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Synthesis, characterization and biological evaluation of some metals complexes with new Azo-Schiff ligand

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***Abstract*---**In this research the ligand 5-((3-(1-((5-bromopyridin-2yl)imino)ethyl)-2-hydroxyphenyl)diazenyl)-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (BHAP)was used to prepare new complexes with elements Cu(II) ,Ni(II), Pt(II),Co(II) ,Cr(III). The new ligand was analyzed by partial analysis of the elements (C.H.N). UV- visible and infrared rays(FTIR), spectroscopy H^1NMR, C^{13}NMR and mass spectrometry. The complexes were prepared after determining the optimum pH and concentration values by examining the spectra (UV - Vis) of the solutions of these complexes to find out the pH (3-10), concentration ($1*10^{-4}$-$5.5*10^{-4}$) and molar ratios that agree with Beer-Lambert's law. The chemical measurement of the complexes was known with respect to the ligand ratio, which was verified by spectroscopy technique, after choosing the maximum absorption of the solutions of those complexes, where the ligand ratio (1:1) was achieved for all complexes. The properties of the prepared complexes were identified by (FTIR), ultraviolet and visible rays, molar conduction, atomic absorption, magnetic sensitivity and elemental analysis techniques) C.H.N. Through the results obtained from physical, chemical and spectroscopic techniques, the geometrical shape of these complexes was suggested as octahedral. All these complexes were evaluated against two types of pathogenic bacteria (positive and negative).

***Keywords*---**Azo, Schiff base, ligand, complexes, anti-bacterial activity.

Introduction

In recent years, coordination chemistry has gained wide interest in chemistry for its rapid development in the scientific aspect of preparing and diagnosing coordination complexes. These complexes have been used in wide fields in medicine[1], industry[2], agriculture, as well as in the field of pollution[3]. Among the many ligands, azo compounds and Schiff bases are among the most important organic ligands involved in the formation of many coordination complexes by giving them electrons to metallic elements [4]. The Heterocyclic Azo compounds are characterized by being well-coordinated ligands with metal ions[5,6] because they contain electron-donating atoms such as oxygen, sulfur, and nitrogen, in addition to the azo bridge group (-N=N-), therefore, we find that azo compounds spread widely and received applications and uses in the industrial, biological and medical fields[7,8] · as well as in the field of analytical chemistry, where they were used as reagents in quantitative and qualitative analysis[9],because of the color characteristic that bestows on these compounds as they were exploited in spectroscopic studies. One of the important compounds (pyrazolin-5-one) that has received attention by many researchers because of its biological activity and effectiveness as a result of the presence of more than one donor atom of electronegative pairs[10], as well as the presence of heterogeneous aromatic rings that are compensated by other groups, which have the ability to form chelating bonds with metals to form coordination complexes with biological activity[11].

Experimental Section

All solvents and chemical materials were purchased commercially from Sigma–Aldrich and Fluka and were of the highest analytical grade. The Fourier transformation infrared Bruker ALPHA FT-IR, Faculty of Science, University of Kufa, was used to record infrared spectra. At Shahid Beheshti University in Iran, NMR spectra in DMSO-d6 were obtained using a Bruker spectrometer (125MHz for ^{13}C NMR, and 500MHz for ^{1}H NMR respectively). The Electro Thermal Melting Point Apparatus from the United Kingdom is used to measure melting points. The acidity function of the prepared solutions was measured using a device (PH-meter),720, WTW 82362. The UV-visible spectra were measured to scan the spectra of the ligand and its metal complexes to construct calibration curves using a device (UV-Visible Spectrophotometer) and the absorption of the solutions was measured at wavelengths(Shimadzu-UV-1700). Microelemental analysis (C.H.N) has gained on a (Eure EA 3000 Elemental analyzer), Mass spectra in Agilent mass spectrometer 5975 quadropole analyser.

