وقائع المؤتمر العلمي الثالث لكلية العلوم جامعة ديالى ٦-٢٠١٢/٥/٧

DIYALA JOURNAL FOR PURE SCIENCES

Synthesis and Characterization of new Tridentate Schiff Base

Phenyl-2-(2-hydroxybenzylidenamino)benzoate and its complexes with some metals Ions

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Key word Schiff base , benzyliden , salicylaldehyde, benzoate

The aim of this work is synthesis and characterization of the tridentate Schiff base ligand (HL) containing (N and O) as donor atoms type (ONO. (HL) phenyl 2-(2-hydroxybenzylidenamino)benzoate

This ligand was prepared by the reaction of (phenyl- 2-aminobenzoate) with salicylaldehyde under reflux in ethanol and few drops of glacial acetic acid which gave the ligand (HL).

The prepared ligand was characterized by (FT IR,UV–Vis) spectroscopy, Elemental analysis of carbon, hydrogen and nitrogen (C.H.N.) and melting point.

The ligand was reacted with some metal ions under reflux in ethanol with (1 metal :2 ligand)mole ratio which gave complexes of the general formula:

$[M(L)_2]$, M = Mn^{II}, Fe^{II} , Co^{II}, Ni^{II}, Cu^{II}, Zn^{II} , Pd^{II}, Cd^{II}, Hg^{II}

The Products were found to be solid crystalline complexes, which have been characterized through the following techniques:

Molar conductivity .Spectroscopic method [FTIR and UV-Vis], additional measurement magnetic susceptibility, Chlorid content and Program [Chem office–CS. Chem.–3D pro 2006]was used.

Our research also includes studying the bio–activity of the some compounds prepared against a kind of bacteria three of which were negative to gram dye (Proteus mirabilis, *Klebsiella pneumonia, Escherichia coli*), and one was positive to gram dye *(Staphylococcus aureus*). Some of the compound showed inhibitive activity against some of bacteria under consideration.The magnetic moment coupled with the electronic spectra suggested an octahedral geometry for all the complexes.

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تحضير وتشخيص قاعدة شيف جديدة ثلاثية السن phenyl-2-(2-hydroxybenzylidenamino)benzoate ومعقداتها مع بعض الأيونات الفلزية

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جامعة بغداد / كلية التربية - ابن الهيثم / قسم الكيمياء

بغداد – الاعظمية

كلمات مفتاحية :

قاعدة شيف ، بنزيليدين ، بنزويت ، سالسلدهايد

الخلاصـــة

يتضمن البحث تحضير وتشخيص الليكاند الجديد (HL) قاعدة شيف ثلاثية السن الحاوية على النتروجين والأوكسجين كذرات واهبة للالكترونات نوع (ONO).

الليكاند: phenyl-2-(2-hydroxybenzylidenamino) benzoate(HL)

حضر هذا الليكاند من خلال تفاعل محلول من السالسالديهايد مع فنيل- ٢-امينو بنزويت تحت التصعيد الرجوعي في الايثانول وقطرات من حامض الخليك الثلجي. تم تشخيص هذا الليكاند بوساطة طيف الأشعة تحت الحمراء (FTIR)وطيف الاشعة المرئية-فوق البنفسجية(UV–Vis)وقياسات درجة الانصهاروالتحليل الدقيق للعناصر (C.H.N).

تم مفاعلة الليكاند مع مجموعة من ايونات العناصر الفلزية تحت التصعيد الرجوعي في الايثانول بنسبة مولية (١:٢ فلز – ليكاند) إذ أعطى التفاعل المعقدات ذوات الصيغ العامة[$M(L)_2$:]

أذ أن $M = Mn^{II}, Fe^{II}, Co^{II}, Ni^{II}, Cu^{II}, Zn^{II}, Pd^{II}, Cd^{II}, Hg^{II}$

المعقدات المحضرة بلورات صلبة درست من النواحي الآتية: الاستقرارية الحرارية، التوصيلية الكهربائية المولارية، الذوبانية، تقدير النسبة المئوية للأيون الفلزي في المعقدات بوساطة مطيافية الامتصاص الذري، الدراسات الطيفية: وتضمنت أطياف (الأشعة تحت الحمراء، الأشعة فوق البنفسجية– المرئية، الخواص المغناطيسية ومحتوى الكلور) مع استعمال البرنامج .

(Chem Office– Cs. chem– 3D pro 2006) في رسم اشكال المعقدات.

