Study Spectroscopic and Characterization of Mixed Ligand Complexes of Ni(II) with New Azo- Schiff-Base Ligand and Its Activity as Antibacterial

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ABSTRACT

The study includes preparation and characterization of mixed azo-linked Schiff-base ligand with Ni(II) complexes. The starting material was isolated from the mixing of 4-amino antipyrine diazonium salt with 4-nitro aniline a 1:1 mole ratio in water. In this work, the formation of azo-linked Schiff-base ligand are reported. The complexes were prepared by mixing the azo-linked Schiff-base ligand as primary ligand, and sodium pyrophosphate as secondary ligands using metal: ligand in (1:1) mole ratio with molecular formula $[Ni(C_{24}H_{20}O_4N_6)Cl_2]$, $[Ni(C_{24}H_{20}O_4N_6)(Pyph)]$. These complexes were characterized by spectral methods FTI.R, (U.V–Vis), mass spectroscopy, molar conductivity and melting point measurements. The complexes product gave square planer geometry.

Keywords :

Nickel

Stoichiometry

syntheses complexes

4-((E)-(2-(((E)-2-hydroxybenzylidene)amino)-5-nitrophenyl)diazenyl)-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one

Validation parameters

Spectrophotometric determination

INTRODUCTION

Azo compounds and Schiff base are important compounds in preparing many of metal complexes for their stability and biological activity[1-3]. Several of nickel complexes with mixed ligands, which showed stability and high efficacy towards many types of bacteria and fungias previous studies of ligand derivatives of the azo-linked Schiff-base of high efficacy have proven to contain two effective groups: azathomethine and azo(4-6).Were prepared the mixed ligand complexes of Ni (II) using 4-((E)-(2-(((E)-2-hydroxybenzylidene)amino)-5-nitrophenyl)diazenyl)-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-

onesodium pyro phosphate. The prepared complexes characterized by elemental analysis, conductance measurement, UV-Visible, FT-IR and Mass spectral.

MATERIALS AND INSTRUMENTATION :

NICKEL (II) chloride $NiCl_2.6H_2O$, 4-amino antipyrine, 4-nitro aniline, 2-hydroxy benzaldehyde, Tetra Sodiumpyrophosphate, potassium dihydrogenphosphate,aceton, DMSO and ethanol were supplied by Aldrich and BDH company. Melting points were measured with an electro thermal (Mettler Toledo MP90 melting points system). Infrared

spectra have been carried out with the use of a Shimadzu, FT.IR-8400S. The compounds' electronic spectra have been recorded with the use of a double-beam (U.V-Vis) spectrophotometer type U.V (Shimadzu-160A), Electrical conductivity measurements were recorder at (25ºC) for (0.001M) solution of the samples in DMSO with the use of (HANNAEC214-Conductivitymeter), , Mass spectra have been recorded with the use of the device LC-MS QP50A: shimadzu(E170Ev).

Synthesis of Azo-schiff ligand ($C_{24}H_{20}O_4N_6$):

4-amino antipyrine (0.5 g, 2.5 mmol) was dissolved in ethanol(10 ml) and hydrochloric acid (2.0 ml) mixture with stirring, a clear solution was obtained. Temperature of (0–5) ^{o}C have been kept, then aqueous solution of sodium nitrite (1g) dissolved in (10 ml) water, was dropwise slowly added, keeping the temperature below 5°C, followed by mixture stirring for half hour, using ice bath the pH was adjusted to 5-6 using potassium dihydrogenphosphate buffer. 4-nitro aniline(0.35g, 2.5 mmol) was dissolved in 10 ml ethanol, cooled by ice bath to (0–5) ^{o}C . the last solution was mixed with cooling with (solution1), the mixture resulted was stir redat (0–5) ^{o}C continually for 1h, the precipitate resulted was then filtered after drying, a reddish brown solid precipitate was obtained, Yield (73.7%), melting point 180°C.

  A solution of the derivative[(E)-4-((2-amino-5-nitrophenyl)diazenyl)-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one] (0.704g, 2 mmol) in 25 ml aceton was mixed with(0.244 g, 2 mmol) of 2-hydroxy benzaldehyde dissolved in (10 ml) ethanol. After adding glacial acetic acid (3-5) drops, the mixture resulted was refluxed for 2h, filtered off, and after washing by ethanol and drying; it was obtained a brown product. The product solid was recrystallized using methanol. Yield: 81%, melting point(193-195°C).

Synthesis of complexes:

1-preparation of[Ni($C_{24}H_{20}O_4N_6$)Cl$_2$] complex:

About 0.5 mmol(0.119g) NiCl$_2$.6H$_2$O, dissolved in 10 mL distilled water and 20ml buffer solution was mixed with 5 mmol (0.228g) of 4-((E)-(2-(((E)-2-hydroxybenzylidene)amino)-5-nitrophenyl)diazenyl)-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one, dissolved in 30 mL acetone. The two solutions were mixed and the pH was adjusted to 6 and refluxed at 65 ^{0}C for 4 h. The solution was cooled and filtered off, washed repeatedly by ethanol and then by dried diethyl ether.

