

Preparation and Identification Complexes of Some Transition Metals with 4,4'-Bis(2-Hydroxy phenyl azo)- 3,3'-Dimethylbenzidine [BHADB] and Study of Its Biological Activity

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Abstract:

This study included synthesis of ligand 4,4'-Bis(2-Hydroxy phenyl azo)- 3,3'-Dimethylbenzidine (BHADB), the ligand was prepared from reaction 3,3'-Dimethylbenzidine with 2-hydroxy phenol. Metal complexes of this ligand were prepared by reaction of transition metal chloride salt of Ni(II), Co(II) and Cu(II) with prepared ligand in ethanol. Characterization of the ligand and its complexes were investigated by FT-IR and UV-visible spectroscopy in addition to conductivity measurement. The molar ratio of ligand-metal were found to be (1:1) for Ni(II) complex and Co(II) complex, and (1:2) for Cu(II) complex. The spectral studies showed the geometry around the Ni(II) and Co(II) ions are distorted tetrahedral, the Cu(II) ion is octahedral. Also includes, the study of biological effect for these complexes on four deferent pathogenic species: (*Streptococcus faecalies*, *Staphylococcus aureus*), (*Escherichia coli*, *Klebsiella pneumonia*) the first and second species are Gram positive while the others are Gram negative (by using agar well diffusion method). Finally, it was found that these compounds show different activity of inhibition on the growth of the bacteria.

الخلاصة:

يتضمن هذا البحث تحضير ليكند (BHADB) من تفاعل 3,3'-Dimethylbenzidine مع 2-hydroxy phenol، حضرت المعقدات الفلزية لهذا الليكند من تفاعل كلوريدات العناصر الانتقالية Ni(II)، Co(II) و Cu(II) في مذيب الايثانول، وتم تشخيص هذه المعقدات بواسطة تقنية مطيافية الاشعة تحت الحمراء ومطيافية الاشعة فوق البنفسجية - المرئية وقياسات التوصيلية المولارية. وبالاعتماد على طريقة النسب المولية تمت معرفة النسبة بين الليكند- فلز وكانت (1:1) لمعقد Ni(II) ومعقد Co(II) و (2:1) لمعقد Cu(II). ومن خلال الدراسات اعلاه اقترحت الاشكال الفراغية لمعقد Ni(II) و Co(II) الرباعي السطوح المشوه، ومعقد Cu(II) ثنائي السطوح. يتضمن البحث ايضاً دراسة بعض جوانب التأثير البايولوجي للمعقدات المحضرة في نمو اربع اجناس بكتيرية ممرضة الاولى والثانية منها موجبان لصبغة كرام وهما:

(*Streptococcus faecalies*, *Staphylococcus aureus*)

والاثنان الاخران سالبة لصبغة كرام وهما: (*Escherichia coli*, *Klebsiella pneumonia*) وباستخدام طريقة الحفر بالأكار وجد ان لهذه المركبات فعالية متباينة في تثبيط نمو البكتريا المدروسة.

Introduction:

3,3'-Dimethylbenzidines are used directly in making several dyes and pigments including naphthol AS-G. They are converted into numerous dye and pigment intermediates, as well as into tanning agents, curing agent for polyurethane elastomers and polymers⁽¹⁾. Some 3,3'-Dimethylbenzidines are used in the detection of metals, thiocyanates, nitrites and chlorine in water⁽¹⁾. Benzidine-based dyes are defined as dyes that contain benzidine attached to other substituents by diazo linkages⁽¹⁾. Dyes based on 3,3'-dimethylbenzidine contain (3,3'-dimethylbenzidine) attached to other substituents by diazo linkages. Dyes based on 3,3'-dimethoxybenzidine contain (3,3'-dimethoxybenzidine) attached to other substituents by diazo linkages⁽¹⁾. Thousands of workers involved in the production of dyes, textile, paper, and leather goods potentially are exposed to dyes based on benzidine, 3,3'-dimethylbenzidine and 3,3'-dimethoxybenzidine. Because more than one of these dyes may be found concurrently in the same industry, it is difficult to count exposed workers and to define the extent of exposure to any specific dye⁽¹⁾. Since it was well known that benzidine is a carcinogenic and mutagenic compound, it has become important to replace the

dyestuffs and corresponding intermediates based on benzidine or its homologues^(2,3). One important method involves the use of arylamine compounds introduced as bridged groups between the two benzene rings of benzidine⁽⁴⁾. Synthetic dyes which include a wide range of aromatic water-soluble dispersible organic colorants are used extensively in textile, paper, printing, cosmetic and food industries⁽⁵⁾. Azo-dyes with the heterocyclic diazo-component form coloured complexes with many metal ions in solution. Great number of the spectrophotometric methods based on these reactions were developed and used in analytical chemistry⁽⁶⁾. Coordination of ligand through nitrogen and oxygen provide models for metal-ligand binding sites in several enzyme⁽⁷⁾. Complexes of zinc have a medical importance for uses in the treatment of diabetes⁽⁸⁾. Nagar and co-workers⁽⁹⁾ have been reported the synthesis, characterization and antibacterial activity of some transition metal complexes with cis-3,7-dimethyl-2,6-octadien semicarbazone (CDOSC) of the general formula $[M(\text{CDOSC})_2\text{Cl}_2]$, where; (M= Cu^{II} , Co^{II} , Ni^{II} , Cd^{II} , Hg^{II} , Mn^{II} , Cr^{III} and Fe^{III}). Complexes, as well as the ligand, were screened for their antibacterial activity. The complexes exhibit strong inhibitory action against Gram (+) bacteria staphylococcus aureus and Gram (-) bacteria Escherichia coli. The antibacterial activities of the complexes are stronger than that of the ligand (CDOSC). In present study reports the preparation and identification of ligand (BHADB), and some its metal complexes and studding the biological activity for their complexes.