كما تم دراسة الفعالية البايولوجية ضد بعض أنواع البكتريا ثلاثة منها سالبة لصبغة كرام : (Gram negative) وهي

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واحدة موجبة لصبغة كرام Gram *(Proteus mirabilis,Klebsiella pneumonia, Escherichia coli)* أظهر قسم من المركبات المحضرة فعالية تثبيطية ضد بعض أنواع positive تمثل (*Staphylococcus aurens)* البكتريا قيد الدرس. قيم العزوم المغناطيسية والأطياف الالكترونية لجميع المعقدات دلت على أن جميع المعقدات لها بنية ثماني السطوح.

Introduction: 1.

Metal complexes of Schiff bases are extensively studied due to synthetic flexibility, selectivity and sensitivity towards a variety of metal atoms [1].They are found useful in catalysis, in medicine as antibiotics and anti-inflammatory agents and in the industry as anticorrosion [2-5].Among the ligands the linear or cyclic Schiff bases obtained by the condensation of primary amines with carbonyl compounds and their metal complexes find a variety of applications including biological, clinical, analytical and industrial, in addition to their important role in catalysis and organic synthesis [6,7].The central metal ion in these complexes acts as active sites and thereby successfully catalyzes chemical reactions [8].Metallic and luminescence probes with microsecond decay time have numerous potential applications in the biophysical and clinical sciences. Ruthenium(II) MLCT compounds display long luminescence life time and are extremely photo stable [9,10].

2-(8-hydroxyquinolinyl)-5-aminomethyl]-3-(4-chlorophenyl)-3(H)-quinazolin-4-one ligand called HACQ(HL4) was studied. Anthranilic acid was converted into N- chloroacetyl anthranilic acid and then to 2-chloromethyl–3(4-chloro phenyl)-3(H)-quinazoline-4-one.This compound was finally condensed with 5-amino-8-hydroxyquinoline for the preparation of this HACQ ligand. The transition metal chelates of Cu(II), Ni(II), Zn(II), Mn(II) and Co(II) of this ligand HACQ were prepared and characterized by Metal-Ligand (M:L) ratio[11].

The tridentate of benzhydrazone derivatives ligand containing ONO donor atoms can be synthesized easily by reacting benzhydrazide with any aldehyde or ketone. [12]

This paper reports the synthesis and characterization of a new ligand derived from the reaction of Salicylaldehyde and phenyl-2-amino benzoate and its complexes.

2. Experimental

All chemicals used were of reagent grade (supplied by either Merck or Fluka , and used as supplied.

2.1. Chemicals and Instrumentals

a -Metal salts ($MnCl_2.4H_2O$,$FeCl_2.4H_2O$, $CoCl_2.6H_2O$, $NiCl_2.6H_2O$, $CuCl_2.2H_2O$, $ZnCl_2$,$PdCl_2$,$dCl_2.2H_2O$,and $HgCl_2$.Salicylaldehydes, 2-phenyl-amino benzoate ($C_{13}H_{11}NO_2$) , ethanol, methanol and dimethylforamaide and KBr from (BDH).

Instrumentation

UV-Vis spectra were recorded on a (Shimadzu UV- 160A) Ultra Violet-Visible Spectrophotometer. IR- spectra were taken on a (Shimadzu, FTI R- 8400S) Fourier Transform Infrared Spectrophotometer (4000- 400) cm^{-1} with samples prepared as KBr discs.. Microelemental analysis (C.H.N) were performed in Al-Mustansiriyah university – college of science While metal contents of the complexes were determined by atomic absorption(A.A)technique using a Shimadzu AA 680G atomic absorption spectrophotometer.. Conductivities were measured for 10^{-3}M of complexes in ethanol at 25°C using (Philips PW- Digital Conductimeter). Magnetic measurements were recorded on a Bruker BM6 instrument at 298°K following the Farady's method . In addition melting points were obtained using (Stuart Melting Point Apparatus). The proposed molecular structure of the complexes were drawing by using chem. office prog, 3DX (2006).

2.2Preparation of Schiff Base:

The Schiff base was Prepared by standard method (Figure. 1), In a round bottom flask .The Schiff base phenyl-2-(2- hydroxyl benzyliden amino) benzoate was prepared by adding 25cm^3 of Salicylaldehyde ethanolic solution (0.73gm; 6m mol) to the same volume of ethanolic solution of phenyl-2-amino benzoate (1.28 gm; 6m mol). The mixture was stirred for 3hrs at (65° C). The resulting solution was evaporated under vacuum to remove the solvent. The Product was collected by filtration, washed several times with ethanol and recrystallized from hot ethanol and air dried . The melting point of the product found to be 170 °C The color of the product is orange. 94.14 %. Anal. Calcd for ligand(HL) C = 33.03%, H = 5.19%, N = 10.27%; Found: C = 33.23%, H = 5.34%, N = 10.21%.