2-preparation of[Ni($C_{24}H_{20}O_4N_6$)(Pyph)] complex:

About 0.5 mmol(0.119g) NiCl$_2$.6H$_2$O, dissolved in 10 mL distilled water and 20ml buffer solution was mixed with 5 mmol (0.228g) of 4-((E)-(2-(((E)-2-hydroxybenzylidene)amino)-5-nitrophenyl)diazenyl)-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one, dissolved in 30 mL acetone, stirring and the pH was adjusted to 6 and the solution was refluxed for half hour at 45ºC. 0.5 mmol (0.223g) of Na$_4$P$_2$O$_7$.10H$_2$O has been added to the mix and refluxed for 4h at 65ºC under constant stirring. The complex was precipitated which has been separated by filtration. The produced complex has been washed thoroughly with ethanol, then with diethyl ether.

3-Determination of linear range

2.0 mL of 1.0 × 10^{-3}M 4-((E)-(2-(((E)-2-hydroxybenzylidene)amino)-5-nitrophenyl) diazenyl)-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one was pipetted into each of ten 10mL volumetric flask containing 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0 mL of Ni(II) (1.0×10^{-3}M). The solutions were made up to 10 mL by phosphate buffer solution of pH (6). After 30 minutes, the absorbance of the solutions were measured at 624 nm and plotted against Ni(II) concentration in □gmL^{-1}.

4-2. Sensitivity of the method

The sensitivity of the method was tested by evaluation of both the limit of detection (LOD), limit of quantification (LOQ) and Sandell's sensitivity [18, 19]. The limit LOD is the lowest amount of the investigated compound in a sample that can be detected, but not necessarily quantified with an acceptable uncertainly. LOD of a method is an important factor if quantitative measurements are to be made at concentrations close to it [18]. The LOD was calculated from the calibration graph of the Ni(II)-$C_{24}H_{20}O_4N_6$system where the intercept (y_B) can be calculated. The following equations were used:

$$SD = \frac{\sqrt{(\sum[yi - \hat{y}]^2)}}{n-2} \qquad (1)$$

$$LOD_{abs} = y_B + 3SD \qquad (2)$$

LOD$_{abs}$ was transferred to LOD value by the equation of the regression line.

$$LOD_{abs} = slope\ (LOD) + y_B \qquad (3)$$

Where yi and $\hat{y}$ are the measured absorbance and the absorbance from the regression equation; respectively. SD is the standard deviation of absorbance from the regression line (equation **2**), LOD$_{abs}$ is the limit of detection of the absorbance, y_B is the intercept of the calibration line and LOD is the detection limit expressed in concentration unit. The limit of quantification (LOQ) is the lowest concentration of compound that can be measured in a sample matrix at an acceptable level of accuracy and precision. The LOQ is generally useful parameter than LOD. The LOQ is relevant only in trace analytical methods when measurements are being at concentrations close to that limit. The LOQ is always higher than the LOD and is often taken as fixed multiple of the LOD (usually double times higher than LOD value). The LOQ is determined by the same way as LOD:

$$LOQ = yB + 10SD \qquad (4)$$

 or calculated directly from the LOD[8]:

$$LOQ = 3.3 \times LOD \qquad (5)$$

Sensitivity of the proposed methods is also determined by calculating Sandell's sensitivity ($\mu g/cm^2/0.001$ Abs unit), which can be defined as smallest weight of

substance that can be detected in column of unit cross section[9].

RESULTS AND DISCUSSION :

Characterization of Azo-schiffligand and Ni(II) Complexes

Solubility:

Solubility of ligand and two prepared complexes has been tested in various solvents, show complete solubility at both(DMSO, DMF, CH_3OCH_3, CH_3CH_2OH) Table1.Some of the physical characteristics have been listed in Table 2.

Molar conductance complexes :

The molar conductance in DMSO solvent of complexes [Ni($C_{24}H_{20}O_4N_6$)Cl_2], [Ni($C_{24}H_{20}O_4N_6$)(Pyph)] lie in the (0.7, 1.7) S.cm^2.mole^{-1} respectively, indicating their non-electrolytic behavior.

FT-IR spectral data:

IR spectral data of ligand and two complexes $C_{24}H_{20}O_4N_6$, [Ni($C_{24}H_{20}O_4N_6$)Cl_2] and [Ni($C_{24}H_{20}O_4N_6$)(Pyph)] summarized in Table 3, Fig1(a,b,c). The IR spectra of two prepared complexes have been compared to the prepared complexes of free ligand. The detected band at 3444 cm^{-1} that was assigned to the stretching frequency of OH group of the free ligand4-((E)-(2-(((E)-2-hydroxybenzylidene)amino)-5-nitrophenyl)diazenyl)-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one, this band was shifted to lower frequency at (3325) cm^{-1} in spectra of two prepared complexes; this shift refers to coordination of OH group via O atom with metal ions Ni(II). The band at 1666 cm^{-1} stretching vibration that denotes for C = N group of free ligand has been shifted to lower frequency at (1647cm^{-1}) in the complexes' spectra, which shows that the coordination between O atom of (C=N) group and metal ions has occurred. In spectra of complex [Ni($C_{24}H_{20}O_4N_6$)(Pyph)] the band shows υ(P = O) appeard at 972 cm^{-1} in spectrum of complex. The change in intensity and shape of band (υP=O) was attributed to coordination of pyrophosphate ion with metal ions Ni(II), via two O atoms.The infrared spectra of Ni(II) complexes showed new two bands that aren't present in the free ligand spectrum, those bands are located at 513, 532 cm^{-1} and at 435 cm^{-1} that are attributed to υ(M-N) and υ(M-O), respectively.

