with the parent free ligands against bacterial and fungal species.

**Key words:** Azo 2,6-diaminopyridine, transition metal complex, antibacterial activity.

#### 1. INTRODUCTION

Coordination chemistry of transition metal complexes with azo ligands is an important and fascinating branch of chemistry. The coordination compounds including azo ligands are of great importance and play a pivotal role in industry, technology and life

processes [1-4]. The azo compounds possesses suitable bonding characteristic due to presence of -N=N group and can form varieties of metal complexes with transition metal ions with unusual structural and magnetic properties [5-9]. Diaminopyridine are important class of

organic compounds, mostly used to synthesize of dyes, cosmetics, drugs and explosives. Recently, 2,4-diaminopyridine was used as pharmacological agent for the relaxation of muscle used in anesthesia and can increase the transmission of musculoskeletal in some pathological conditions. In addition, 3,4-diaminopyridine was used as a drug to treat Lambert-Eaton syndrome (a rare autoimmune disorder characterized by muscle weakness) as described for multiple sclerosis [10]. Furthermore, 4-aminopyridine as a pyridine derivative was used to inhibit the K-channel in the neutral membranes, and also to prolong the nerve action potential [11].

2,6-diaminopyridine (2,6-DAP) is used to synthesize hair dye and energetic compounds [12]. Furthermore, it was reported that amino pyridine and diaminopyridine are present in many biologically significant molecules such as folate. Antifolate drugs, and cytosine derivatives [13]. Many coworkers reported the use of 2,6-diaminopyridine in synthesis macro cyclic ligand for chelation with metal ions like Ni(II), Cu(II), Cr(III), La(III), Pb(II), Cd(II) and Zn(II) which obviously open up fascinating area of research in coordination chemistry [14]. Complexations of amino pyridylazo with transition metals have been studied [15]. Heterocyclic azo dyes have been widely applied as reagent for determination of microamount of transition elements [16] and are used as indicators in direct and indirect titration of metal ions with EDTA [17]. In view of these reports, we are interested in synthesis of X-arylazo 2,6-diaminopyridine (X= p-NO<sub>2</sub>, -H) ligands (L<sub>1</sub>, L<sub>2</sub>) and its corresponding metal chelates. The newly synthesized products

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of the free ligands and its metal complexes with Co(II), Ni(II) and Cu(II) are screened for their biological activity against bacterial species (*Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*) and fungi (*Candida albicans*), which show inhibition activities in metal chelates more than free ligands.

## 2. EXPERIMENTAL

### 2.1. Materials and reagents

All chemicals that used in this study are of the highest purity from trading suppliers such as Merck; BDH and Aldrich they include 2,6-diaminopyridine, aniline, *p*-nitro aniline, CoCl<sub>2</sub>.6H<sub>2</sub>O, NiCl<sub>2</sub>.6H<sub>2</sub>O and CuCl<sub>2</sub>.2H<sub>2</sub>O. All chemicals are used without additional purification. The organic solvents such as absolute methanol and ethanol, DMF and DMSO are purchased from Alpha Aesar.

### 2.2 Instrumentation

Melting points were identified in the capillary tube using (Gallen Camp) electrothermal melting point instrument (fine controlled, measured temperature up to 370 °C) and elemental microanalysis of the separated solid chelates and the antibacterial and antifungal activities were performed at the microanalytical Centre, Cairo University. The molar conductance of synthesized solid chelates in DMF was performed using WPA CM35 conductivity meter cell fitted with platinized electrodes. Infrared spectra were recorded on the Perkin-Elmer FT-IR type 1650 spectrophotometer type scale in wave number area 4000–400 cm<sup>-1</sup>. Measurement of solid reflectance spectra on a Shimadzu 3101pc spectrophotometer. <sup>1</sup>H-NMR studies were recorded on Bruker DPX 400 spectrometer (300.068787 MHz) and

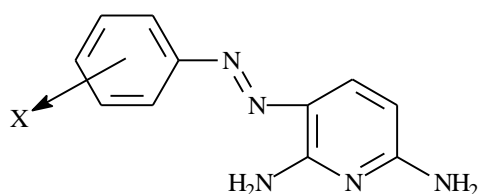
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DMSO was used as the internal reference solvent. The electron impact (EI) mass spectra (MS) at 70 eV of the tested compounds had been done using MS-5988 GS-MS Hewlett-Packard instrument. The thermal analysis TGA was implemented in a dynamic nitrogen atmosphere (10mL. min<sup>-1</sup>) with a heating rate of 10°C min<sup>-1</sup> using DTG-50 H Shimadzu.

## 2.3 Methods

### 2.3.1. Synthesis of free ligands

The azo dye ligands Figure (1) were synthesized by diazotization coupling reaction of (4-nitro aniline 1.38g, 0.01mol) for L<sub>1</sub> and (aniline 0.93g, 0.01mol) for L<sub>2</sub> in 30 ml of distilled water and 5ml of concentrated hydrochloric acid. The resulting mixture were stirred and cooled to 0°C, and then a solution of (0.69g, 0.01mol) of sodium nitrate in 25ml of distilled water was added drop wisely and stirred for 30 min at 0°C. The resulting cold diazonium chloride solutions were added dropwise to an alkaline solution of (1.09g, 0.01mol) 2,6diaminopyridine in 150ml of ethanol while stirring continuously at 0-5 °C. The mixtures formed were stirred continuously for 1h at 0-5°C in ice-bath and left in refrigerator overnight. The mixture was acidified to pH = 6. The crude products were filtered off and washed with cold distilled water and were purified by recrystallization and the purity were confirmed by elemental analysis.



Where: X = *p*-NO<sub>2</sub> (L<sub>1</sub>), H (L<sub>2</sub>)

**Figure (1):** structure of free ligands (L<sub>1</sub> and L<sub>2</sub>)

### 2.3.2. Preparation of metal complexes

The metal complexes were prepared by dissolving (0.005 mol) of ligands (L<sub>1</sub> and L<sub>2</sub>) in hot ethanol (50 ml) and added drop wisely with stirring to a stoichiometric amount of 1:1 (M:L) molar ratio to (0.005 mol) with CoCl<sub>2</sub>.6H<sub>2</sub>O, NiCl<sub>2</sub>.6H<sub>2</sub>O and CuCl<sub>2</sub>.2H<sub>2</sub>O. The reaction mixture was refluxed for 40 min and left overnight. Solid compounds formed were filtered, washed with distilled water until the solution is colorless and washed with 10ml hot ethanol-water mixture (1:1) to remove any traces of the unreacted materials. The solid complexes dried at 70°C for several hours and stored in desiccator over P<sub>2</sub>O<sub>5</sub>.

### 2.4. Biological activity

The modified Kirby-Bauer disc diffusion method [18] was used to determine the antimicrobial activity of the tested samples. Pfaller examined 100µl of bacteria or fungi that were tested and found that they had grown in 10ml of fresh media until they reached a count of approximately 10<sup>8</sup> cells/ml for bacteria and 10<sup>5</sup> cells/ml for fungi. 100µl from microbial suspension to spread on to agar plate corresponding to the stew that was kept. The isolated colonies of each organism that may be playing a pathogenic role from primary agar plates should be selected and tested for allergy by disc diffusion method. Among the many available media, NCCLS recommends Agar Mueller-Hinton because of its results in good batch-to- batch clone. The Disc Diffusion method for filamentous fungi tested by using approved standard method (M38-A) developed. To evaluate the susceptibility of the fungus to antifungal agent. Disc diffusion method for developed by National Committee for Clinical

laboratory standards using approved standard method (M44-P). Plates incubated with filamentous fungi such as *Aspergillus flavus* at 25°C for 48 hours; Gram (+) bacteria as *Staphylococcus aureus*; Gram (-) bacteria as *Escherichia coli*, they were incubated at 35-37 °C for 24- 28 hours and yeast as *Candida albicans* incubated at 30 °C for 24- 28hours, then the diameters of the inhibition zones were measured in millimeters with the calibrated calipers of National Committee for clinical Laboratory Standards. Standard discs of tetracycline (antibacterial agent) and amphotericin B (antifungal agent) have served as positive controls for antimicrobial activity but filter discs impregnated with 10µl of solvent (distilled water, Chloroform, DMSO) were used as negative control [19].