2.3.General Method for Preparation of the Complexes

All complexes were prepared by dissolving (0.634gm; 2mmole) of phenyl-2-(2-hydroxybenzylidenamino)benzoate(HL)in (30ml)ethanol.The solution was added gradually with stirring to(20ml)solution of the respective metal(II) chloride (1mmole). The mixture was stirred for(2-3)hrs at 65° C).The resulting solution was evaporated under vacuum to remove the solvent. the colored complexes separated out in each case. The Product was filtered, washed several times with ethanol and recrystallized from hot ethanol and air dried at room temperature .

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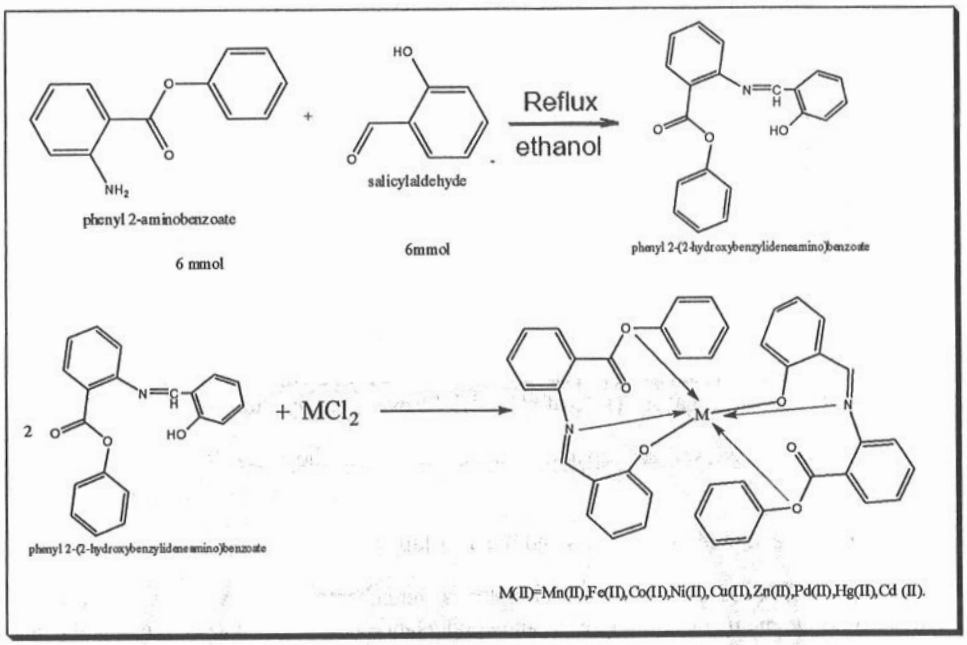

(Figure. 1)Schematic representation of synthesis of HL ligand and its complexes

2.4. Antimicrobial activities

Antimicrobial activities of the ligand and the complexes have been carried out against the pathogenic bacteria like Escherichia coli, Bacillus subtilis, Staphylococcus aureus, and Pseudomonas aeruginosa, using nutrient agar medium by disc diffusion method8. The test solution were prepared in DMSO and soaked in filter paper of 5 mm diameter and 1mm thickness. These discs were placed on the already seeded plates and incubated at 37 °C for 24 h. The diameters (mm) of the inhibition zone around each disc were measured after 24hours. Streptomycin was used as standard. [12,13] .

3. Results and Discussion.

phenyl-2-(2-hydroxybenzylidenamino)benzoate(HL)(Figure. 2)*was* prepared by reacting equimolar amounts of Salicylaldehyde and phenyl-2-amino benzoate in ethanol.

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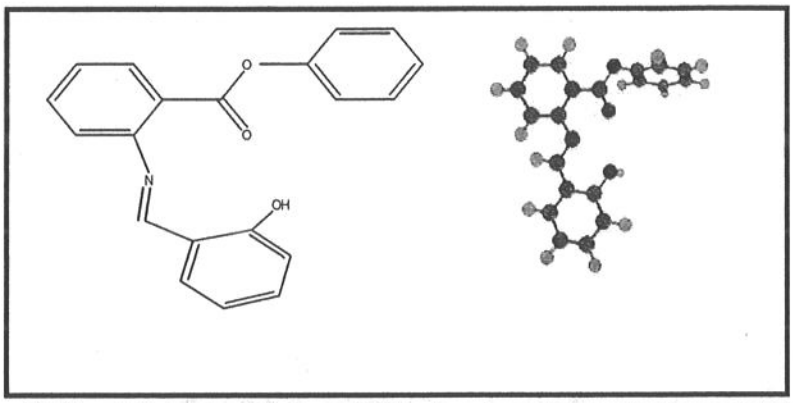

(Figure. 2) Chemical structure of phenyl-2-(2-hydroxybenzylidenamino)benzoate (HL)

The complexes were prepared by direct reaction of the ligand (HL) with the metal (II) chlorides in ethanol.