U.V-Vis Spectral data :

The U.v-vis spectral data of ligand and Ni(II) complex were summarized in Table 4, Fig2. In each case the spectrum showed intense absorption peak in the u.v region at (262)nm and (288) nm refers to (*π→π) electronic transition for intra-ligand, at (349) nm and (418) refers to (n*→ π) electronic transition. The spectra of complexes displayed absorption peak at (419)nm (23866) cm^{-1}, (415 nm) (24096cm^{-1}), respectively, which may be attributed to LMCT, while the peak at (598)nm (16722)cm^{-1}, (624)nm(16025)

cm^{-1} in spectra of [Ni($C_{24}H_{20}O_4N_6$)Cl$_2$, [Ni($C_{24}H_{20}O_4N_6$)(Pyph)]complexes respectively, can be assigned to($^3A_2g{\rightarrow}^3T_2g$) (Lever, 1984) respectively. All (d-d) electronic transitions for two Ni(II) complexes [Ni($C_{24}H_{20}O_4N_6$)Cl$_2$, [Ni($C_{24}H_{20}O_4N_6$)(Pyph)] suggest low-spin Square planner geometry about center ion Ni(II) ($4d^8$-configuration).

Mass spectra of ligand and Ni(II) complexes :

Mass spectrum of ligand ($C_{24}H_{20}O_4N_6$) is shown in **Fig. 3 a**. Mass spectrum of this ligand molecular weight equals (456) gave a parent peak at m/z = 455(M+). Mass spectrum of complex [Ni($C_{24}H_{20}O_4N_6$)Cl$_2$ is shown in **Fig 3 b**. Mass spectrum of this complex (molecular weight equals 585.7) gave a parent peak at m/z = 585 (M+). Mass spectrum of complex [Ni($C_{24}H_{20}O_4N_6$)(Pyph)] is shown in **Fig. 3 c**. Mass spectrum of this complex (molecular weight equals 734.7) gave a parent peak at m/z = 733 (M+).

Linear range

The linear range of Ni(II)-$C_{24}H_{20}O_4N_6$complex wasevaluated by measuring the absorbance of Ni-$C_{24}H_{20}O_4N_6$ complex at different concentration of Ni(II) (0.00-12.0 □g/mL) and fixed concentration of $C_{24}H_{20}O_4N_6$ (4.65□gmL^{-1}) at 624 nm; **Fig. 4** The relation between absorbance and Ni(II) concentration was found to be linear in the concentration range 0.58-8.5□g/mL.(linear range). The linearity of calibration graphs was proved by the high values of the correlation coefficient (R^2= 0.9959) and the small value of the intercept (0.0593). The apparent molar absorptivity of the Ni(II)- $C_{24}H_{20}O_4N_6$ complex at 624 nm was evaluated (0.602 X 10^3 Lmol^{-1}cm^{-1}).

Sensitivity of the proposed Method

The sensitivity of the method was tested by evaluation of both the limit of detection (LOD), limit of quantification (LOQ) and Sandell's sensitivity [7-9]. The LOD was calculated from the calibration graph of the Ni(II)- $C_{24}H_{20}O_4N_6$ system where the intercept (y_B) can be calculated. LOD, calculated from y_B + 3S$_B$ (99% confidence)was (0.0651□gmL^{-1}). The LOQ is determined by the same way as LOD. Where LOQ = yB + 10SD (see Appendix 1 or the experimental section), or calculated directly from the LOD (LOQ = 3.3 × LOD) . LOQ of Ni(II) using $C_{24}H_{20}O_4N_6$ was calculated from the LOD to be : LOQ = (0.07856□gmL^{-1}). The Sensitivity of the proposed methods is also determined by calculating Sandell's sensitivity (μg/cm^2/0.001 Abs unit), which can be defined as smallest weight of substance that can be detected in column of unit cross section[8,10].Sandell's sensitivity was evaluated and found to be 0.0963□g.cm^{-2}. The values of LOD, LOQ and Sandell's sensitivity are very low which reflect the high sensitivity of the proposed method.