The agar Mueller –Hinton, which has been thoroughly tested for training and PH. Further the depth of the agar in plate is a factor that must be considered in the disc diffusion method. This method is well documented and standard zones of inhibition have been determined for susceptible and resistant values. Blank paper disks (Schleicher and Schuell, span) with a diameter of 8.0 mm were mixed with 10µl of tested concentration of the stock solutions. When the filter paper disc

is filled with chemicals tested on agar, the chemical moves from the disc into the agar. This Diffusion will place the chemical in the agar only around the disc. The solubility of the chemical and its molecular size will determine the size of the area chemical infiltration around the disc. If an organism is placed on the agar, it will not grow in the area around the disc if it is exposed to the chemical. This uncultivated area of no growth around the disc is known as zone of inhibition or clear zone. For the disc diffusion, the zone diameters were measured. Matar found that, Agar -based methods such as E-test and disc diffusion can be good alternatives because they are simpler and faster than broth-bases methods [20].

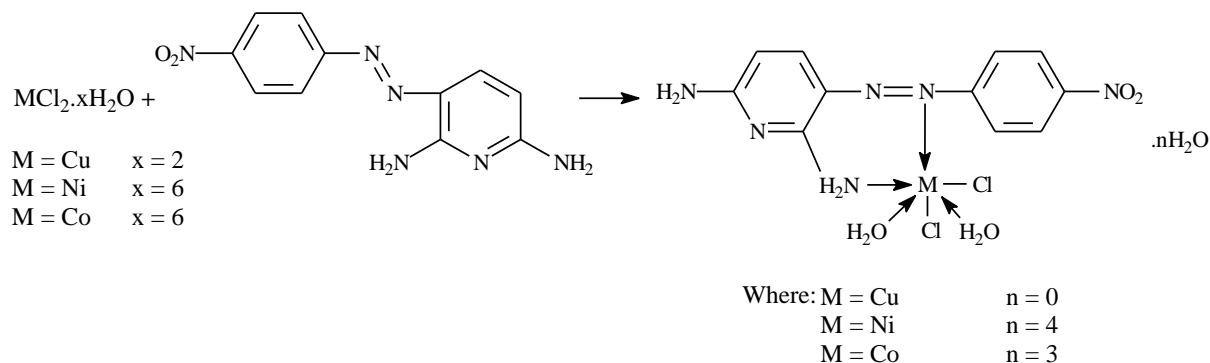
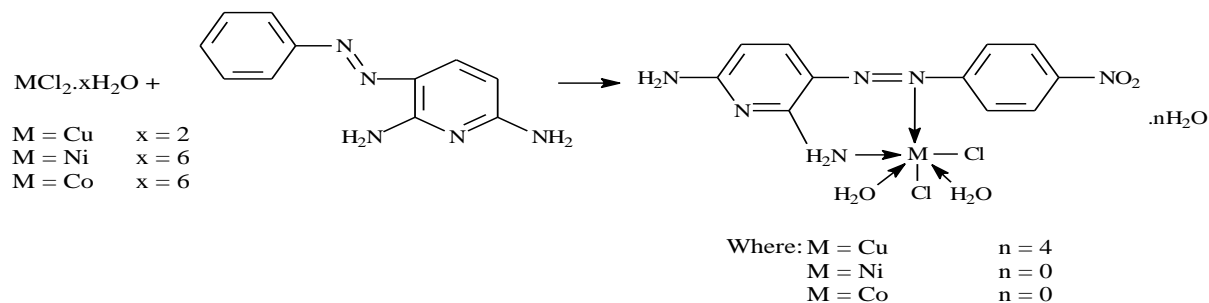
### 3. RESULTS AND DISCUSSION

#### 3.1. *Elemental analysis and physical properties*

Elemental analysis data for free ligands and its corresponding transition metal chelates agreed with the theoretical values within limit of experimental error; as shown in Table (1). These analytical data confirm the proposed general formulae of the prepared compounds Figure (2, 3).

**Table (1).** Physical properties of arylazo ligands ( $L_1, L_2$ ) and their new complexes of molar ratio (1:1) of transition metal cation ( $M=Co(II), Ni(II)$  and  $Cu(II)$  chlorides).

Compound (Molecular formula)	Color	m.p. °C	% Found (Calcd)			$\mu_{\text{eff}}$	$\Omega^{-1}$ mol <sup>-1</sup> cm <sup>2</sup>	Formula weight
			C	H	N			
$L_1$ (P-NO <sub>2</sub> ) C <sub>11</sub> H <sub>10</sub> N <sub>6</sub> O <sub>2</sub>	Red	245	50.97 (51.16)	3.81 (3.87)	32.01 (32.35)	--	--	258
$L_2$ (H) C <sub>11</sub> H <sub>11</sub> N <sub>5</sub>	Yellow	218	61.04 (61.97)	5.13 (5.16)	32.35 (32.49)	--	--	213
[CoC <sub>11</sub> H <sub>10</sub> N <sub>6</sub> O <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub> Cl <sub>2</sub> ].3H <sub>2</sub> O	Red	303	27.59 (27.62)	3.84 (3.76)	17.52 (17.57)	5.05	8.3	477.83
[NiC <sub>11</sub> H <sub>10</sub> N <sub>6</sub> O <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub> Cl <sub>2</sub> ].4H <sub>2</sub> O	Red	286	26.61 (26.66)	4.11 (4.03)	16.91 (16.94)	3.07	9.7	495.7
[CuC <sub>11</sub> H <sub>10</sub> N <sub>6</sub> O <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub> Cl <sub>2</sub> ]	Red	279	30.78 (30.8)	3.32 (3.27)	19.54 (19.60)	2.1	6.6	428.48
[Co C <sub>11</sub> H <sub>11</sub> N <sub>5</sub> (H <sub>2</sub> O) <sub>2</sub> Cl <sub>2</sub> ]4H <sub>2</sub> O	Black	312	29.35 (29.31)	4.27 (4.22)	15.58 (15.55)	4.81	7.5	450.29
[Ni C <sub>11</sub> H <sub>11</sub> N <sub>5</sub> (H <sub>2</sub> O) <sub>2</sub> Cl <sub>2</sub> ]	Black	297	34.71 (34.85)	3.90 (3.96)	18.54 (18.48)	2.92	5.8	378.71
[CuC <sub>11</sub> H <sub>11</sub> N <sub>5</sub> (H <sub>2</sub> O) <sub>2</sub> Cl <sub>2</sub> ]	Brown	280	34.42 (34.39)	3.96 (3.91)	18.34 (18.24)	1.9	6.15	383.73

**Figure (2):** Equation for the prepared metal complexes of p-nitrophenylazo 2,6 diaminopyridine.**Figure (3):** Equation for the prepared metal complexes of phenylazo-2,6 diaminopyridine.

### 3.2. Molar conductivity measurements

Table (1) shows the values of molar conductance of the complexes. It is concluded from the results that molar conductivity values of Co (II), Ni (II) and Cu (II) ions in DMF at 30°C are found to be 5.8-9.7 $\Omega^{-1} \text{ mol}^{-1} \text{ cm}^2$ . It is clear from these data that metal chelates are non - ionic by nature and non electrolytes [21].

### 3.3. FT-IR spectroscopy

The characteristic IR data of the proposed structures of the azo ligand and its metal complexes were suggested by using a comparison technique of the IR spectra of the free ligands and its metal complexes were listed in Table (2).

#### 3.3.1 IR spectra of $L_1$ and its metal complexes

IR spectra of p-nitro phenylazo 2,6 diaminopyridine(  $L_1$ ), exhibit bands at 3419 - 3366  $\text{cm}^{-1}$  which are attributed to  $\nu(\text{N-H})$  stretching vibration of C(2)-, C(6)- $\text{NH}_2$  of the pyridine ring. The strong band at 1337 $\text{cm}^{-1}$  region can be assigned to the  $\nu(\text{NO}_2)$ . The stretching band of C-H stretch aromatic group appears as a strong band at 3089 $\text{cm}^{-1}$ . The strong band at 1455  $\text{cm}^{-1}$  is due to  $\nu(\text{N=N})$  stretching were in good agreement with the structure proposed of the ligand. In addition, the out-of -plane C-H bending vibration at 897 $\text{cm}^{-1}$ , can be used to assign the positions of substituents on the aromatic ring consistent with meta disubstituted ring (two amino groups in 2,6 diaminopyridine) of free ligand[22].