*3.*1.Characterization of Schiff bases and metal complexes:

The Schiff base ligands and their complexes were characterized by using, FT-IR, UV-Vis spectroscopy , magnetic susceptibility and conductance measurements and elemental analysis for Schiff base only

3.2. Physical properties of the prepared complexes:

Table (1) shows the physical data for the prepared complexes which show different melting points, All complexes are colored, non-hygroscopic and thermally stable solids indicating a strong metal-ligand bond. The complexes are insoluble in water and benzene but soluble in common organic solvents such as ethanol, methanol , acetone, chloroform ,DMF and DMSO.

*3.*3. Atomic Absorption :

The atomic absorption measurements (Table-1) for all complexes gave approximated values for theoretical values.

3.4. Molar Conductance :

The observed molar conductance (Table 1) values measured in ethanol in 10^{-3}M solution lie in the

(2.38-14.6) Ω^{-1} cm^2 mol^{-1} range, indicted that the complexes are non-electrolytes[14]

3.5.Fourier transform infrared spectra:

Infrared Spectral measurement: Infrared spectral data have been reported by research groups working on the synthesis of Schiff bases their metal complexes. The characteristic bands in Schiff base are used to determine the binding modes which found in the system are υ (C=N), υ (O-H), υ (C-O-C)(M-O), υ (M-N).

A summary of characteristics IR bands is shown in Table 2 (Figure. 3). Generally, the band at 1640 cm^{-1} are assigned to the existence of υ (HC=N-) group of the azomethine for ligand and in complexes are in the range of 1620-1625 cm^{-1}.This band gets shifted to lower frequency in the complexes, indicating the coordination through azomethine nitrogen to metal atom [15-16].υ(C=O) in the ligand and it's complexes appear at 1659 cm^{-1}. The band at3039cm^{-1} assigned to υ (O-H) in the IR spectrum of ligand and complexes. At range (3232-3549) C^{-1}. The IR spectrum of free ligand [HL] appered a band at(1240 cm^{-1}) refers to the stretching frequency of υ(C- O) phenolic group which splited at range (1153-1130 cm^{-1}) and shifted at range (1230-1220 cm^{-1}) to lower frequency in IR spectra for all complexes , this shifting indicated the coordination between O atom of phenlic group and metal ion [17]. Finally , the stretching frequency of ester group υ (C-O-C) asym. Was shifted to high frequency in IR spectra for all complexes at range (1261-1265) when it comparison with that in free ligand . This shift refers to coordinate between (C-O-C) through O atom with metal ions .In the IR spectra of prepared complexes the new band at (509 -524) and (435-447) cm^{-1} due to υ (M-N) and υ (M-O) vibrations respectively. [18,19]

3.6. *Electronic spectra & Magnetic studies* :

The UV-Vis spectral data of the free ligands (HL) and all metal complexes are listed in (Table-3).The UV-Vis spectrum of the ligand (HL) (Figure. 4) shows three peaks at 235 nm (42553 cm^{-1}), 299 nm(33445 cm^{-1}) and 358 nm (28169 cm^{-1})assigned to ($\pi - \pi^*$), ($n - \pi^*$) , and, ($n - \pi^*$) electronic transitions respectively). The two absorption peaks in the region(223-291)and the peak at range (345-355)nm for all complexes assigned to ligand field (L-F),and charge transfer (C-T) respectively), while the peaks in the region (685-943) nm which assigned to (d-d) electronic transition which are a good evidence for octahedral geometry about M (II) ion for all complexes [20 -23]

The measured magnetic moment (μ_{eff}) for Mn(II) , Fe(II) , Co(II) , Ni(II) and Cu(II) complexes are shown in Table (3) , All prepared complexes exhibit magnetic moment values which can be a normal values for high-spin complexex when it compared with that has been found in literature [24].

3.7. The composition of the complexes

The composition of the complexes formed in solution has been established by job method , .In this cases the results reveals (1:2) metal to ligand ratio. A chosen plots of were represented in (Figure. 5). (Table-4) summarize the results obtained as a conditions for the preparation of the complex Ni(II) with HL.

3.8. Antibacterial Activities:

Antibacterial activity of ligand and [$Pd(L)_2$], [$Hg(L)_2$]and[$Cd(L)_2$]

complexes are recorded in Table ٥. (Figure. 6) It has been observed that the metal complex have a high activity than ligand against same organisms under the identical experimental condition.