Accuracy and Precision

Accuracy is an important requirement of analytical methods. It can be defined as the closeness between the true or accepted reference value and the obtained value [8]. The results summarized in **Table.6** show high accuracy of the proposed method, as indicated by the percentage recovery (97.27%-100.4%) and %Er (0.4-2.7%) values. On the other hand, precision is a measure of how close results are to one another. Precision also expressed as the closeness of agreement between independent test results obtained under stipulated conditions. Precision usually expressed as standard or relative standard deviations of the replicate analysis [10-16]. Hence the precision of the proposed spectrophotometric method using the Ni-$C_{24}H_{20}O_4N_6$ complexes investigation was measured as percentage relative standard deviation (RSD%). Inter-day (reproducibility) and intra-day (repeatability); **Table.6**

The relative standard deviations were found to be very small (0.3-1.5)% indicating reasonable repeatability and reproducibility of the proposed method[15-21]; **Table.6.**

The antibacterial activity

Table.7 explain the comparison between the inhibition of control(4-((E)-(2-(((E)-2-hydroxybenzylidene)amino)-5-nitrophenyl)diazenyl)-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one) and the inhibition of complexes ($C_{11}$and C_{12})

The role of these compounds may be linked and destroyed the cell wall of microbes or stopped replication of microbial DNA [22, 23]. The differences in the inhibitory effect related to the chemical synthesis of each compound.

The suggested structural formula of prepared Ni(II) complexes

According to the results obtained frommolar conductance, FTIR, U.V-Vis, and Lc-Mass, the suggested structural formula of complexes were shown in **Table 5**.

Table 1 : Solubility of ligand and Ni (II) complexes

No	Compounds	DMSO	DMF	Aceton	C_2H_5OH
1	$C_{24}H_{20}O_4N_6$	+	+	+	0
2	[Ni($C_{24}H_{20}O_4N_6$)Cl_2]	+	+	+	0
3	[Ni($C_{24}H_{20}O_4N_6$)(Pyph)]	+	+	+	0

Where: (+) = Soluble and () = sparingly soluble

Table 2: Some physical properties of Ni (II) complexes

Complexes	M-wt g/mol	Yield%	Colour	m.p.°C
$C_{24}H_{20}O_4N_6$	456	75	Brown	193-195
[Ni($C_{24}H_{20}O_4N_6$)Cl$_2$]	585.7	88	Blackish brown	220-223
[Ni($C_{24}H_{20}O_4N_6$)(Pyph)]	734.7	92	Blackish brown	227-230

Table 3 : Important IR spectral data of complexes

No	OH	C=O	N=N	P=O	C=N	M-N	M-O
L	3444	1678	1404	-----	1666	-----	-----
1	3325	1678	1404	-----	1647	513	-----
2	3325	1678	1404	972	1647	532	435

Table 4 : Electronic spectral data of Ni (II) complexes

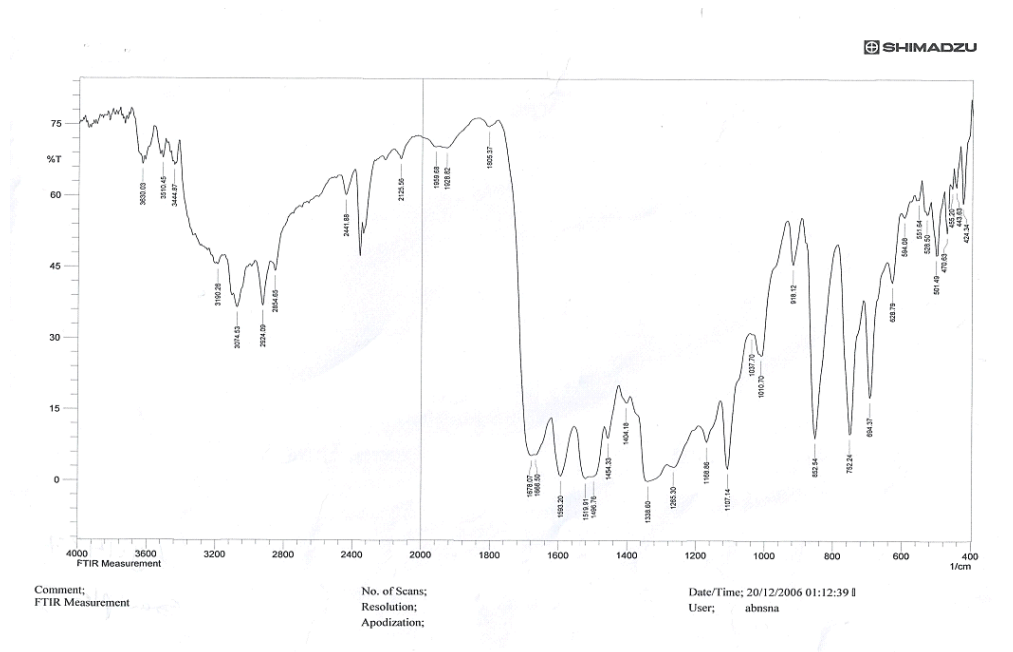| No.	Compounds	λmax nm	Wave number	Transition Assignment
1	Ligand(**L**)	262 288 349 418	38167 34722 28653 23923	$\pi{\rightarrow}\pi^*$ $\pi{\rightarrow}\pi^*$ $* \pi{\rightarrow}n$ $* \pi{\rightarrow}n$
2	[Ni($C_{24}H_{20}O_4N_6$)Cl$_2$](**C$_{11}$**)	261 276 320 419 598	38314 36231 31250 23866 16722	$\pi{\rightarrow}\pi^*$ $\pi{\rightarrow}\pi^*$ $* \pi{\rightarrow}n$ C.T $^3A_2g{\rightarrow}^3T_2g$
3	[Ni($C_{24}H_{20}O_4N_6$)(Pyph)](**C$_{12}$**)	261 279 336 415 624	38314 35842 29761 24096 16025	$\pi{\rightarrow}\pi^*$ $\pi{\rightarrow}\pi^*$ $* \pi{\rightarrow}n$ C.T $^3A_2g{\rightarrow}^3T_2g$