On complexation, a new band at 3750  $\text{cm}^{-1}$  appear due to presence of water of crystallization. The shift of  $\nu(\text{N-H})$  band in the region at 3386-3327 $\text{cm}^{-1}$  in complexes support the contribution of N-

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atom of  $\text{NH}_2$  group in complex formation. Furthermore, the  $\nu(\text{N=N})$  stretching was suffer a blue shifted during complexation to 1449,1441 and 1440  $\text{cm}^{-1}$  for Co(II), Ni(II) and Cu(II). These bands shifted as a result of the formation of coordination bond between the metal and azo -nitrogen atom [23,24]. Stretching band of pyridine ring observed 1028  $\text{cm}^{-1}$  shows no change on complexation indicating that the pyridine does not bind to metal [25]. The stretching band of the coordinated water molecules  $\nu(\text{H}_2\text{O})$  was observed at 795, 820, 806  $\text{cm}^{-1}$  for Co(II), Ni(II) and Cu(II) respectively. The new bands appeared at range 595, 596 and 615  $\text{cm}^{-1}$  attributed to  $\nu(\text{M-N})$  stretching vibrations for Co(II), Cu(II) and Ni(II) respectively [26,27]. Finally, a peak assigned to out-of-plane C-H bending vibrations of metasubstituents in 2,6-diaminopyridine ring were blue shifted to 851, 854 and 857 $\text{cm}^{-1}$  for Co(II), Ni(II) and Cu(II) respectively. This also confirmed that nitrogen atom of amino group of ligand was used to formed coordinated with metal [28].

#### 3.3.2. IR spectra of $L_2$ and its metal complexes

IR spectrum of phenylazo 2,6 diaminopyridine ( $L_2$ ) exhibit two bands in the region 3404-3348  $\text{cm}^{-1}$  that are assignable to  $\nu(\text{NH}_2)$  stretching vibration of two amino group of pyridine ring. The weak band at 3056  $\text{cm}^{-1}$  region can be assigned to the  $\nu(\text{C-H})$  stretching aromatic. And the strong band in the region 1450  $\text{cm}^{-1}$  is due to the  $\nu(\text{N=N})$  mode were in good agreement with the structure proposed of the ligand.

The vibration spectra of the prepared complexes of  $L_2$  exhibit a weak bands at

**E-mail:** [president@aun.edu.eg](mailto:president@aun.edu.eg)

around 3863,3855  $\text{cm}^{-1}$  due to the  $\nu$  (OH) stretching as a result of presence of crystallized water in complexes. The lower shift of  $\nu$  (N-H) band in the region at 3305-3383 $\text{cm}^{-1}$  in complexes support the contribution of N- atom of  $\text{NH}_2$  group in complex formation. On basis of the suggested formulae of the solid chelates deduced from the micro – analytical result, the absence of  $\nu$  (NH) band in the spectra of Ni – $\text{L}_2$  chelates is presumably due its obscurity under the broad band of water molecules. The strong band assigned for C-H aromatic was red shifted to 3187,3072 and 3136  $\text{cm}^{-1}$ . Stretching band for  $\nu$  (N=N) is shown at 1450  $\text{cm}^{-1}$  in the spectra of free ligand and suffer a blue

shift during complexes at 1407,1414 and 1442  $\text{cm}^{-1}$  for Co(II), Ni(II) and Cu(II) respectively. The lower frequency shift of this band confirms our suggesting that N=N is taking part in coordination. The presence of coordinated water was confirmed by appearance of  $\nu$  ( $\text{H}_2\text{O}$ ) stretching at 824, 827 and 821 $\text{cm}^{-1}$  for Co(II), Ni(II) and Cu(II) respectively. Finally, peaks assigned for out-of-plane C-H bending vibration of meta substituents in 2,6 diaminopyridine ring at 900  $\text{cm}^{-1}$  were shifted to 789,870 and 894  $\text{cm}^{-1}$ , this confirmed that nitrogen atom of amino group of ligand was used to formed coordinated with metal[28].

**Table (2)** Infrared spectrum data of *p*-nitrophenylazo( $\text{L}_1$ ) and phenylazo 2, 6 diaminopyridine( $\text{L}_2$ ) and its metal chelates (band maxima in  $\text{cm}^{-1}$ )

Compound	$\nu(\text{H}_2\text{O})$	$\nu$ (N-H)	$\nu(\text{C-H})$ Aromatic	$\nu(\text{N=N})$	$\nu(\text{NO}_2)$	pyridine	C-H out of plane	$\nu(\text{M-N})$
$\text{L}_1$	.....	3419b 3366s	3089s	1455s	1337s	1028s	897w	.....
Co – $\text{L}_1$ complex	3750w 795w	3403s 3327w	3046s	1449s	1336s	1026s	851s	595w
Ni- $\text{L}_1$ complex	820w	3418s	2990w	1441s	1333s	1026s	854s	615w
Cu- $\text{L}_1$ complex	806m	3412s 3386s	3046 br	1440s	1371s	1024s	857s	596w
$\text{L}_2$	.....	3404s 3348b	3056w	1450s	.....	1065w	900br	...
Co – $\text{L}_2$ complex	3863w 824s	3408s 3305s	3187s	1407s	.....	1064w	789s	606w
Ni- $\text{L}_2$ complex	3855w 827w	3410s	3072s	1414w	.....	1059s	870s	605w
Cu- $\text{L}_2$ complex	821s	3383s 3325b	3136w	1442s	.....	1068s	894s	594w

Band property: s=strong, m= medium, br=broad, w= weak

### 3.4. <sup>1</sup>H-NMR Measurements

The <sup>1</sup>H NMR spectrum of *p*-nitrophenyl azo 2,6-diaminopyridine (L<sub>1</sub>) and phenyl azo 2,6-diaminopyridine (L<sub>2</sub>) and its metal chelates were measured in DMSO-d<sup>6</sup> as a solvent. Chemical shifts at δ = 8.05- 8.86 ppm of ring proton for L<sub>1</sub> and δ = 7.25- 7.68 ppm of ring protons for L<sub>2</sub>, respectively. The signals of the four protons of the two amino groups of free ligands were not observed in <sup>1</sup>H-NMR spectrum because they are acidic hydrogen atoms, which could exchange with deuterium atoms of solvent [22]. Proton signal of coordinated water exhibit at δ = 3.27-4.30 ppm which in agreement with elemental analysis. The ring protons of free ligands were appeared as a multiplet 7.25 – 8.86 ppm. On complexation little downfield shifts in signal of ring protons due to desheilding effect of the metal ion as M-N bond formed [27].

### 3.5. Electronic Spectral Data and Magnetic Susceptibility Measurements

The most stereochemistry of the synthesized Co(II), Ni(II) and Cu(II) complexes of ligands L<sub>1</sub>, L<sub>2</sub> were given by its magnetic moment. The effective magnetic moment of Co(II), Ni(II) and Cu(II) complexes were measured in DMSO solution at room temperature. The effective magnetic moment (μ<sub>eff</sub>) of Co(II) complexes were seen at 5.05, 4.81 B. M for L<sub>1</sub> and L<sub>2</sub> respectively which suggested the high spin six-coordinated octahedral arrangement of ligand molecules around the metal ion [29-32]. The diffuse reflectance spectra give bands at 12, 480, 18, 320 and 12, 400, 18, 320 cm<sup>-1</sup> for L<sub>1</sub> and L<sub>2</sub> Co(II) complexes, respectively. The observed bands are set to the transitions <sup>4</sup>T<sub>1g</sub>(F) → <sup>4</sup>T<sub>2g</sub>(F) (ν<sub>1</sub>), <sup>4</sup>T<sub>1g</sub>(F) → <sup>4</sup>T<sub>1g</sub>(P) and

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<sup>A</sup><sub>2g</sub>(F)(ν<sub>2</sub>), respectively, suggesting that there is an octahedral structure around the Co(II) ion [30-32]. The Ni(II) complexes have effective magnetic moment (μ<sub>eff</sub>) value of 3.07, 2.92 B.M. indicating a spin free octahedral [30-31] configuration. These geometries were also supported by electronic transitions. Their diffused reflectance spectra display band at ν<sub>1</sub> (12,880 cm<sup>-1</sup>) <sup>3</sup>A<sub>2g</sub>(F) → <sup>3</sup>T<sub>2g</sub>(F) (17, 200 cm<sup>-1</sup>) ν<sub>2</sub>, ν<sub>3</sub> (20, 240 cm<sup>-1</sup>) <sup>3</sup>A<sub>2g</sub>(F) → <sup>3</sup>T<sub>1g</sub>(P). Reflectance spectra of Cu(II) chelate show bands at 15,850 and 20,400 cm<sup>-1</sup>. This can attributed to the <sup>2</sup>B<sub>1g</sub> → <sup>2</sup>B<sub>2g</sub>, <sup>2</sup>B<sub>1g</sub> → <sup>2</sup>E<sub>g</sub> transition. The magnetic moment of the synthesized metal complex was measured and found equal to (2.1, 1.9 B.M) which falls within the range commonly observed for octahedral Cu(II) complexes [29-32].