Synthesis Azo compound (3-acetyl-2-hydroxy-5-methylphenyl)diazenyl)-1-phenyl-2,4-dihydro-3H-pyrazol-3-one

The ligand was synthesized according to the general[12] method by dissolving of (1.751 g, 0.01 mol) 3-amino-1-phenyl-2-pyrazolin-5-one in a mixture consisting of (3 mL) of con. hydrochloric acid, (10 mL) ethanol and (10 mL) of distilled water. The solution mixture was cooled under to (5 ^{o}C) then (0.69 g) (0.01 mol) sodium nitrite in (10 mL) distilled water solution was added dropwise with stirring in order to obtain the dizonium salt solution. After 20 min the dizonium solution

was slowly added to a cooled basic ethanolic solution of (1.501 g, 0.005 mol) of 2-hydroxy-5-methylacetophenone and to obtain the azo compound. The brown colored mixture was neutralized by dilute hydrochloric acid, sodium hydroxide and the solid precipitation was filtered off and washed several times with distilled water then left to dry at room temperature and recrystallized from with hot absolute ethanol, yield (75%) and m.p ^{o}C (158-160), scheme (2-1), represents the formation reaction of azo compound. In this step of the reaction, the dysonium salt was prepared according to the following equation:

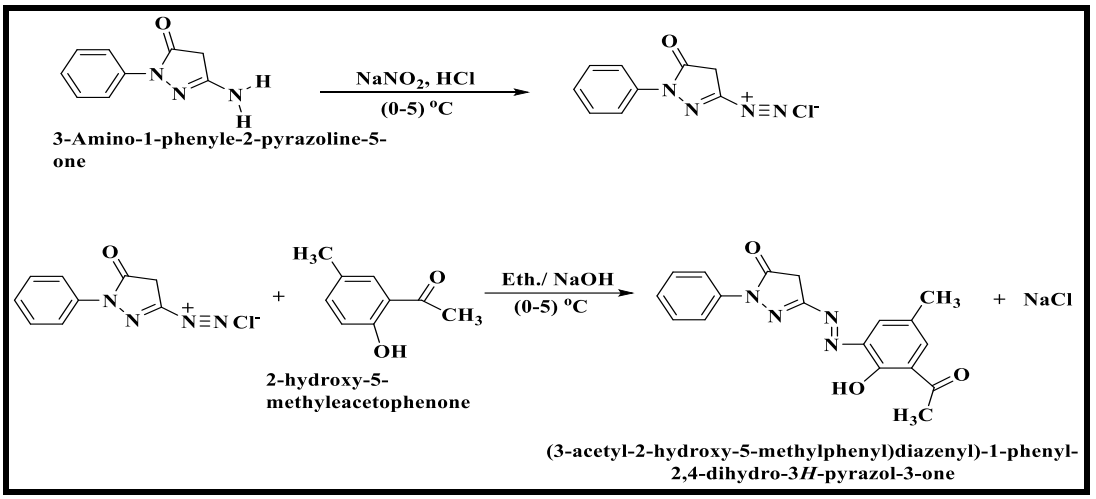


Fig.1: Synthesis Azo compound

Synthesis new ligand azo-Schiff base (BHAP) (5-bromopyridin-2-yl)imino)ethyl)-2-hydroxy-5-methylphenyl)azo)-1-phenyl-2,4-dihydro-3H-pyrazol-3-one

In general, the Schiff base compound was prepared by the reaction of the (3.361 g, 0.01 mol) from azo compound a above in paragraph (2.4.1) in 20 ml of absolute ethanol after adding 3-4 drops of glacial acetic acid with (1.73 g, 0.01 mol) 2-amino-5-bromopyridine, the reaction mixture was refluxed for (5 hrs.), the progress of the reaction was followed by TLC using hexane–ethanol (3:7) (v/v) as eluent. After completion, the resulting mass was recrystallized from ethanol when a pale brown crystalline material was formed, melting point was found to be 168-170 ºC, and the yield was 81%. R_f (0.59). The scheme (2-1), represents the formation reaction of the ligand[13].