Figure 1a. FT-IR spectrum of ligand (4-((E)-(2-(((E)-2-hydroxybenzylidene)amino)-5-nitrophenyl)diazenyl)-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one)
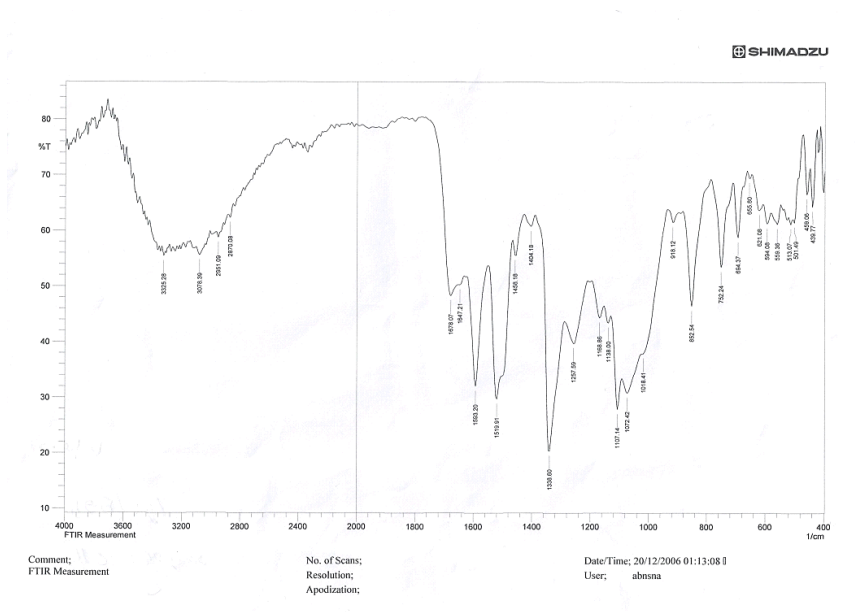