### 3.6. Mass spectra

#### 3.6.1. Mass spectra of [Co(C<sub>11</sub>H<sub>10</sub>N<sub>6</sub>O<sub>2</sub>)Cl<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>].3H<sub>2</sub>O

The electron impact mass spectra (EI-MS) of the newly prepared complexes are recorded at 70 eV and investigated. The electron ionization (EI-MS) mass spectrum for [Co(C<sub>11</sub>H<sub>10</sub>N<sub>6</sub>O<sub>2</sub>)Cl<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>].3H<sub>2</sub>O shows a signal at m/z = 477 (mole mass = 477.2, RI = 29%) this signal may be referred to the appearance of main molecular weight. This metal chelate is broken in three pathways which are presented in scheme (1). Pathway I shows a signal at m/z = 388 (mole mass = 387.2, RI = 32%) due to loss of five molecules of water, followed by a signal at m/z = 320 (mole mass = 319.2, RI = 28%) due to loss of C<sub>3</sub>H<sub>4</sub>N<sub>2</sub> fragment. Pathway II shows a signal at m/z = 300 (mole mass = 301, RI = 28%) due to loss of three molecules of water and nitrobenzene. The signal at m/z

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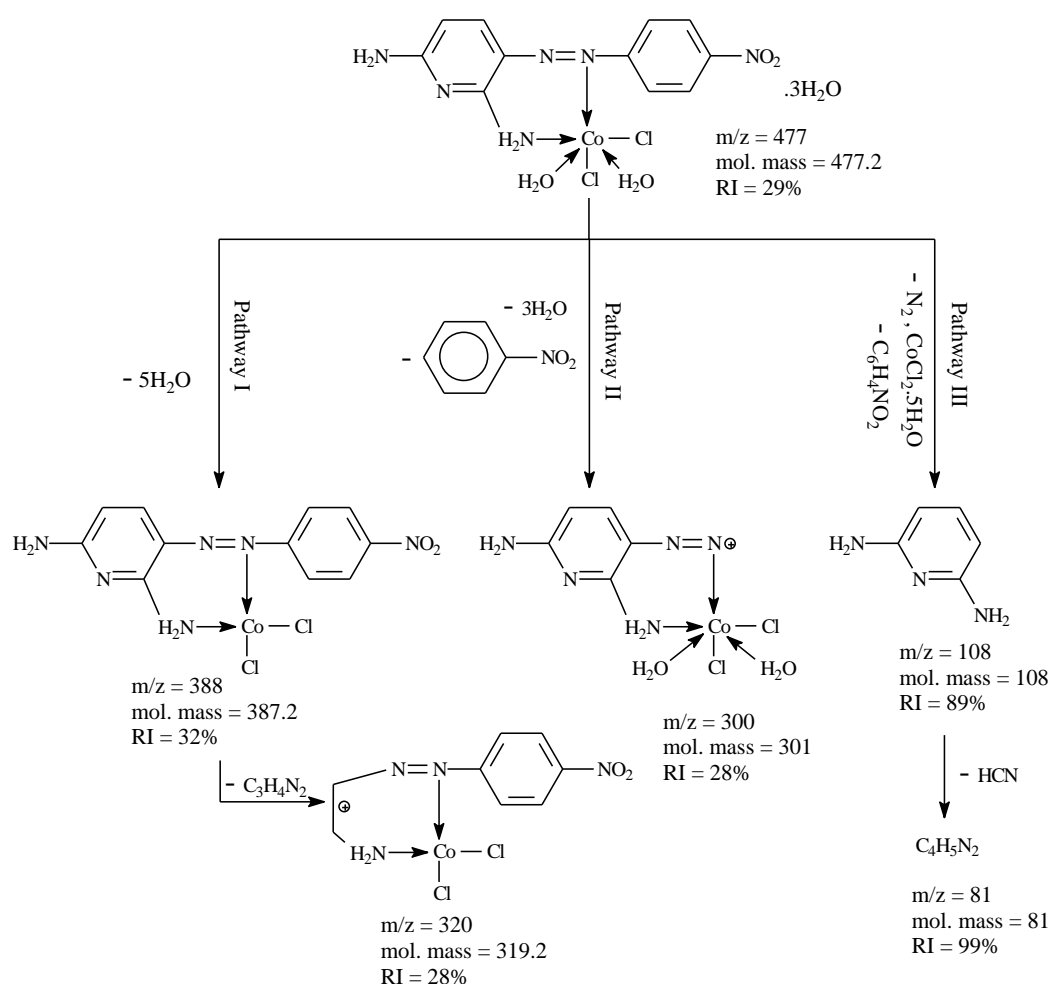


=108 (mole mass = 108, RI = 89%) due to loss of cobalt chloride, five water molecules,  $N_2$  and  $C_6H_4NO_2$ , followed by loss of HCN molecule leaving a fragment gave a signal at  $m/z = 81$  (mole mass = 81, RI = 99%).

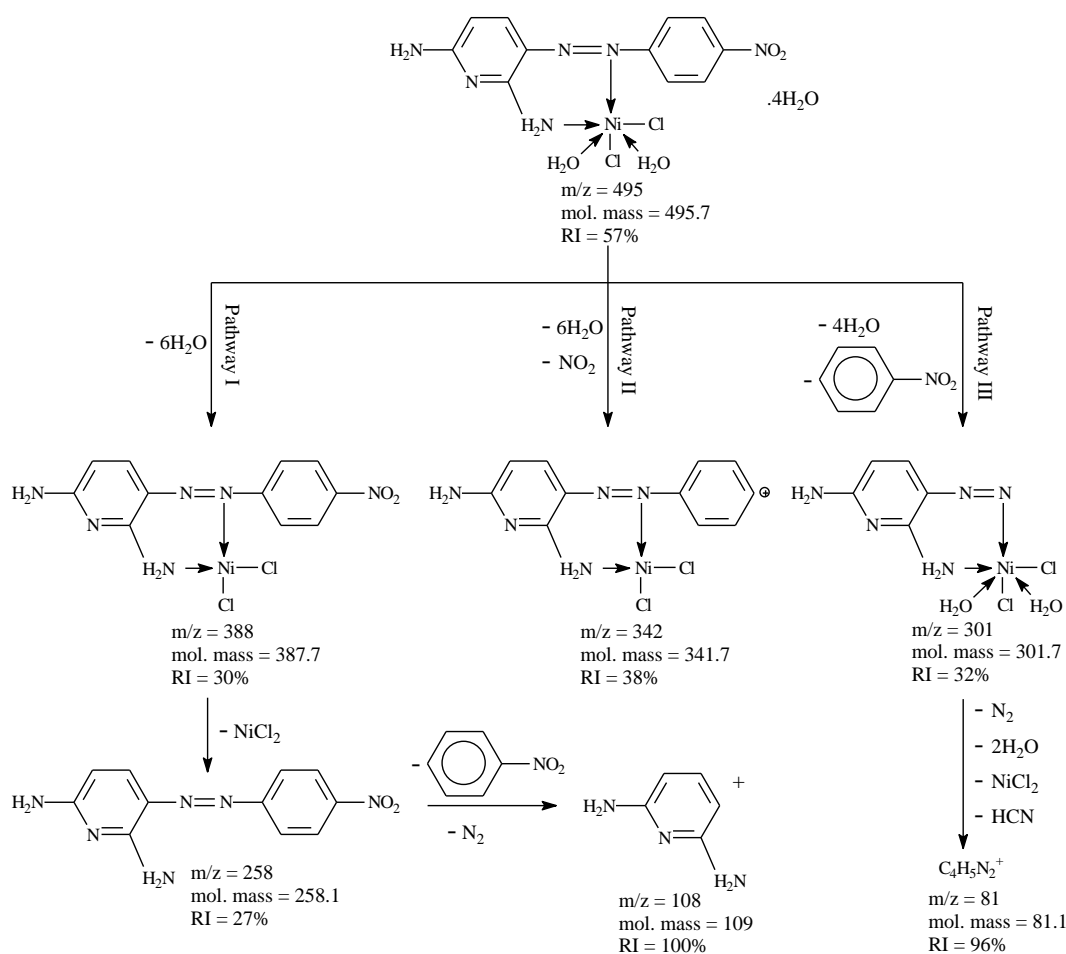
### 3.6.2. Mass spectra of $[NiC_{11}H_{10}N_6O_2Cl_2(H_2O)_2].4H_2O$