Experimental:

Reagents were purchased from Fluka and BHD chemical company. IR spectra were recorded as KBr discs using a shimadzu 8300 FT-IR spectrophotometer in rang $(4000-400)\text{cm}^{-1}$. Electronic spectra of prepared compounds were measured in the region $(200-1000)\text{nm}$ for (10^{-3}M) solution in DMSO at (30°C) using Centra 5. Electrical conductivity measurements of the complexes were recorded at (30°C) for 10^{-3}M solution of the sample in DMSO using WTW-Terminal 740 digital conductivity meter. Melting points were determined on Gallen Kamp capillary melting point apparatus and were uncorrected.

1- Preparation of Ligand [BHADB]:

Ligand was prepared according to the following general procedure⁽¹⁰⁾. 3,3'-Dimethylbenzidine (0.53g, 2.5mmole) was dissolved in (25ml) of water and (15ml) of concentrated hydrochloric acid. The filtered solution was diazotized below (5°C) with (20ml) of aqueous [1.0M] sodium nitrate. The result diazonium chloride solution was mixed with phenol (0.47g, 5mmole) dissolved in (50ml) alkaline ethanol cooled below (0°C) . After leaving in the refrigerator for 30minutes. The precipitate was cooled and collected by filtration. The precipitate was washed several timed using ethanol and dried in desicator.

2- Preparation of complexes:

A solution of ligand [BHADB] (0.14g, 0.32mmole) in ethanol (15ml) was placed in becar. A solution of copper(II) chloride dehydrates (0.11g, 0.64mmole) in (15ml) ethanol was added drop-wise with stirring. The pH of the reaction mixture was adjusted to $\approx (9)$ by using the sodium hydroxide. The resulted mixture was left at room temperature for 30minutes. A solid complex was obtained, collected by filtration and dried to give (80%) yield of the title complex, m.p. $(240-242)^\circ\text{C}$. A similar method to that mentioned in preparation of Cu(II) complex was used to prepare the complexes of ligand with $[\text{Ni}^{\text{II}}, \text{Co}^{\text{II}}]$ ions. The pH of the reaction mixture was adjusted to $\approx (9)$ by using the sodium hydroxide. All complexes were soluble in DMF and DMSO solvents. Table (1) appears some physical properties of the prepared complexes.

Table (1): *Some physical properties of the prepared complexes.*

Complexes	M.P. (°C)	Color	Yield (%)	Conductivity in DMSO S.mol ⁻¹ .cm ²
[NiLCl (H ₂ O)]	258-260	Brown	78	9.35
[CoLCl (H ₂ O)]	250-252	Red	83	8.66
[Cu ₂ LCl ₄ (H ₂ O) ₄]	240-242	Green	80	6.45

3- Antibacterial Activity:

The agar-well diffusion method according to Perez *et. al.* ⁽¹¹⁾ was used to screen the antibacterial activity of each extract. Aliquots of (20mL) of Müeller Hinton agar (Oxoid®, Basingstoke, Hampshire, England) were poured into 9cm Petri dishes in order to evaluate bacterial growth. The agar inoculated with (100µL) of (18-24)hour nutrient broth cultures of the following bacteria (Gram positive: *Streptococcus faecalis* and *Staphylococcus aureus*) and (Gram negative: *Escherichia coli*, and *Klebsiella pneumoniae*). Four holes, equidistant from each other and from the edge of the plate, were bored into the solidified seeded agar using sterile glass borers (8mm in diameter). The plates were incubated at (37°C) for 30 minutes before introducing (50µL) of each complex solution into each holes using micropipette. Holes containing distilled water alone were included on each plate as control. The plates were refrigerated at (4°C) for 30 minutes to allow for diffusion before incubating at (37°C) for 24 hours. The produced zones of inhibition were measured for each organisms screened.

Results and Discussion:

IR spectra:

The (I.R.) spectra of the ligand is shown in Fig.(1) and the (I.R.) spectra of the complexes Ni^{II}, Co^{II} and Cu^{II} are shown in Figs.(2), (3) and (4) respectively. The assignments of the characteristic bands are summarized in Table (2). The broad band at (3200)cm⁻¹ in the free ligand spectrum Fig.(1) which assigned to ν(-OH) stretching, the lower frequency for ν(-OH) due to hydrogen bonding ⁽¹²⁾. The band at (1500)cm⁻¹ assigned to ν(-N=N-) stretching, the band at (1145)cm⁻¹ attributed to phenolic ν(C-O) ⁽¹³⁾. The (I.R.) spectra for nickel(II) complex and cobalt(II) complex Fig.(2) and Fig.(3) respectively, shows bands at (3615 and 3620)cm⁻¹ assigned to free ν(-OH) stretching respectively ⁽¹²⁾, the strong bands at (1470)cm⁻¹ attributed to ν(-N=N-) stretching. The band at (1145)cm⁻¹ assigned to phenolic ν(C-O) stretching. This band remains in the same region in free ligand and the two complexes, and the another side the phenolic ν(C-O) stretching was shifted to higher frequency and appeared at (1230 and 1240)cm⁻¹ in nickel(II) complex and cobalt(II) complex respectively, indicated a weak linkage between oxygen and carbon ⁽¹⁴⁾. This shift confirms the participation of phenolic oxygen in the (C-O-M) bond ⁽¹⁵⁾. A new bands at the range (470-480)cm⁻¹ attributed to ν(M-N) and the range (570-580)cm⁻¹ attributed to ν(M-O) stretching respectively ^(16,17). While the IR spectrum of copper(II) complex Fig.(4). Show the broad band at (3410)cm⁻¹ due to ν(H₂O) stretching of water molecule, which that lattice water molecules ⁽¹⁸⁾. The band at (1450)cm⁻¹ attributed to ν(-N=N-) stretching. The phenolic ν(C-O) stretching appeared at (1380)cm⁻¹ in this complex was shifted to higher frequencies ⁽¹³⁾. A new bands at (470 and 510)cm⁻¹ can be attributed to ν(M-N) and ν(M-O) stretching respectively ^(16,17).