Figure 1b. FT-IR spectrum of [Ni($C_{24}H_{20}O_4N_6$)Cl$_2$
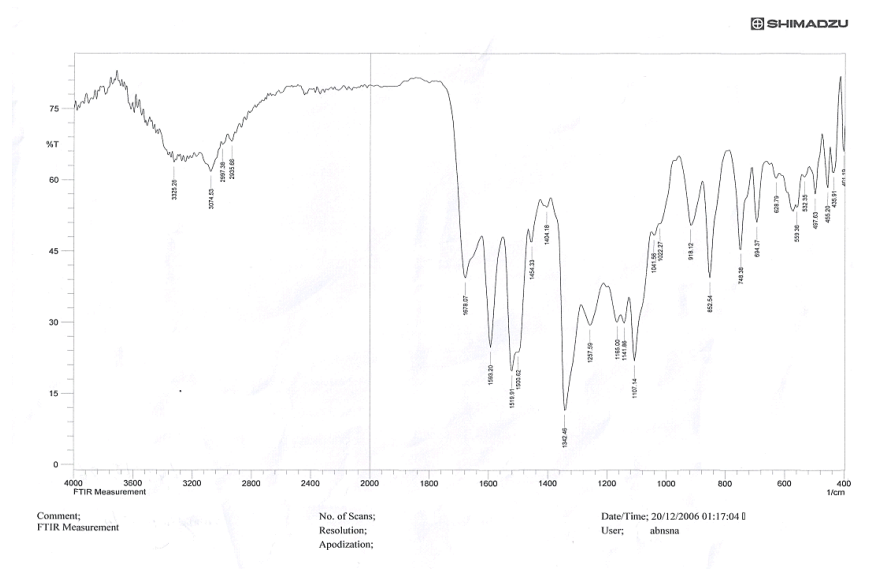
Figure 1c. FT-IR spectrum of [Ni(C$_{24}$H$_{20}$O$_{4}$N$_{6}$)(Pyph)]
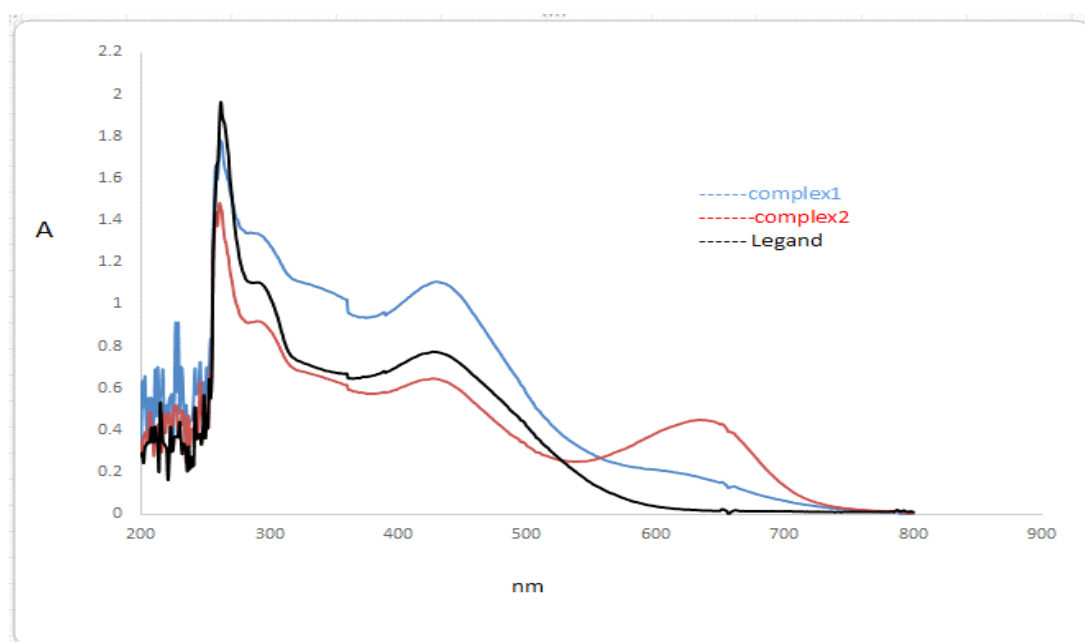
Fig2 : Electronic spectrum of ligand and complexes
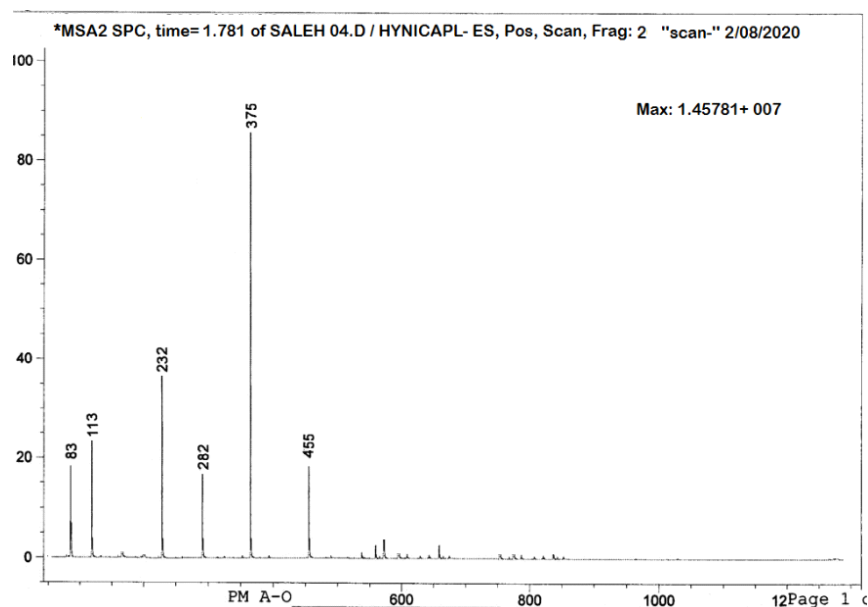
Fig3. :Mass spectrum of ligand [$C_{24}H_{20}O_4N_6$]
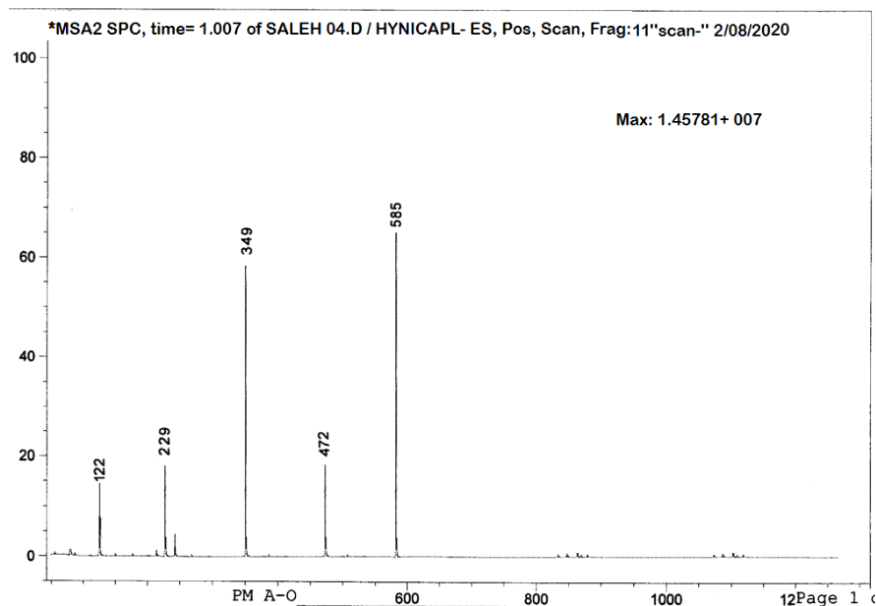
Fig3.a :Mass spectrum of [Ni($C_{24}H_{20}O_4N_6$)Cl_2]