The mass fragmentation of  $[NiC_{11}H_{10}N_6O_2Cl_2(H_2O)_2].4H_2O$  after ionization of neutral molecule at 70eV consists of three principle pathways as shows in scheme (2). The signal at  $m/z = 495$  (mole mass = 495.7, RI = 57%) may be referred to the appearance of its molecular

weight. Pathway I shows signal at 388 (mole mass = 387.7, RI = 30%) is followed by signals at  $m/z = 258$ , 108 (mole mass = 258.1, 109, RI = 27%, 100%) due to loss of water molecules,  $NiCl_2$  and nitrobenzene. Pathway II shows a signal at  $m/z = 342$  (mole mass = 341.7, RI = 38%) due to loss of six molecules of water and  $NO_2$ . Pathway III shows signal at  $m/z = 301$  (mole mass = 301.7, RI = 32%) due to loss of four molecules of water and nitrobenzene, followed by signal at  $m/z = 81$  (mole mass = 81.1, RI = 96%) which may be referred to loss of two water molecules,  $-N=N-$ , nickel chloride and HCN molecules.



**Scheme (1):** The mass fragmentation pathways of Co (II) chelate with L<sub>1</sub>



**Scheme (2):** The mass fragmentation pathways of Ni(II) chelate with  $L_1$

### 3.6.3 Mass spectra of $[Cu(C_{11}H_{10}N_6O_2)Cl_2(H_2O)_2]$

The mass fragmentation of  $[Cu(C_{11}H_{10}N_6O_2)Cl_2(H_2O)_2]$  chelate after ionization of neutral molecule at 70eV consists of four principal pathways as rationalized in scheme (3). The signal that appears at  $m/z$  428 (mole mass = 428.48, RI = 14%) may be referred to the appearance of the main molecular weight of metal chelate. Pathway I shows appearance of the signal at  $m/z$  = 305 (mole mass = 306, RI = 18%) due to loss of nitrobenzene. Pathway II shows a signal at  $m/z$  = 258 (mole mass = 259, RI = 12%) due to the loss of copper chloride and two coordinated water molecules, followed by

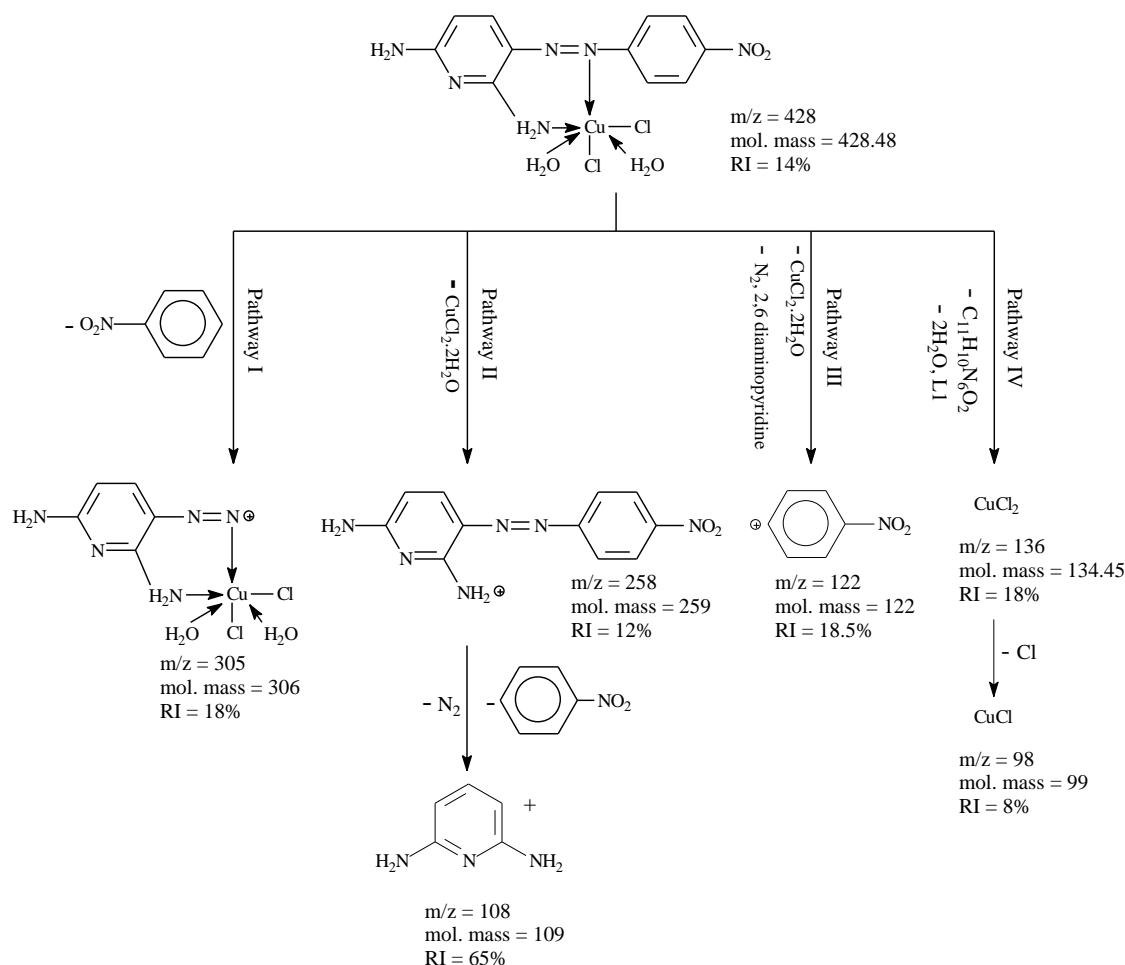
elimination of  $N_2$  gas and 4-nitrobenzene with a signal at  $m/z$  = 108 (mole mass = 109, RI = 65%). Pathway III shows a signal at  $m/z$  = 122, mole mass = 122, RI = 18.5%) this fragment may be referred to the loss of  $2H_2O$ , 2,6-diaminopyridine,  $N_2$  and copper chloride. The final pathway give a signal at  $m/z$  = 136 (RI = 18%) due to loss of  $L_1$  and two coordinated water molecules, followed by loss of chloride ion forming  $CuCl$  at  $m/z$  = 98 (mole mass = 99, RI = 8%).