Table(2): *Characteristic IR (cm⁻¹) absorption bands of the ligand and its complexes*

Compound	ν (-OH)	ν (C-O)	ν (-N=N-)	ν (M-O)	ν (M-N)	ν(H ₂ O) lattice water	ν(H ₂ O) coordinate water
[BHADB]	3200	1145	1500	---	---	---	---
[NiLCl (H ₂ O)]	3615	1230	1470	570	470	3410	670
[CoLCl (H ₂ O)]	3620	1240	1470	580	480	3400	650
[Cu ₂ LCl ₄ (H ₂ O) ₄]	---	1380	1450	510	470	3410	680

UV-Visible:

The UV-Visible spectrum of the ligand, Fig.(5) shows twin absorption peak at (215nm, 46511) cm^{-1} ($\epsilon_{\text{max}}= 486 \text{ molar}^{-1}\text{cm}^{-1}$) and (248nm, 40322) cm^{-1} ($\epsilon_{\text{max}}= 606 \text{ molar}^{-1}\text{cm}^{-1}$) and absorption peak at (389nm, 25706) cm^{-1} ($\epsilon_{\text{max}}= 1643 \text{ molar}^{-1}\text{cm}^{-1}$) assigned to ($\pi \rightarrow \pi^*$) and ($n \rightarrow \pi^*$) transitions respectively ⁽¹³⁾. While the electronic spectra of the complexes of Ni^{II}, Co^{II} and Cu^{II}. Figs.(6-8) exhibited the following data . The nickel(II) complex shows peak at (390nm, 25641) cm^{-1} ($\epsilon_{\text{max}}= 1490 \text{ molar}^{-1}\text{cm}^{-1}$) assigned to intraligand charge transfer and the shoulder at (525nm, 19047) cm^{-1} ($\epsilon_{\text{max}}= 500 \text{ molar}^{-1}\text{cm}^{-1}$) attributed to d-d transition type ${}^3A_{1g}^{(F)} \rightarrow {}^4B_{1g}^{(F)}$ which that similar to that showed of distorted tetrahedral d^8 complexes. The cobalt(II) complex shows peak at (256nm, 39062) cm^{-1} ($\epsilon_{\text{max}}= 1258 \text{ molar}^{-1}\text{cm}^{-1}$) assigned to intraligand charge transfer and the peaks at (400nm, 25000) cm^{-1} ($\epsilon_{\text{max}}= 450 \text{ molar}^{-1}\text{cm}^{-1}$) and (494nm, 20242) cm^{-1} ($\epsilon_{\text{max}}= 411 \text{ molar}^{-1}\text{cm}^{-1}$) attributed to d-d transition type ${}^4A_2^{(F)} \rightarrow {}^4T_2^{(F)}$ and ${}^4A_2^{(F)} \rightarrow {}^4T_1^{(P)}$ respectively. Corresponding with previous studies of distorted tetrahedral complexes. While the copper (II) complex shows peak at (256nm, 39062) cm^{-1} ($\epsilon_{\text{max}}= 1614 \text{ molar}^{-1}\text{cm}^{-1}$) assigned to intraligand charge transfer and the peaks at (393nm, 25445) cm^{-1} ($\epsilon_{\text{max}}= 272 \text{ molar}^{-1}\text{cm}^{-1}$) and (524nm, 19083) cm^{-1} ($\epsilon_{\text{max}}= 260 \text{ molar}^{-1}\text{cm}^{-1}$) attributed to d-d transition type ${}^2B_{1g} \rightarrow {}^4E_g$ and ${}^2B_{1g} \rightarrow {}^2B_{2g}$ respectively. Attached with the octahedral geometry around the metal ion d^9 complexes ⁽¹⁹⁾.

Conductivity measurements

The complexes are non-electrolytes as shown by their conductivity in DMSO at room temperature, the values are in the range 6.45- 9.35 $\text{S.mol}^{-1}.\text{cm}^2$. (Table1).

Stoichiometric study:

The estimation of the complex structure was carried out by using mole-ratio method (Fig.1,2,3) in spectrophotometric technique. The results were referred to the (metal : ligand) ratios for nickel(II) complex and cobalt(II) complex were (1:1) and the ratio for copper(II) complex was (2:1) at $\text{pH} \approx 9$.