Elemental microanalysis of ligand (BHAP): The results of elemental microanalysis were in good agreement with the proposed molecular formula($C_{23}H_{19}BrN_6O_2$): theoretical C; 56.22, H; 3.90*, N;* 17.10, O; 6.51 and experimental: C; 56.31, H; 3.84, *N;* 17.06, O; 6.62. Mass Spectrum for the Azo Ligand (PABH)*:* The mass spectrum data for azo Schiff base ligand, the molecular ion peaks are shown at (491.4) whereas molecular weight of prepared ligand is (491.51)

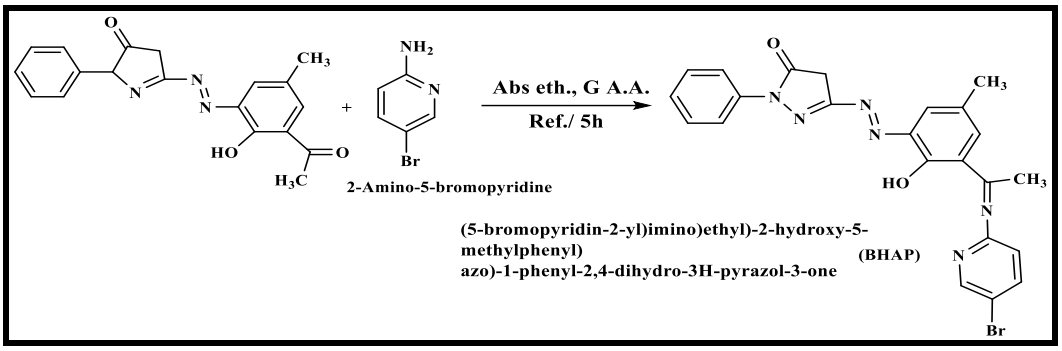


Fig.2: Synthesis ligand Azo-Schiff base (BHAP)

Prepare standard solutions of ions

Standard solutions were prepared at a concentration of (0.001M) in a volumetric flask of 100 ml capacity by dissolving 0.001 mol of metal salt in ethyl alcohol and then completing the volume to the mark. From this solution, standard solutions were prepared by successive dilution in the same way above, standard solutions of ions (Pt, Cr, Cu, Co, Ni) were prepared, taking into account the molecular weight of each element, and from these solutions other standard solutions were prepared by successive dilution.

Preparation of Buffer Solutions

Buffer solutions, covering the pH values from (3) to(10), of ammonium acetate (0.01mole) were prepared by dissolving (0.7708g) of CH3COONH4 in one liter of doubly distilled deionized water. The required pH was obtained by the addition of either ammonium hydroxide solution or glacial acetic acid.

The effect of the acid function:

Solutions of the metal ions under study were prepared with the prepared ligand with a concentration of (0.0005) molar for each of the metal and ligand. A wide range of acidic functions were selected, ranging from (PH=3-10) to the previously prepared buffer solution by dissolving ammonium acetate in distilled water and measuring the absorbance for each complex at higher wavelengths.

Calibration curve

The calibration curve was determined for solutions of metal ion complexes with the prepared ligand by studying a wide range of concentrations ranging from ($1*10^{-4}$ - $5.5*10^{-4}$ M) and the following figures show the calibration curves for each complex, including the range of concentrations that apply with Beer's law and calculating the molar absorption coefficient, the correlation coefficient, and from these values we conclude that The spectroscopic method has good sensitivity for the determination of these metals.

The size of the ligand

In order to find M:L ratio, the absorption of many metal – ligand solutions mixture were obtained at optimum pH using the following method mole Ratio method.

Synthesis of (BHAP)-Solid complexes

The solid complexes were prepared from the reaction of the ligand (BHAP) with metal chloride salts in (1:1) mole ratio heated under reflux in ethanol to produce pure complexes, where isolated in good yield, Cr(III), Co(II), Ni(II), Cu(II) and Pt(II).

Study of the solubility of complexes:

Astudy was conducted to find out the solubility of these prepared complexes as well as the ligand in some polar and non-polar solvents, namely water, ethanol, methanol,DMF, DMSO, di ethyl ether, petrolium ether and aceton,by taking a very small amount of each complex and placing it in a test tube, adding a small volume of the solvent and observing the dissolution of the complex in it.