### 3.6.4. Mass spectra of $[CoC_{11}H_{11}N_5(H_2O)_2Cl_2]4H_2O$

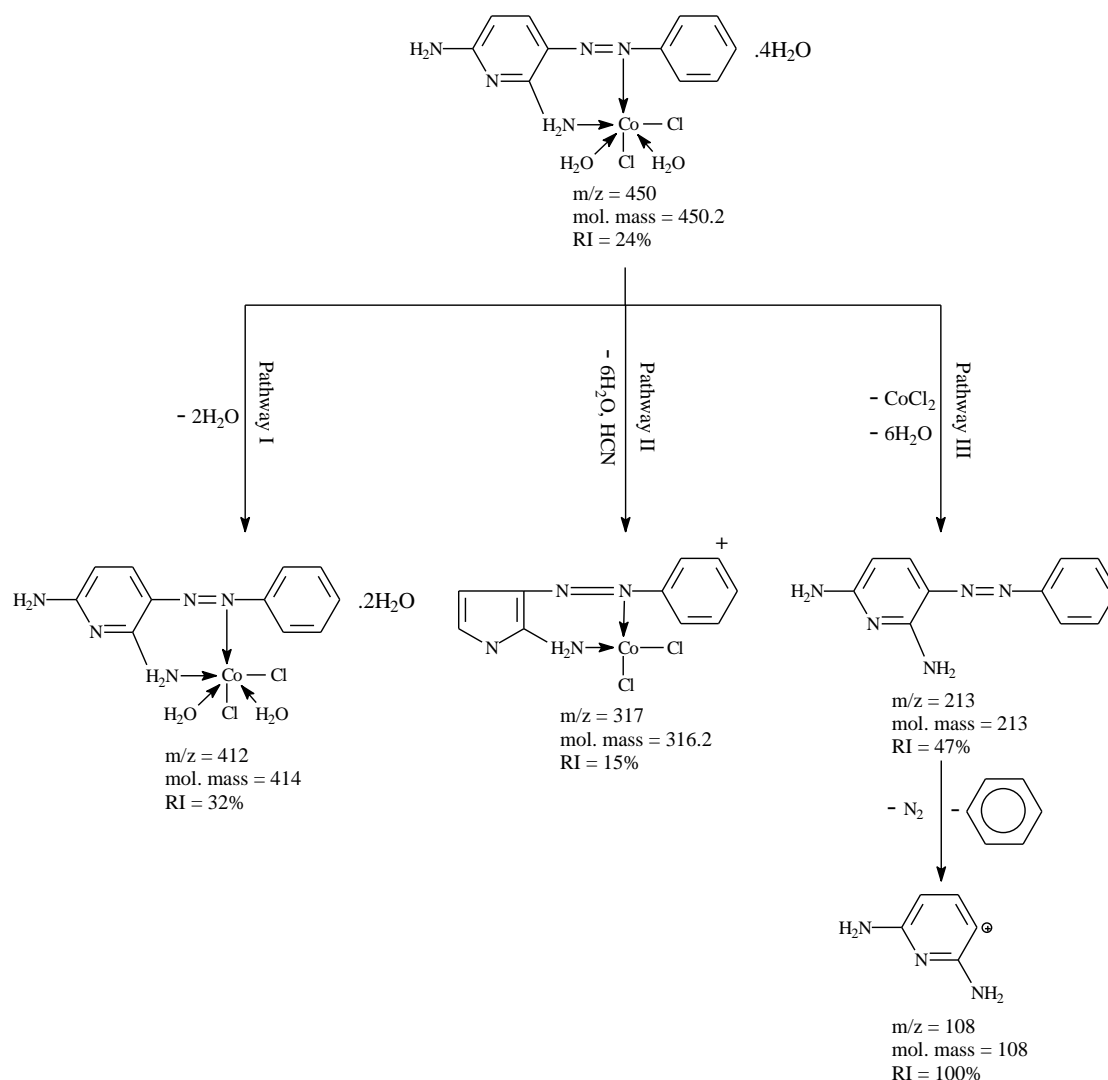
The electron mass spectrum for  $[CoC_{11}H_{11}N_5(H_2O)_2Cl_2]4H_2O$  chelate

consists of three principal pathways presented in scheme (4). The signal at  $m/z = 450$  (mole mass = 450.2, RI = 20%) refers to the main molecular weight. Pathway I, the fragment at  $m/z = 412$  (mole mass = 414, RI = 32%) refers to the loss of two coordinated water. Pathway II show a signal at  $m/z = 317$  (mole

mass=316.2, RI = 15%) which attributed to loss of six molecules of water and HCN. Pathway III shows a signal at  $m/z = 213$  (mole mass= 213,RI = 47%) due to loss of six molecule of water and cobalt chloride. This step is followed by loss of  $-N=N-$  and phenyl ring give signal at  $m/z = 108$  (mole mass=108,RI = 98%).



**Scheme (3):** The mass fragmentation pathways of Cu(II) chelate with  $L_1$

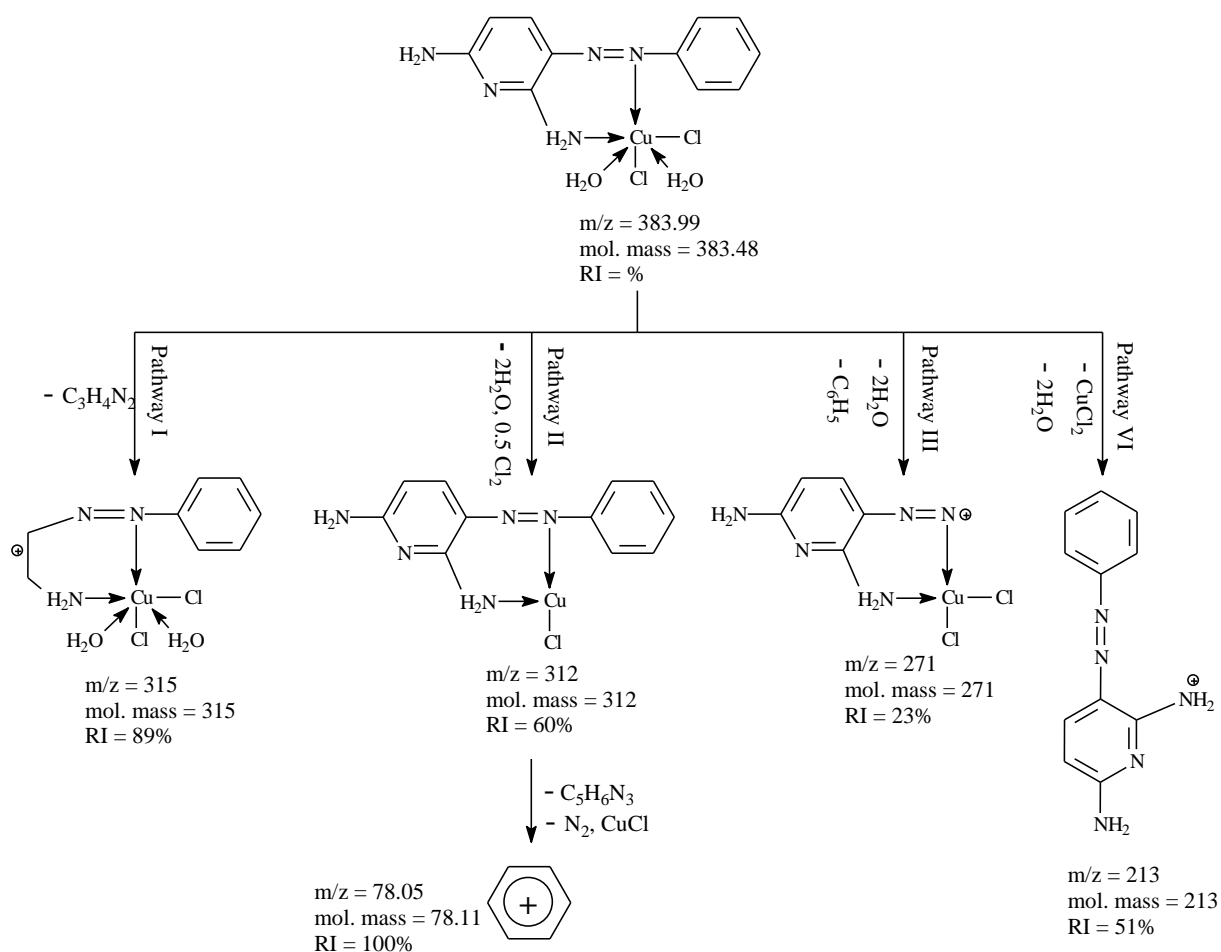


**Scheme (4):** The mass fragmentation pathways of Co(II) chelate with L<sub>2</sub>

### 3.6.5 Mass spectra of [Cu(C<sub>11</sub>H<sub>11</sub>N<sub>5</sub>)Cl<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>]

The mass fragmentation of [Cu(C<sub>11</sub>H<sub>11</sub>N<sub>5</sub>)Cl<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>] chelate consists of four principal pathways presented in scheme(5). The signal at  $m/z = 383.99$  (mole mass = 383.48, RI = 9%) refers to the main molecular weight. Pathway I shows a signal at  $m/z = 315$  (mole mass = 315, RI = 89%) due to loss of C<sub>3</sub>H<sub>4</sub>N<sub>2</sub>. Pathway II, the fragment at  $m/z = 312$

(mole mass = 312.03, RI = 60%) refers to the loss of  $\frac{1}{2}\text{Cl}_2$  and two coordinated water molecules. This step is followed by loss of N<sub>2</sub>, 2, 6-diaminopyridine and CuCl with  $m/z = 78.05$  (mole mass = 78.11, RI = 100%). Pathway III shows signal at  $m/z = 271$  (mole mass = 271, RI, 23%) as loss of two hydrated water molecules and phenyl ring. Pathway IV (RI = 51%) attributed to loss of copper chloride hydrates fragment ion of mole mass = 213.



**Scheme (5):** The mass fragmentation pathways of Cu(II) chelate with  $L_2$

### 3.7. Thermal analyses (TGA)

The TGA thermal analyses data of the synthesized metal chelates are tabulated in Table (3, 4).