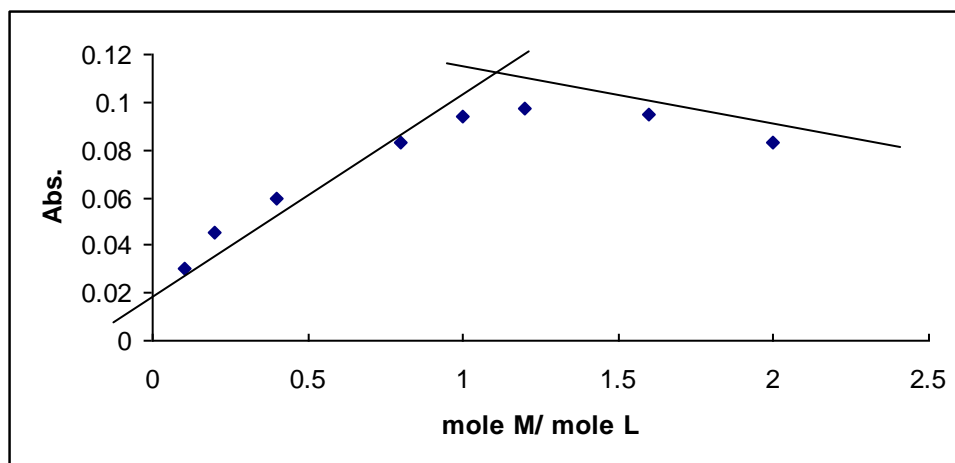


Fig.(1): Mole-Ratio of Ni(II) complex $[M] = [L] = 1 \times 10^{-4}$, $\text{pH} \approx 9$

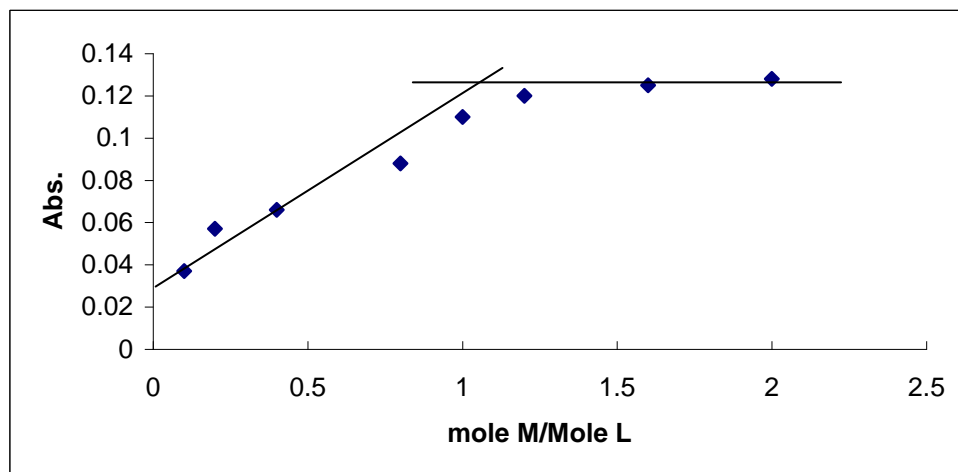


Fig.(2): Mole-Ratio of Co(II) complex $[M] = [L] = 1 \times 10^{-4}$, $pH \approx 9$

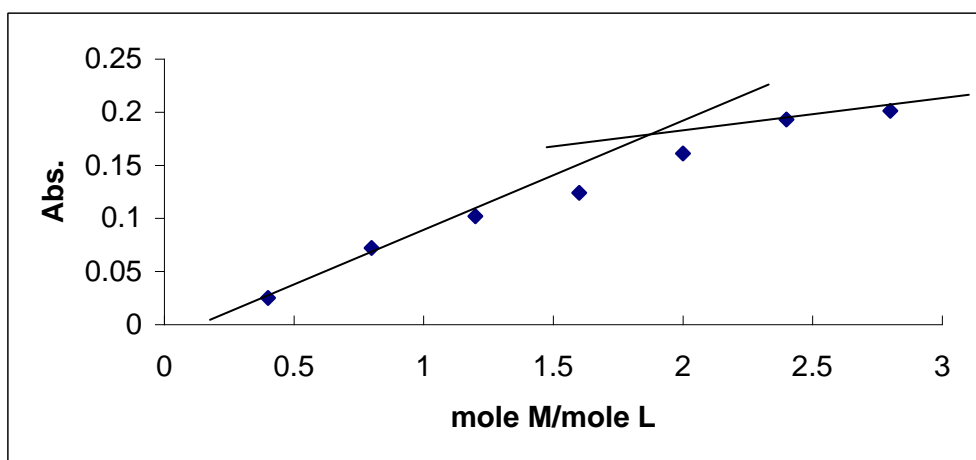


Fig.(3): Mole-Ratio of Cu(II) complex $[M] = [L] = 1 \times 10^{-4}$, $pH \approx 9$

According to these results the structural formula of prepared complexes may be proposed in Fig.(4) and Fig.(5).

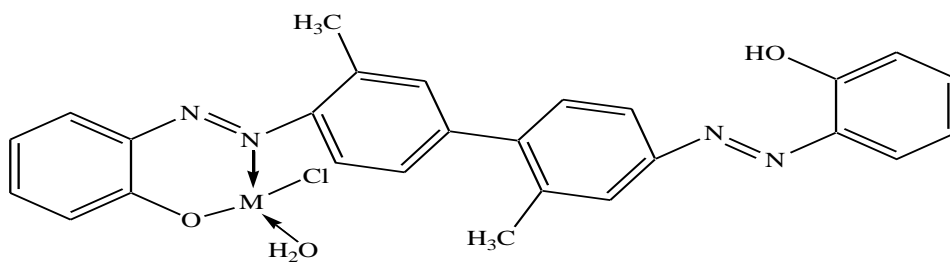


Fig.(4): The structural formula of nickel(II) complex and cobalt(II) complex.