Study of the biological activity of the ligand and its complexes

The evaluation of ligand and its complexes against two kinds of pathogenic bacteria, one strain of Gram Positive bacteria *(Staphylococcus aureus)* and Gram Negative bacteria (*Escherichia coli) w*ere carried out using agar-well diffusion[5]. Prepared agar and Petridishes were sterilized by autoclaving for (15min) at 120 ^{o}C. The bacteria types were spread out in the dishes and on the surface of Muller Hinton Agar. The medium after herding were put in the Petri dish holes having 6 mm diameter using a cork-borer. The agar plates were surface inoculated uniformly from the broth culture of the tested microorganisms. In the solidified medium suitably spaced apart holes were made all (6mm) in diameter, were filled with 100 µL of the prepared compounds (1mg of the compound dissolved in 1mL of DMSO solvent). These plates were incubated at (37 ^{o}C) for (24 hrs.). After that, the inhibition zones caused by the various compounds on the bacteria were measured by the use of the millimeter ruler.

Consequences and Discussion

The ligand Azo-Schiff(BHAP) is Burnt orange, wich is not soluble in the water but is soluble in some of the solvent.

Table (1): Solubility of Ligand (BHAP) in different solvents

Solvent	L=(BHAP) ($C_{22}H_{17}BrN_6O_2$)
EtOH	+
MeOH	+
DMSO	+
H_2O	-
DMF	+
CCl_4	÷

Diethyl ether	-
Acetone	÷
Petrolium ether	-

(+) soluble, (–) insoluble, (÷) sparingly

The reaction of this ligand with the metal ions gives different color crystals. All complexes are quiet water - insoluble, stable in air, while they are soluble in most organic solvents such as DMSO, DMF, acetone…etc.