It is evident from the above data that the antibacterial activity significantly increased on coordination. This enhancement in the activity may be rationalized on the basis of their structures mainly possessing an additional azomethine bond. It has been suggested that the ligands with nitrogen and oxygen donor systems inhibit enzyme activity. Coordination reduces the polarity of the metal ion mainly because of the partial sharing of its positive charge with the donor groups within the chelate ring system..[23, 25]

4.Conclusion:

On the bases of elemental analysis, molar conductivity , magnetic moment , chloride content measurements and spectroscopic studies (IR, U.V-vis.and Atomic Absorption) for the ligand (HL) and all prepared complexes , we suggested that the ligand (HL) behaves as tridentate on complexation with metal ions via N of (-C=N) group , phenolic O atom and O atom for ester group C-O-C . The suggested structure for all prepared complexes are octahedral geometry .

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Chemical Formula $L=C_{20}H_{14}NO_3$	Formula Weight $g \cdot mol^{-1}$	Color	Melting Point (°C)	Λm $\Omega^{-1} cm^2 mol^{-1}$	Metal % Theory	Metal% Exp	Cl%
$[Mn(L)_2]$	687	Green	173	8.94	8.00	7.68	Nill
$[Fe(L)_2]$	688	Yellowish green	163	14.6	8.13	7.94	Nill
$[Co(L)_2]$	690	Pale brown	188	13	8.40	8.02	Nill
$[Ni(L)_2]$	691	Green	170	8.26	8.53	7.86	Nill
$[Cu(L)_2]$	695.5	Green	154	11.62	9.13	8.86	Nill
$[Zn(L)_2]$	697.4	Pale yellow	169	7.8	9.37	8.74	Nill
$[Pd(L)_2]$	738.4	Greenish brown	172	2.38	14.40	14.02	Nill
$[Cd(L)_2]$	744.4	Greenish brown	175	7.3	15.09	14.83	Nill
$[Hg(L)_2]$	832.5	Brown	162	2.48	24.08	23.75	Nill

Table (1): The physical properties complex.

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Table(2): The Infrared Spectra For the Free Ligand and its Complexes (cm^{-1})

$[Mn(L)_2]$	3232(b)	1659(m)	1261(s) 1165(s)	1620(w)	1230(w) 1130(w)	825(w)	447(s)	510(m)	υ(C=C)aroma. 1577,1512,1435. υ(C-H) aroma. 3053,υ(C-H)imin. 2735,υ(C-N) 1041,r(CO_2) 540
$[Fe(L)_2]$	3414(b)	1659(m)	1261(s) 1165(m)	1625(w)	1220(w) 1153(w)	821(w)	439(w)	509(m)	υ(C=C)aroma. 1573,1512,1435. υ(C-H) aroma. 3039,υ(C-H)imin. 2735,υ(C-N) 1041,r(CO_2) 536
$[Co(L)_2]$	3410(b)	1659(m)	1260(Vs) 1165(m)	1620(w)	1230(w) 1135(w)	825(w)	443(m)	524(w)	υ(C=C)aroma. 1577,1512,1435. υ(C-H) aroma. 3082,υ(C-H)imin. 2735,υ(C-N) 1041,r(CO_2) 551
$[Ni(L)_2]$	3370(b)	1659(s)	1261(s) 1165(m)	1620(w)	1225(w) 1153(w)	821(w)	466(w)	520(w)	υ(C=C)aroma. 1589,1508,1435. υ(C-H) aroma. 3043 ,υ(C-H)imin. 2735,υ(C-N) 1041 .r(CO_2) 540
$[Cu(L)_2]$	3417(b)	1659(s)	1261(Vs) 1165(m)	1620(w)	1220(w) 1153(w)	821(w)	447(w)	515(w)	υ(C=C)aroma. 1589,1509,1435. υ(C-H)aroma.3039,υ(C-H)imin. 2735, υ(C-N) 1041,r(CO_2) 536
$[Zn(L)_2]$	3549(b)	1659(s)	1260(Vs) 1165(s)	1620(w)	1220(w) 1140(w)	821(w)	435(w)	513(m)	υ(C=C)aroma. 1589,1512,1435. υ(C-H)aroma.3074,υ(C-H)imin. 2735,υ(C-N) 1041,r(CO_2) 540
$[p4(L)_2]$	3414(w)	1659(s)	1260(s) 1165(s)	1620(w)	1225(w) 1140(w)	825(w)	439(w)	513(m)	υ(C=C)aroma. 1577,1512,1435. υ(C-H)aroma.3039,υ(C-H)imin. 2735, υ(C-N) 1041 ,r(CO_2) 540
$[C4(L)_2]$	3545(b)	1659(s)	1260(s) 1165(s)	1620(w)	1225(w) 1140(w)	825(w)	443(s)	509(m)	υ(C=C)aroma. 1577,1512,1435. υ(C-H) aroma. 3059 υ(C-H)imin. 2735,υ(C-N) 1041 ,r(CO_2) 540
$[Hg(L)_2]$	3626(b)	1659(m)	1261(b) 1165(m)	1620(Vw)	1230(w) 1130(w)	825(w)	439(m)	516(w)	υ(C=C)aroma. 1577,1512,1435. υ(C-H)aroma.3012,υ(C-H)imin. 2735,υ(C-N) 1041, ,r(CO_2) 540

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Table (3): Electronic Spectra of the ligand and its complexes.