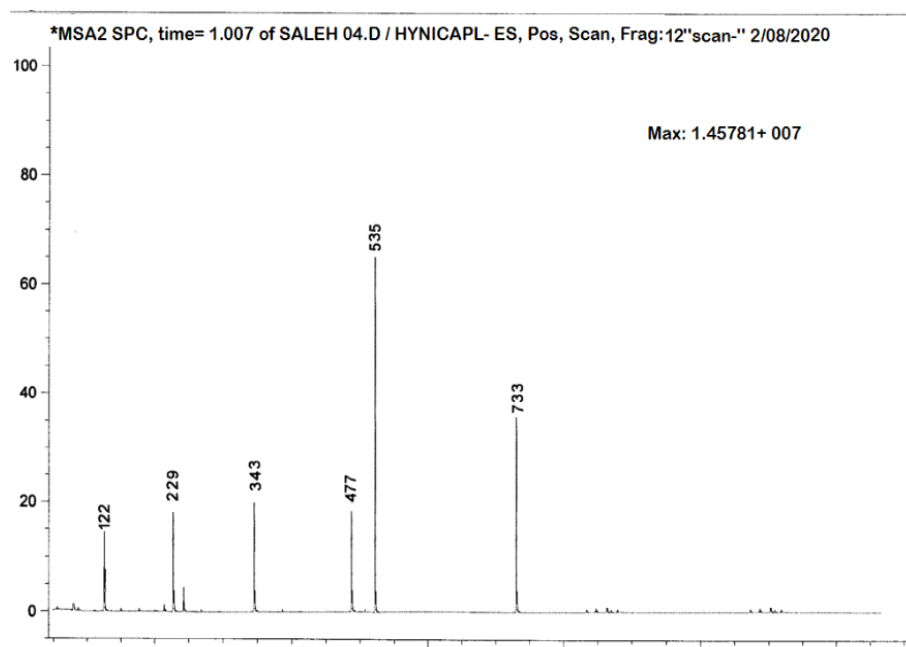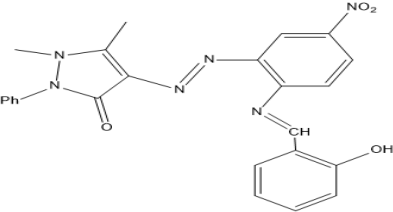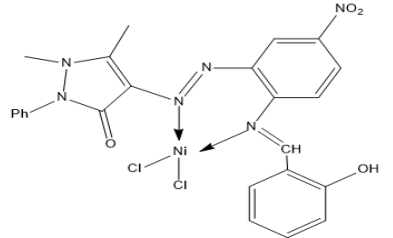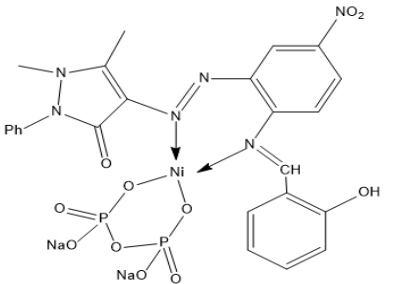
Fig3.c :Mass spectrum of [Ni($C_{24}H_{20}O_4N_6$)(Pyph)]
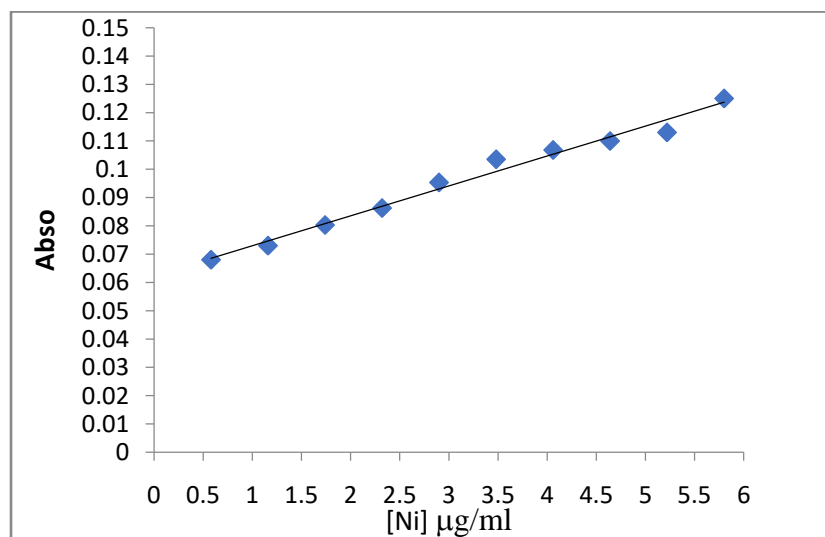
Fig4. Relationship of the absorbance of complex Ni (II) and ($C_{24}H_{20}O_4N_6$) by increasing concentration of Ni (II) in excess ($C_{24}H_{20}O_4N_6$) solution