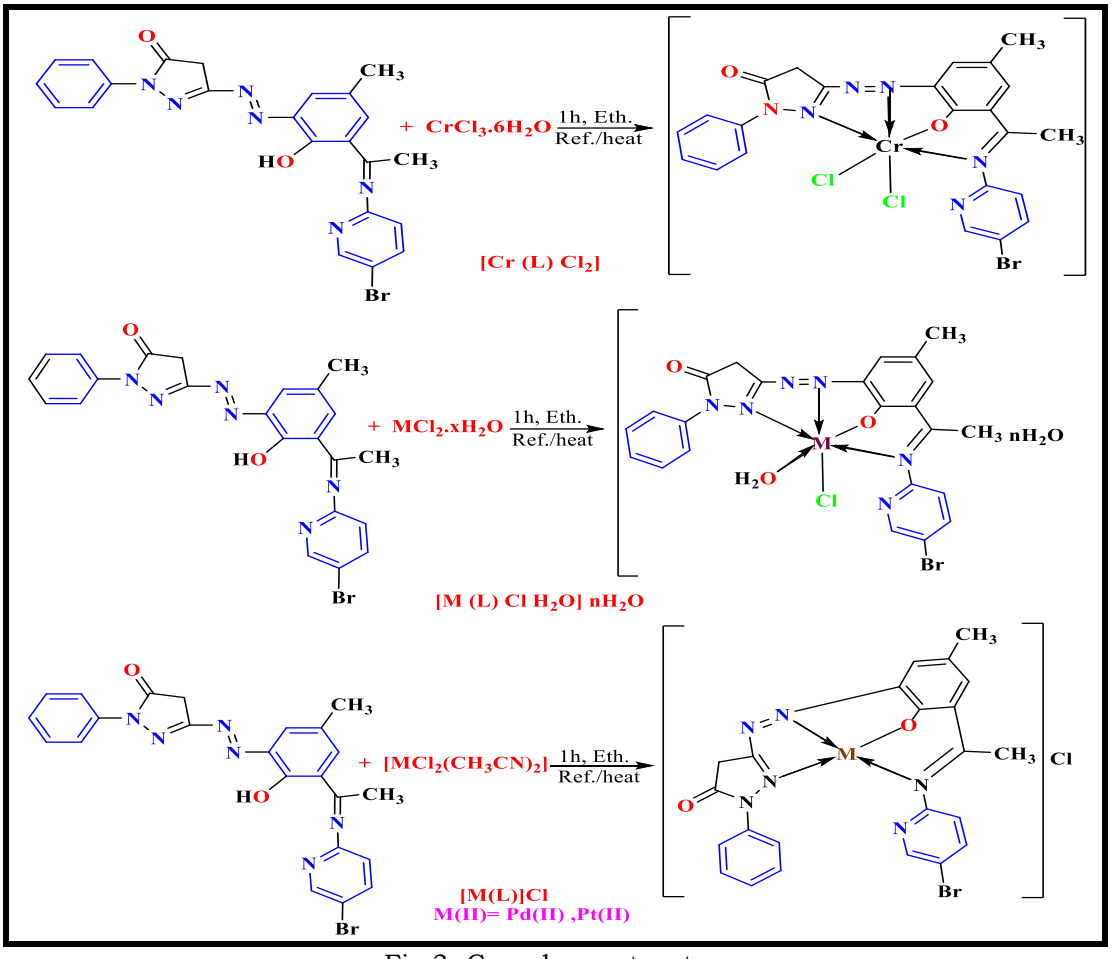


Fig.3: Complexes structure

Influence of pH

The best pH of the complex solutions of metal ions was verified after fixing(λmax)for each complex and measuring the absorbance of the following curve.

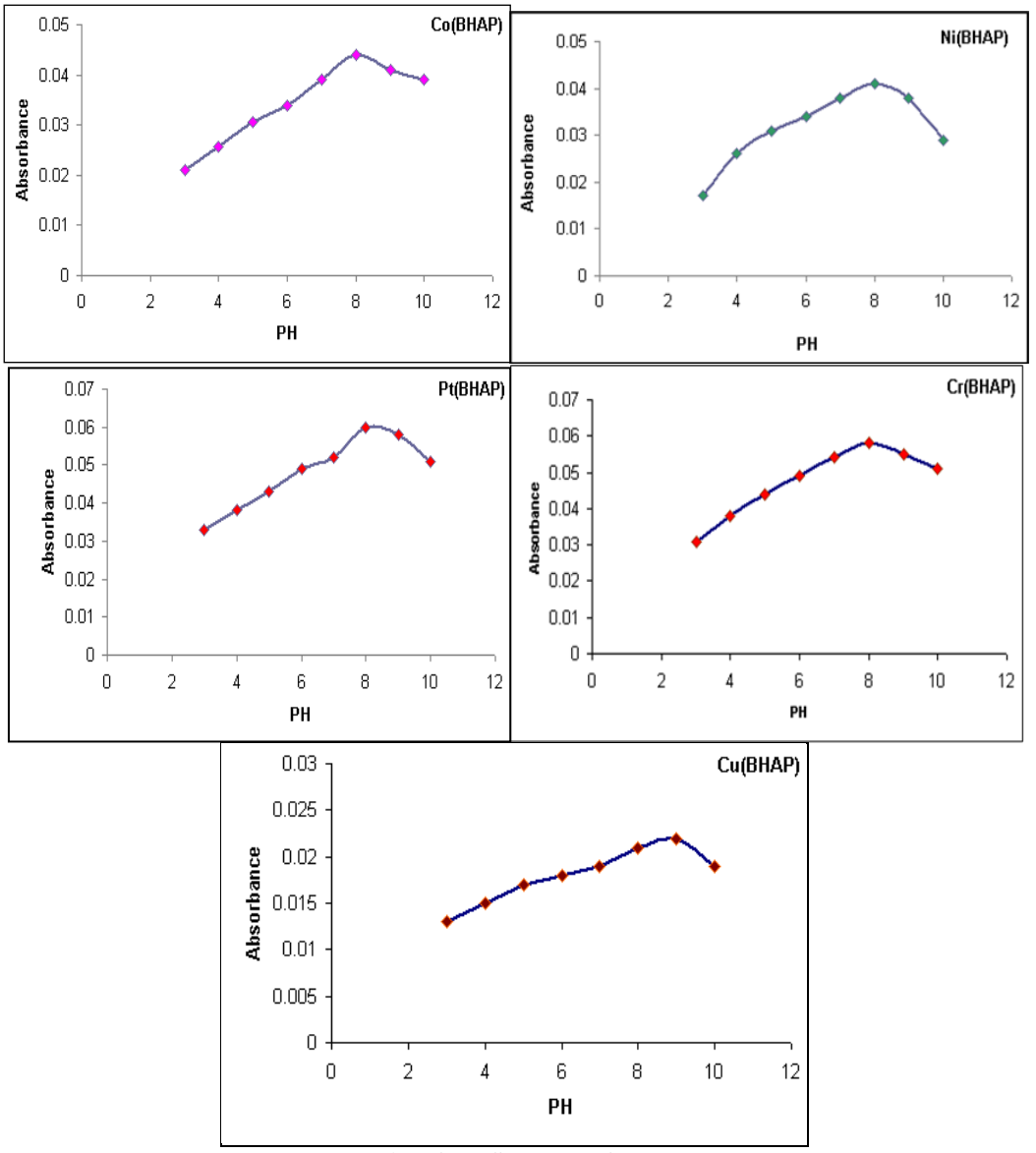
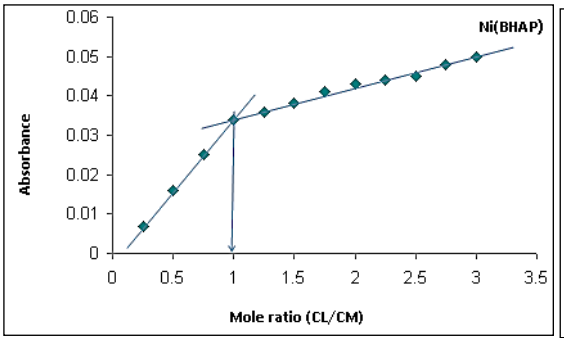
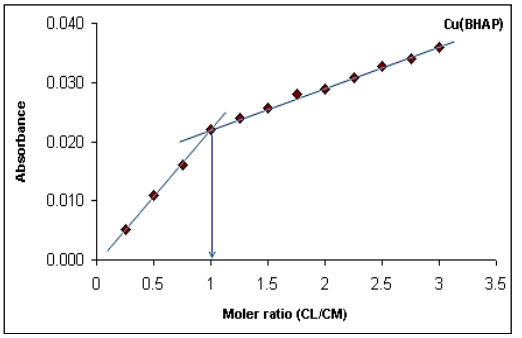