Compounds	λ_{max}	$\bar{\upsilon}$ (Cm)$^{-1}$	ξ_{max} L. mol^{-1}.cm^{-1}	Assignment	μ_{eff} (BM)
[HL]	235	42553	21	$\pi \rightarrow \pi^*$	-
	299	33445	1076	$\pi \rightarrow \pi^*$	
	358	28169	1244	$n \rightarrow \pi^*$	
$[Mn(L)_2]$	223	44843	1821	L-F	5.73
	283	35336	1946	L-F	
	348	28736	965	C-T	
	685	14599	5	$^6A_{1g} \rightarrow {}^4T_{2g}$	
	943	10604	4	$^6A_{1g} \rightarrow {}^4T_{1g}$	
$[Fe(L)_2]$	223	44843	1175	L-F	5.20
	291	34369	900	L-F	
	349	28653	337	C-T	
	804	12438	4	$^5T_{2g} \rightarrow {}^5E_g$	
	1000	10000	4	$^5T_{2g} \rightarrow {}^5E_g$	
$[Co(L)_2]$	227	44053	1542	L-F	4.88
	283	35336	2003	L-F	
	345	28986	1091	C-T	
	873	11455	8	$^4T_{1g} \rightarrow {}^4A_{2g}$	
$[Ni(L)_2]$	228	43860	1662	L-F	3.120
	285	35088	1484	L-F	
	349	28653	670	C-T	
	786	12723	5	$^3A_{2g} \rightarrow {}^3T_{1g}$	
$[Cu(L)_2]$	237	42194	1621	L-F	1.88
	270	37037	2281	L-F	
	355	28169	668	C-T	
	872	11468	42	$^2E_g \rightarrow {}^2T_{2g}$	
$[Zn(L)_2]$		42553	556	L-F	Diamagnetic
	235	35211	1038	L-F	
	284				
$[pd(L)_2]$	223	44843	1841	L-F	Diamagnetic
	283	35336	1858	L-F	
	348	28736	889	C-T	
	776	12887	18	$^1A_{1g} \rightarrow {}^1B_{1g}$	
$[Cd(L)_2]$	263	42372	837	L-F	Diamagnetic
	282	35460	1565	L-F	
	348	28735	891	C-T	
$[Hg(L)_2]$	223	44843	1741	L-F	Diamagnetic
	283	35336	1754	L-F	
	349	28653	β45	C-T	

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Table(4) : Solution preparation and absorbance measurements at 348nm of Ni (II)complex.

Sample no.	V mL [Ligand] $1x10^{-3}$M	V mL Ni(II)] $1x10^{-3}$M	$\frac{Vm}{(Vm+VL)}$	$\frac{VL}{(Vm+VL)}$	Absorbance
0	0	10	1.0	0	0
1	1	9	0.9	0.1	0.554
2	2	8	0.8	0.2	1.065
3	3	7	0.7	0.3	1.476
4	4	6	0.6	0.4	2.001
5	5	5	0.5	0.5	2.4
6	6	4	0.4	0.6	2.75
7	7	3	0.3	0.7	2.13
8	8	2	0.2	0.8	1.825
9	9	1	0.1	0.9	0.215
10	10	0	0.0	1	0

Table (5): Showed the inhibition circle diameter in millimeter for the bacteria after 24 hour incubation paid and 37°C for[$Pd(L)_2$], -[$Hg(L)_2$]and[$Cd(L)_2$].

compounds	*Escherichia coli*	*Proteus mirabilis*	*Klebsiella pneumonias*	*Staphylococcus aurens*
(DMF) Control	10	10	9	10
HL	11	12	11	12
1- [$Pd(L)_2$]	15	18	11	15
2-[$Hg(L)_2$]	24	32	15	20
3-[$Cd(L)_2$]	22	32	17	25

Synthesis and Characterization of new Tridentate Schiff Base Phenyl-2-(2-hydroxybenzylidenamino)benzoate and its complexes with some metals Ions