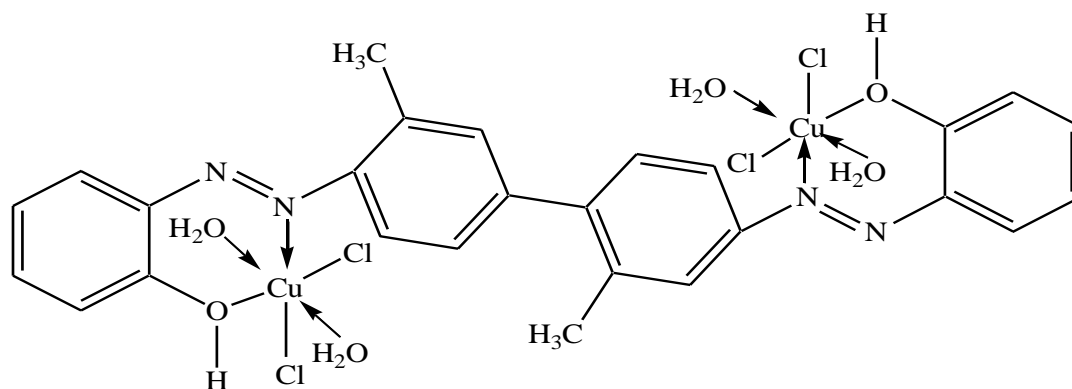


Fig.(5): The structural formula of copper (II) complex.

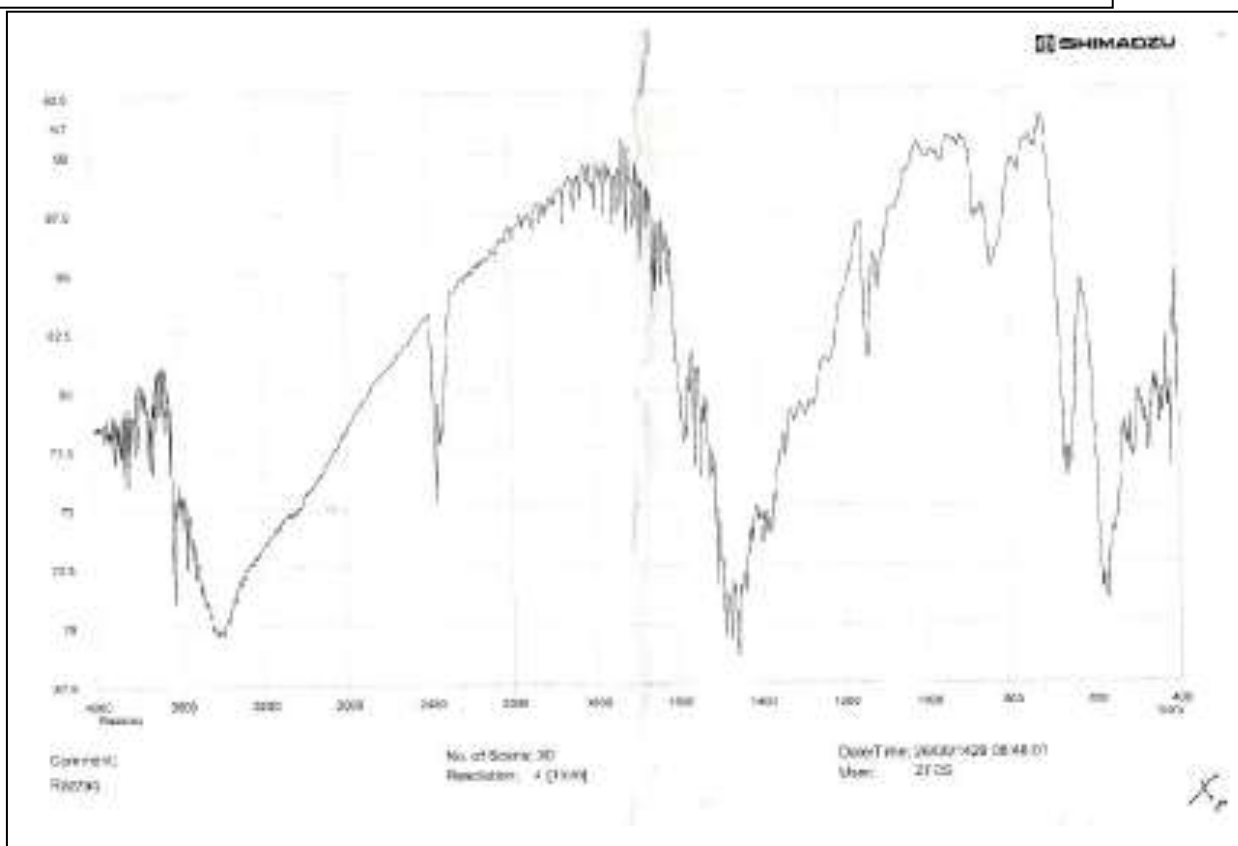
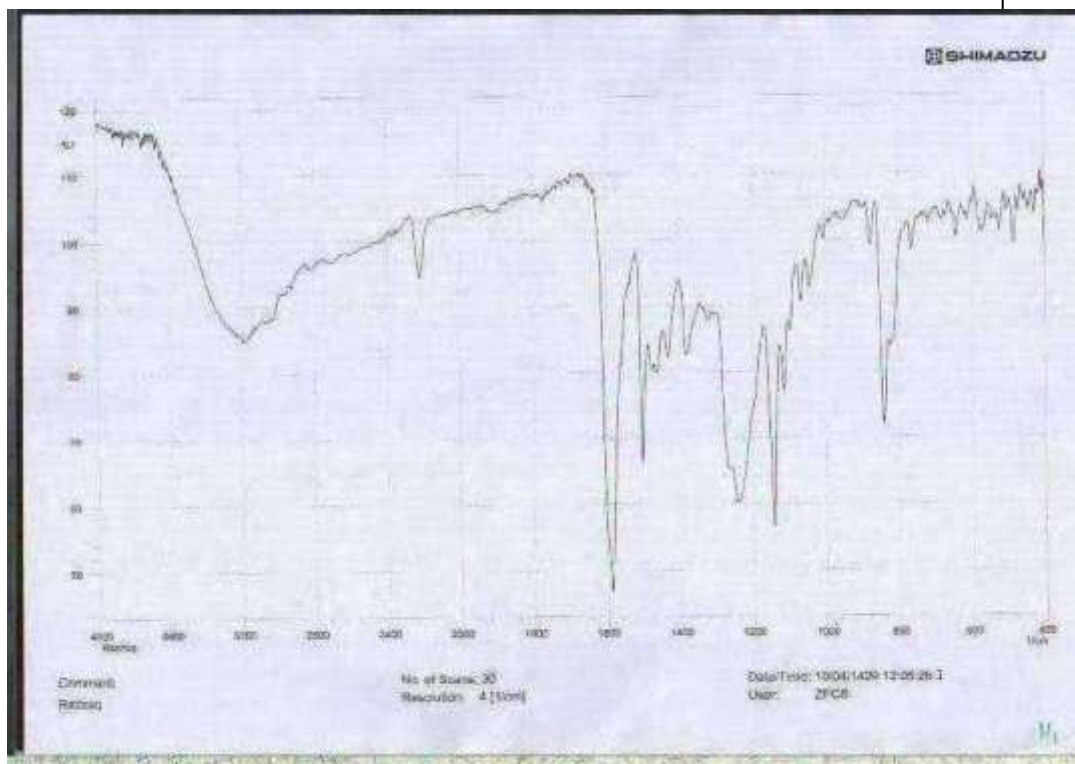
Antibacterial Activity:

The task of antibacterial activity is to show the effect of these complexes Ni^{II} , Co^{II} and Cu^{II} against indicator strains, two gram positive: *Streptococcus faecalis* and *Staphylococcus aureus* ; and two gram negative: *Escherichia coli* and *Klebsiella pneumoniae*. As shown in Table(3) the three complexes Ni^{II} , Co^{II} and Cu^{II} gave a high activity against gram negative indicator strains compared with gram positive bacteria, on which the zone inhibition of *Escherichia coli* toward $\text{Co}(\text{II})$ complex was (14mm) while $\text{Cu}(\text{II})$ complex and $\text{Ni}(\text{II})$ complex gave a zone inhibition (8mm) and (11mm) respectively and *Klebsiella pneumoniae* the zone inhibition of $\text{Co}(\text{II})$ complex was (5mm) and $\text{Cu}(\text{II})$ complex was (9mm) while $\text{Ni}(\text{II})$ complex was (10mm), inhibition the zone of *Streptococcus faecalis* and *Staphylococcus aureus* were weakened when compared with the results of the gram negative tested bacteria. The variability of these results may be attributed to the difference in the method of effect because each of antimicrobial agent inhibit the microbial growth by: inhibition of cell wall synthesis. Disruption of cell membranes, interference with protein synthesis or interference with nucleic acid synthesis ⁽²⁰⁾ from one side and from the other side the difference between structure of gram positive and gram negative bacterium affect on the activity of antimicrobial agents, even on the level species under the gram positive and gram negative bacteria.

Table(3): Biological activity of the 10ppm of the complexes Ni^{II} , Co^{II} and Cu^{II} expressed as zone of inhibition (mm) using agar well diffusion method.

Bacteria	Zone of Inhibition (mm)		
	Ni(II) complex	Co(II) complex	Cu(II) complex
<i>Streptococcus faecalis</i>	5	3	5
<i>Staphylococcus aureus</i>	4	7	4
<i>Escherichia coli</i>	11	14	8
<i>Klebsiella pneumoniae</i>	10	5	9

Figures:



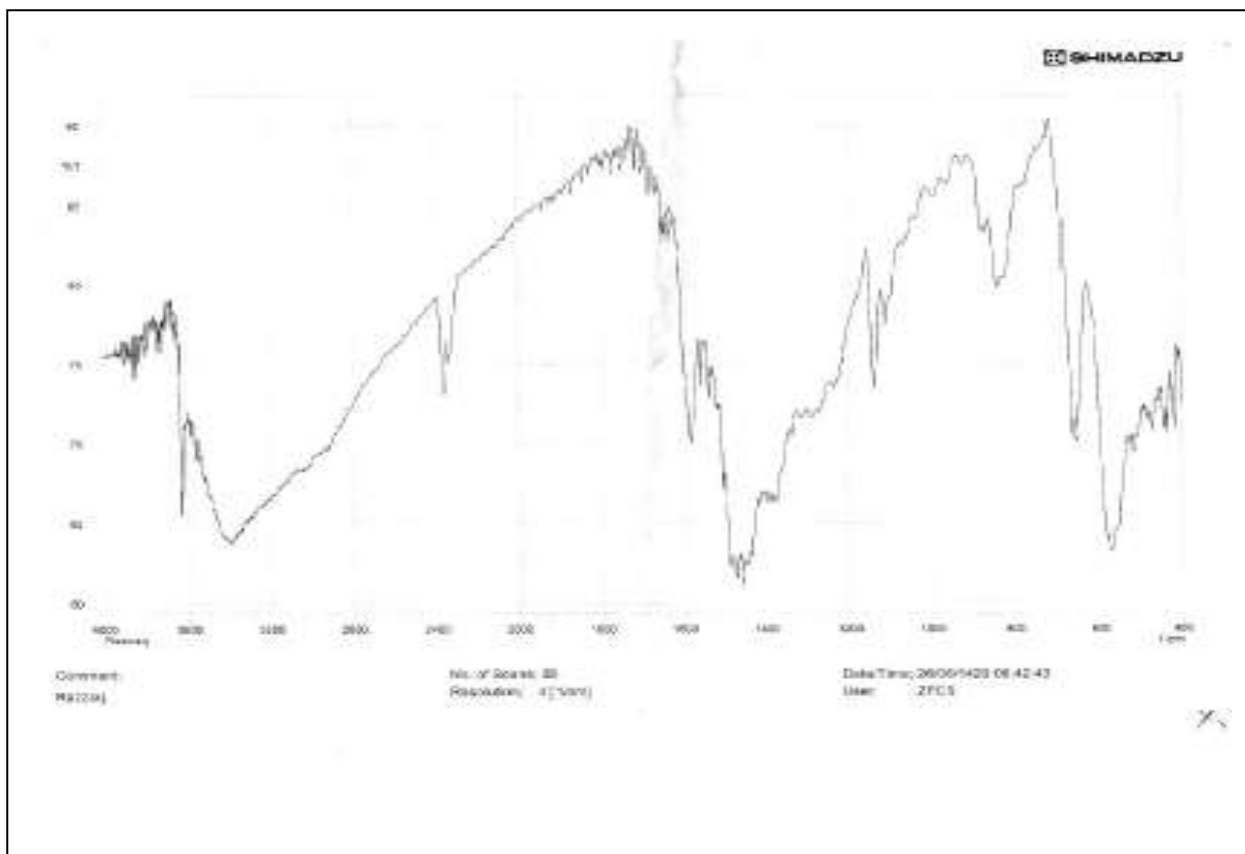


Fig.(3): FT-IR spectrum of Co(II) complex

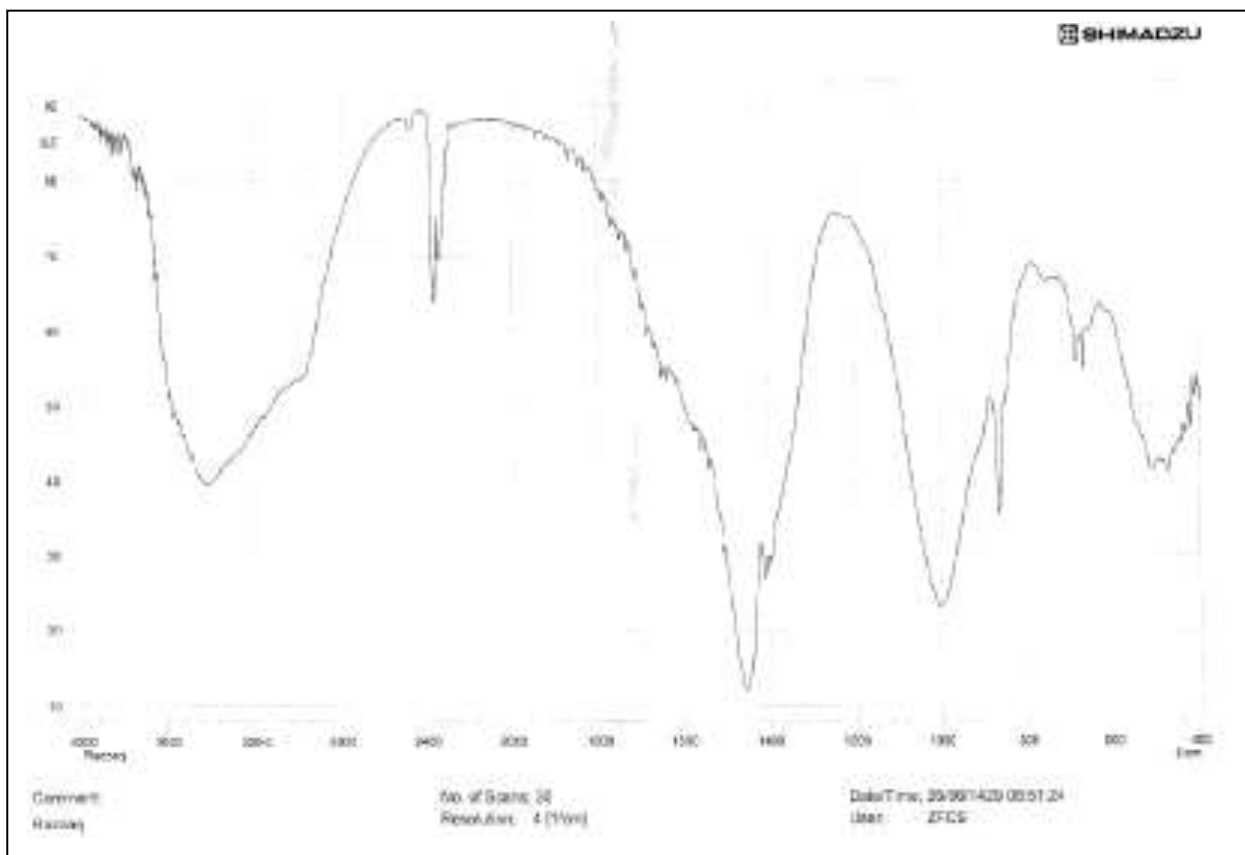


Fig.(4): FT-IR spectrum of Cu(II) complex

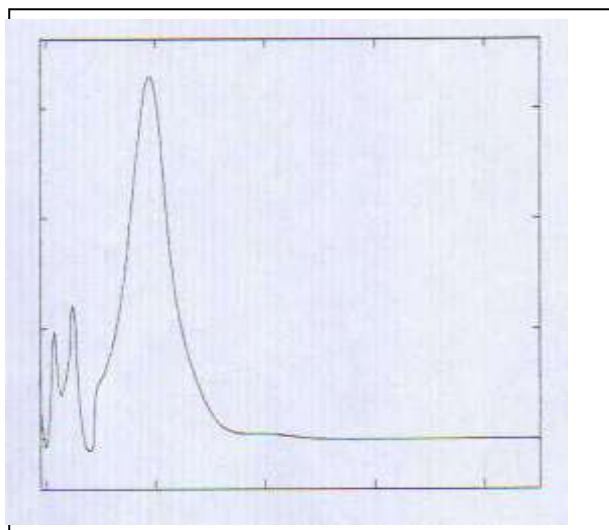


Fig.(5): *UV-Vis. Spectrum of (BHABD)*

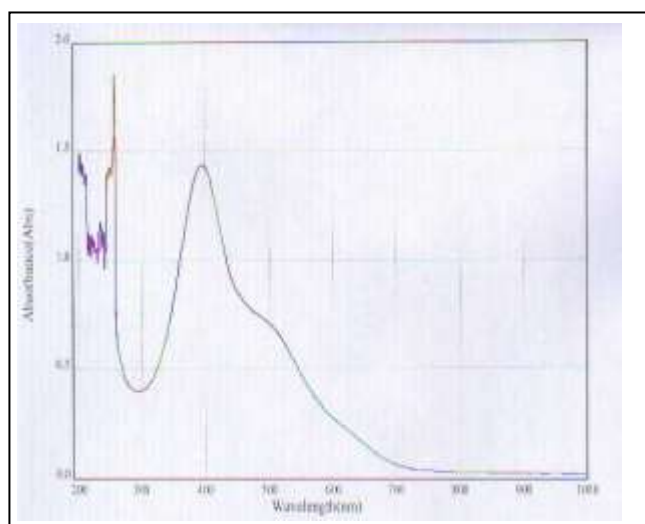