#### 3.7.1. TGA of metal chelates of *p*-nitrophenylazo -2, 6 -diaminopyridine

The thermogram of  $[\text{CoC}_{11}\text{H}_{10}\text{N}_6\text{O}_2(\text{H}_2\text{O})_2\text{Cl}_2] \cdot 3\text{H}_2\text{O}$  chelate exhibit four decomposition steps Table (3). First step corresponds to the loss of five molecules of water of hydration within the temperature range 33-100°C with weight loss 18.8% (calcd = 17.8%) then followed by loss of  $\text{NO}_2$  at 100-176°C with mass loss of 8.7% (calcd = 9.6%), the subsequent steps (176 - 976°C) <http://www.aun.edu.eg>

corresponding to remove the organic part of the ligand leaves the metal chloride as residue. The overall weight loss equal 70.2% (calcd = 72.9 %).

TGA curve of  $[\text{Ni}(\text{C}_{11}\text{H}_{10}\text{N}_6\text{O}_2)\text{Cl}_2(\text{H}_2\text{O})_2] \cdot 4\text{H}_2\text{O}$  chelate shows four steps of decomposition within temperature range 47- 669°C. The first step is attributed to the loss of four molecules of water of hydration and  $\text{NH}_3$  in temperature range 47-135°C (estimated mass loss = 17.6%, calcd = 17.9%). The second stage at 138 - 214°C corresponds to the loss of two coordinated water molecules with an estimate mass loss 8.9% (calcd = 7.2%). The third step at 217-329°C corresponds to loss of nitrobenzene with an

**E-mail:** [president@ aun.edu.eg](mailto:president@ aun.edu.eg)

estimated mass loss 24.6% (calcd 18.3%). Step four within the temperature range 327 – 669°C corresponds to removal of organic part of ligand leaving metal chloride.

Four decomposition steps appear in the thermal analysis of  $[\text{Cu}(\text{C}_{11}\text{H}_{10}\text{N}_6\text{O}_2)\text{Cl}_2(\text{H}_2\text{O})_2]$  chelate within the temperature range 31- 1100°C. The first step of decomposition in the range 31– 114°C corresponding to loss of two coordinated water molecules with an estimated mass loss of 8.4% (calcd 10.7%). The second stage for chelate within the temperature range 115–239°C corresponding to loss of  $\text{NO}_2$  group with a mass loss 10.7% (calcd 10.2%). The subsequent steps within temperature ranged listed in Table (3) corresponds to the removal of the organic part of the ligand and chloride ion leaving  $\text{CuCl}$  as a residue.

### 3.7.2. TGA of metal chelates of phenlazo 2, 6- diaminopyridine

The TGA curve of  $[\text{CoC}_{11}\text{H}_{11}\text{N}_5(\text{H}_2\text{O})_2\text{Cl}_2]4\text{H}_2\text{O}$  chelate shows three steps decomposition within

the temperature range 46- 787°C as illustrated in Table (4 ). The first and second steps of decomposition corresponds to loss of six molecules of water of coordination within temperature range 46-238°C with an estimated mass loss = 24.3% (calculated = 23.9%). The third stage at 250-787°C corresponds to removal of the ligand leaving the metal chloride as a residue.

The thermogram of  $[\text{Cu}(\text{C}_{11}\text{H}_{11}\text{N}_5)\text{Cl}_2(\text{H}_2\text{O})_2]$  shows a three-step decomposition that appears within the temperature range 47-812°C. The first step of degradation occurs within the temperature range 47-185°C and corresponds to loss of two molecules of coordinated water with an estimated mass loss of 7.5% (calculated 9.3%). The second stage for the chelate is observed at the temperature range 187-466°C and can be attributed to the loss of a phenyl ring with a weight loss of 20.1% (calculated 20%). The subsequent steps in the temperature ranges correspond to the removal of the organic part of the ligand that leaves metal chloride as the remainder product.

**Table (3).** Thermoanalytical analyses data for synthesized chelates of ligand ( $L_1$ )

Complex	TG range (°C)	Mass loss calcd (Found)%	Assignment
$[\text{CoC}_{11}\text{H}_{10}\text{N}_6\text{O}_2(\text{H}_2\text{O})_2\text{Cl}_2].3\text{H}_2\text{O}$	33 - 100	18.8(17.8)	Loss of $5\text{H}_2\text{O}$
	100 - 176	9.6 (8.7)	Loss of $\text{NO}_2$
	176 - 233	3.5(3.4)	Loss of $\text{NH}_3$
	233 - 853	41(40.3)	Loss of $\text{N}_2$ , $\text{C}_{11}\text{H}_{11}\text{N}_5$
$[\text{Ni}(\text{C}_{11}\text{H}_{10}\text{N}_6\text{O}_2)\text{Cl}_2(\text{H}_2\text{O})_2].4\text{H}_2\text{O}$	47 - 135	17.9(17.6)	Loss of $4\text{H}_2\text{O}$ , $\text{NH}_3$
	138 - 214	7.2(8.9)	Loss of $2\text{H}_2\text{O}$
	217- 329	24.6(18.3)	Loss of $\text{C}_6\text{H}_5$ , $\text{NO}_2$
	327 - 669	24.2(27.6)	Loss of $\text{N}_2$ , $\text{C}_5\text{H}_5\text{N}_2$
$[\text{Cu}(\text{C}_{11}\text{H}_{10}\text{N}_6\text{O}_2)\text{Cl}_2(\text{H}_2\text{O})_2]$	31 - 114	8.4 (10.7)	Loss of $2\text{H}_2\text{O}$
	115- 239	10.7(10.2)	Loss of $\text{NO}_2$
	239 - 489	21.9 (21.6)	Loss of $\text{C}_6\text{H}_5$ , $\text{NH}_3$
	490 - 1100	36.6(39.2)	Loss of $\text{C}_5\text{H}_6\text{N}$ , $\text{N}_2$ and $\text{Cl}$

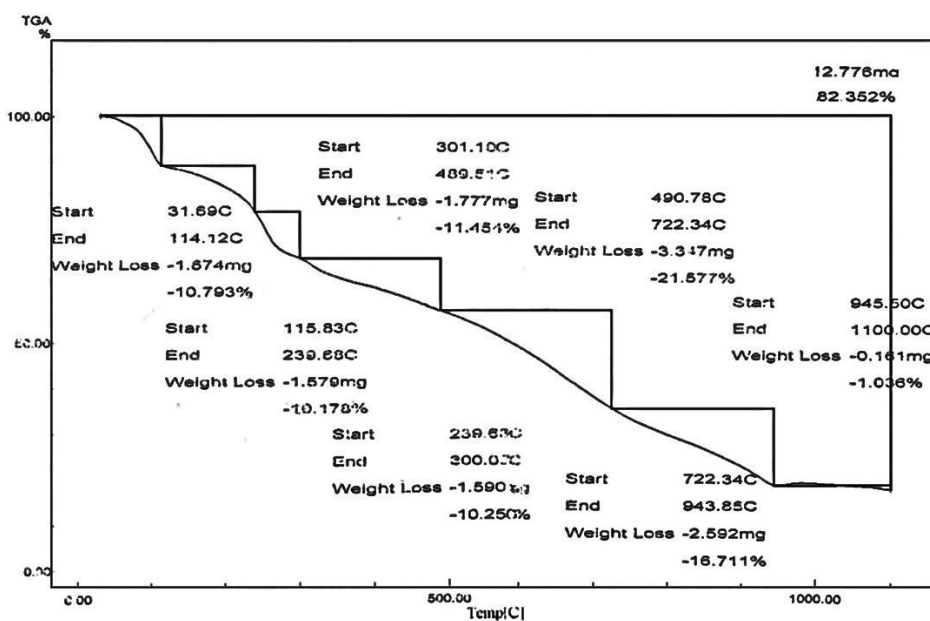


Figure (4): TGA of  $[\text{Cu}(\text{C}_{11}\text{H}_{10}\text{N}_6\text{O}_2)\text{Cl}_2(\text{H}_2\text{O})_2]$

Table (4): Thermoanalytical analyses data of newly synthesized chelates of ligand ( $\text{L}_2$ ).