Taghreed H. Al-Noor , Sajid. M. Lateef , Mazin H. Rhayma

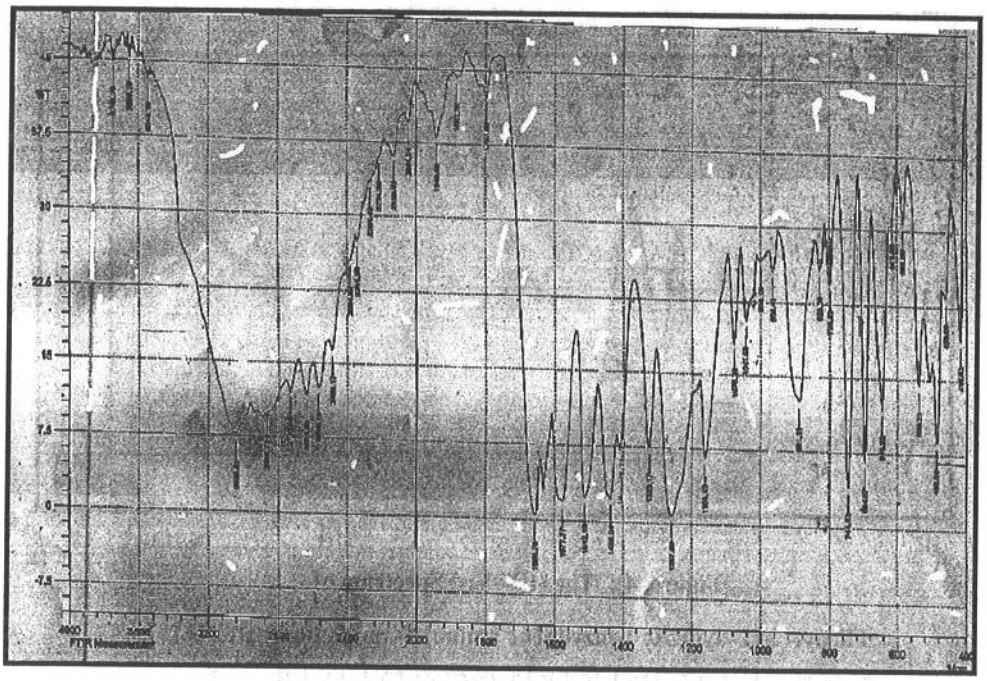

Figure. 3): *The* (FTIR) Spectrum of (Phenyl-2-(2-hydroxybenzylidenamino)benzoate(HL)

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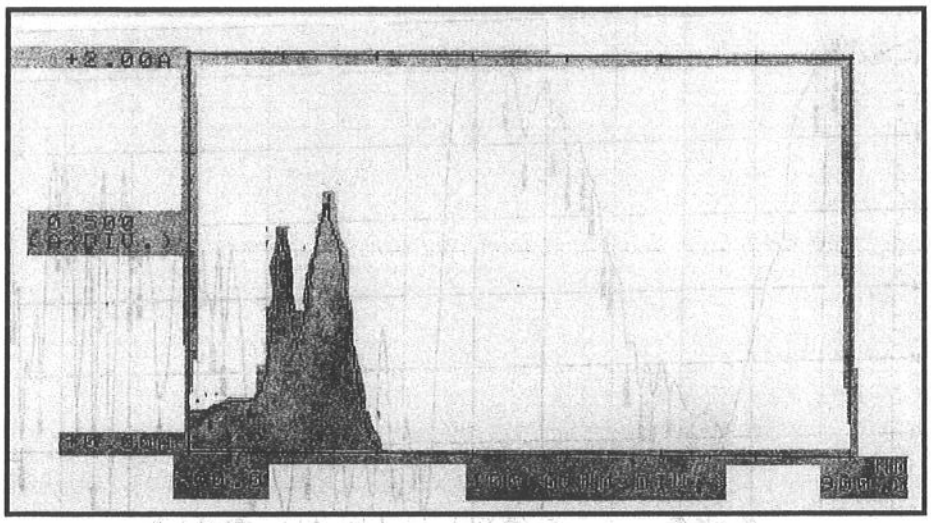

(Figure. 4): The (UV-Vis) Spectrum of Phenyl- 2-(2-hydroxy benzylidene amino)-6-amino benzoate(HL)

Synthesis and Characterization of new Tridentate Schiff Base

Phenyl-2-(2-hydroxybenzylidenamino)benzoate and its complexes with some metals Ions