Fig.(6): *UV-Vis. Spectrum of Ni(II) complex*

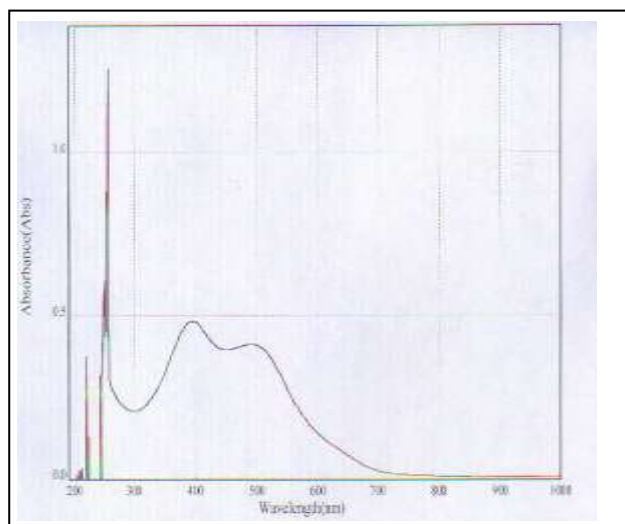


Fig.(7): *UV-Vis. Spectrum of Co(II) complex*

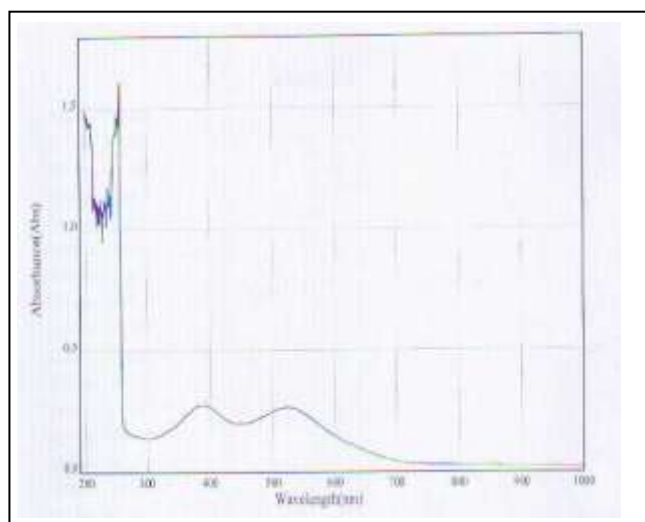


Fig.(8): *UV-Vis. Spectrum of Cu(II) complex*

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