Table 5 :The suggested structural formula of prepared Ni (II) Complexes

No.	Complexes molecular formula	The molecular structure
1- (L)	$C_{24}H_{20}O_4N_6$ 4-((E)-(2-(((E)-2-hydroxybenzylidene)amino)-5-nitrophenyl)diazenyl)-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one	
2- (C$_{11}$)	[Ni($C_{24}H_{20}O_4N_6$)Cl$_2$] (dichloro(4-((E)-(2-(((E)-2-hydroxybenzylidene)amino)-5-nitrophenyl)diazenyl)-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one)) Nickel(II)	
3-(C$_{12}$)	[Ni(C$_{24}H_{20}O_4N_6$)(Pyph)] (pyrophospheto(4-((E)-(2-(((E)-2-hydroxybenzylidene)amino)-5-nitrophenyl)diazenyl)-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one)) Nickel(II)	

Table(6)Analytical parameters (Using (4-((E)-(2-(((E)-2-hydroxybenzylidene)amino)-5-nitrophenyl)diazenyl)-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one)) -Reagent to detrmenation of Ni (II).

| Parameter	Value
$\square_{max}$ nm	624
Molar Absorptivity (Lmol^{-1}cm^{-1})	0.602×10^3
Limit of Detection (µg mL^{-1})	0.0651
Limit of Quantification (µg mL^{-1})	0.07856
pH	6
Slope (mL$\square$g^{-1}cm^{-1})	0.0105
Intercept	0.0593
Correlation Coefficient (R^2)	0.995
Shandell's Sensitivity (µg cm^{-2})	0.0693
SD	0.0019
% RSD	(2.51-5.9)%
% Recovery	(97.25-100.4)%
% Er	(0.3-2.7)%

Table 7. Diameter zone of inhibition (mm) of the(4-((E)-(2-(((E)-2-hydroxybenzylidene)amino)-5-nitrophenyl)diazenyl)-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one) complexes

Comp	Isolated	$1*10^{-3}$	$1*10^{-4}$	$1*10^{-5}$
L	*E. Coli*	5mm	5mm	7mm
L	*S. aureus*	-ve	-ve	9mm
C$_{11}$	*E. Coli*	7mm	11mm	12mm
C$_{11}$	*S. aureus*	9mm	11mm	14mm
C$_{12}$	*E. Coli*	10mm	8mm	15mm
C$_{12}$	*S. aureus*	7mm	8mm	10mm

L:4-((E)-(2-(((E)-2-hydroxybenzylidene)amino)-5-nitrophenyl)diazenyl)-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one

C$_{11}$:(dichloro(4-((E)-(2-(((E)-2-hydroxybenzylidene)amino)-5-nitrophenyl)diazenyl)-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one)) Nickel(II)

C$_{12}$:   (pyrophospheto(4-((E)-(2-(((E)-2-hydroxybenzylidene)amino)-5-nitrophenyl)diazenyl)-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one)) Nickel(II)
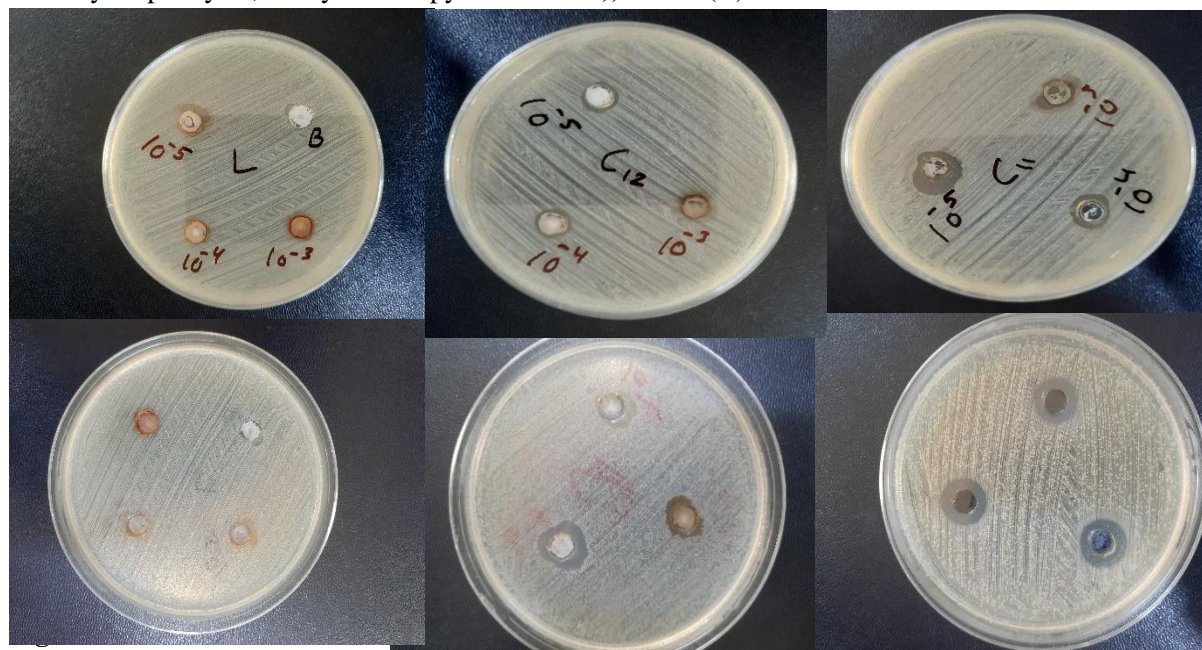
aureus

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