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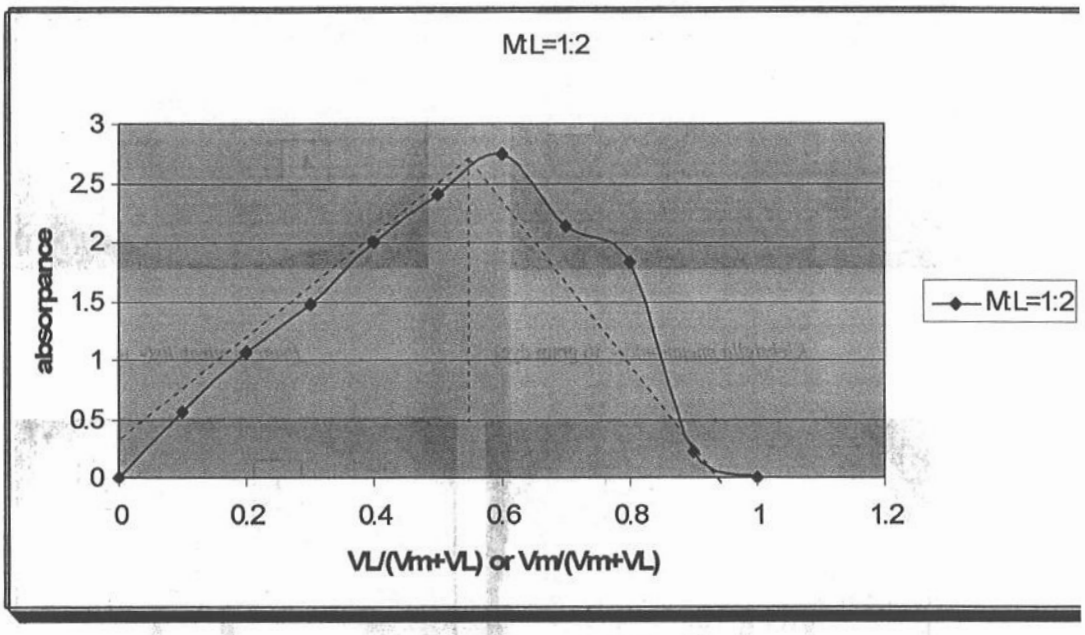

(Figur. 5): Job's plot for HL and Ni (II)complex formed using equimolar concentration

($1x10^{-3}$)M at 348nm

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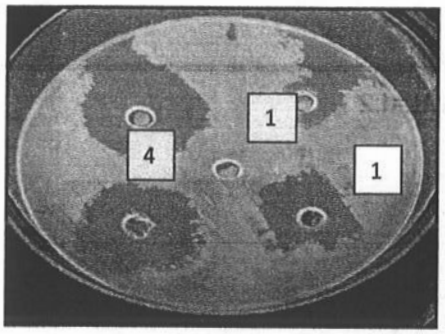
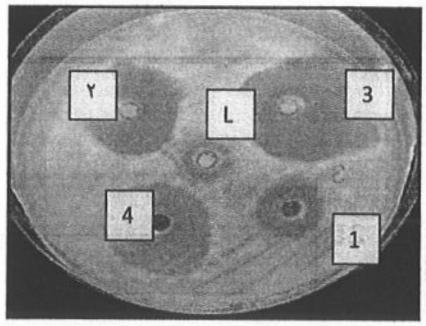
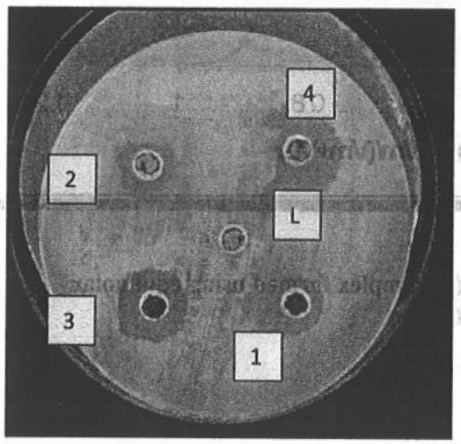


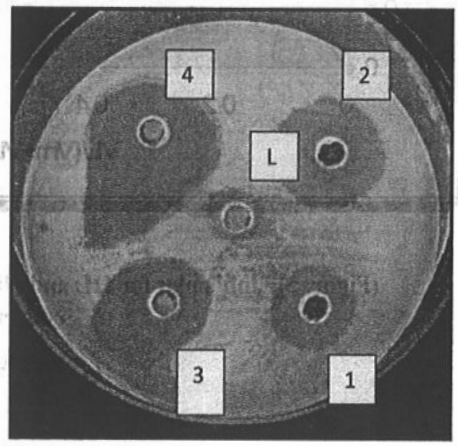
Klebsiella pneumonia(- to gram dye)



Proteus mirabilis(- to gram dye)



Escherichia coli(- to gram dye)



Staphylococcus aureus(+ to gram dye)

(Figure. 6): shows the antimicrobial activity of chemical compounds (ligands,complex ,1,2,3) appear the inhibition zones against some pathogenic bacteria.

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