Complex	TG range (°C)	Mass loss calcd (Found)%	Assignment
$[\text{CoC}_{11}\text{H}_{11}\text{N}_5(\text{H}_2\text{O})_2\text{Cl}_2]4\text{H}_2\text{O}$	46 -127	15.9(15.8)	Loss of $4\text{H}_2\text{O}$
	125- 238	8 (8.5)	Loss of $2\text{H}_2\text{O}$
	250 - 787	47.3 (46.7)	Loss of $\text{C}_{11}\text{H}_{11}\text{N}_5$
$[\text{Cu}(\text{C}_{11}\text{H}_{11}\text{N}_5)\text{Cl}_2(\text{H}_2\text{O})_2]$	47 - 185	9.3(7.50)	Loss of $2\text{H}_2\text{O}$
	187 - 466	20 (20.1)	Loss of $\text{C}_6\text{H}_5$
	467 - 812	44.7(46.3)	Loss of $\text{N}_2$ , $\text{Cl}$ and $\text{C}_5\text{H}_6\text{N}_3$

#### 4. BIOLOGICAL ACTIVITY

Comparison between the biological activity of the synthesized free ligands and their metal complexes with the standards (ampicillin and amphotericin standards for antibacterial and antifungal respectively) towards different organisms were described. The data are listed in Table (5) and shown in Figures (3&4). The free ligands and their metal chelates were

inspected against candida albicans (fungi), Staphylococcus aureus and Bacillus subtilis (G+) and Escherichia coli (G-) to assess their potential antimicrobial agent.

##### 4.1. *p*-nitrophenylazo 2,6diaminopyridine and its complexes

The biological activities of the metal complexes (Table 5) are higher than the free ligands towards gram -positive, gram -negative bacteria and fungi species. In

addition, the biological activity of the complexes follows the order  $\text{Co(II)} > \text{Cu(II)} > \text{Ni(II)}$  against *Bacillus subtilis* and *Escherichia coli* organisms for ( $L_1$ ) and its complexes. At the same time, the biological activity of the complexes follows the order  $\text{Cu(II)} > \text{Co(II)} > \text{Ni(II)}$  against *Staphylococcus aureus*. But with *Candida albicans*, the biological activity follows the order  $\text{Co(II)} > \text{Ni(II)} > \text{Cu(II)}$ .

#### 4.2. Phenylazo 2,6 diaminopyridine and its complexes

The biological activity of the metal complexes of  $L_2$  was higher than that of the free ligand and the biological activity of the follows the order  $\text{Co(II)} > \text{Cu(II)} > \text{Ni(II)}$  against *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* organisms. But with *Candida albicans* the biological activity follows the order  $\text{Ni(II)} > \text{Co(II)} > \text{Cu(II)}$ .

**Table (5):** Biological activity of  $L_1$  and  $L_2$  their metal chelates

Organism sample	Inhibition zone of diameter (mm/mg sample )			
	<i>Staphylococcus Aureus</i>	<i>Bacillus subtilis</i>	<i>Escherichia Coli</i>	<i>Candida albicans</i>
DMSO	0	0	0	0
$L_1$	13	14	10	0
$L_1 + \text{Co(II)}$	19	24	21	19
$L_1 + \text{Ni(II)}$	16	15	15	17
$L_1 + \text{Cu(II)}$	25	23	17	15
$L_2$	13	12	11	0
$L_2 + \text{Co(II)}$	27	26	25	18
$L_2 + \text{Ni(II)}$	16	15	18	20
$L_2 + \text{Cu(II)}$	25	22	19	16



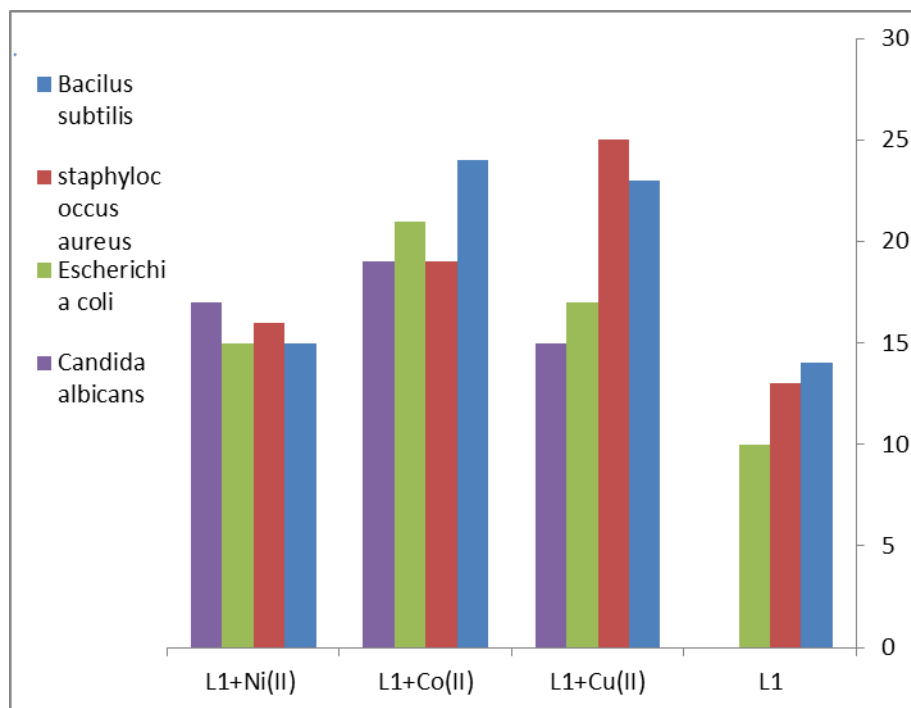


Figure (3): Biological activity of p-nitrophenylazo- 2,6diaminopyridine and its metal complexes.

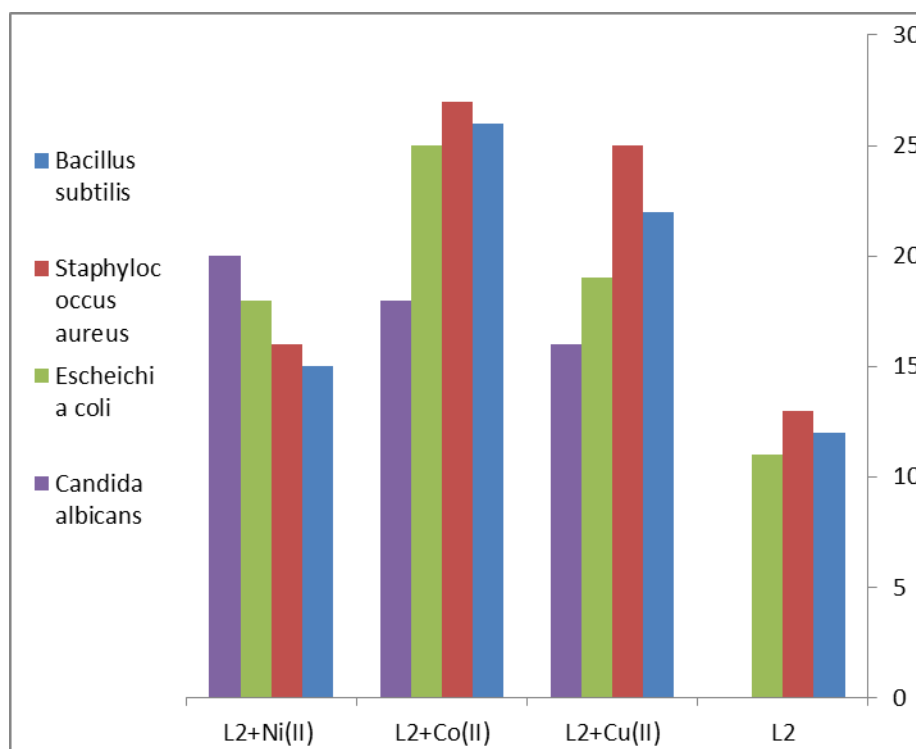


Figure. (4): Biological activity of phenylazo-2, 6diaminopyridine and its metal complexes.

## CONCLUSION

In the present study, the free ligands (L<sub>1</sub>, L<sub>2</sub>) and their Co (II), Ni (II) and Cu (II) complexes were prepared and structurally identified. The structures of the free ligands and their metal chelates were proved by elemental analyses and applying spectroscopic measurements (<sup>1</sup>H-NMR, FT-IR, and mass spectra) and confirmed by thermal analyses. On the basis of their analytical data, we proposed an octahedral geometry for the metal complexes. The synthesized free ligands were found to be biologically active and their metal complexes showed significantly enhanced antibacterial and antifungal activities against microbial strains in comparison to the free ligands.

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**Conflict of interest:** The authors declare that they have no conflict of interest.

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<http://www.aun.edu.eg>

**E-mail:** [president@ aun.edu.eg](mailto:president@ aun.edu.eg)

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