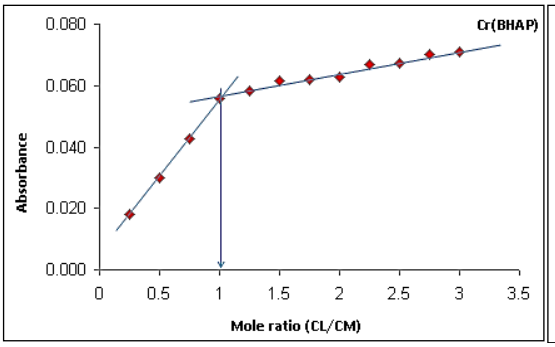
Fig. 5: Influence of pH

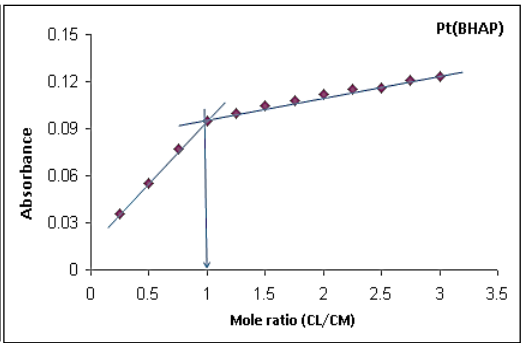
Mole Ratio [Metal:Ligand] Ratio

The molar ratios technique was adopted to determine the size of the ligand against the metal ion by choosing different volumes of the ligand against a fixed volume of the metal ion and calculating the absorbance after fixing the maximum wavelength of the metal ion. The calculations proved through the graph between the absorbance and the selected volumes that the ratio of the metal ligand equals 1 :1 According to the following diagrams:









NMR Spectra for the Ligand(PABH)

The 1H-NMR spectrum of ligand (PABH) in DMSO-d 6 Figure (4) was shows a single peak appeared at δ (10.458) ppm which was attributed to chemical shift of OH proton in the 2-naphthol. A multiplet peak at δ (8.461 - 6.884) ppm that have been as a result of chemical shifts of aromatic protons for aromatic rings of 2-naphthol and phenyl moieties. The doublet signal observed at δ (3.561 - 3.514) ppm were assigned to H-C-H protons on the pyrazolin ring moiety in the ligand. 13C-NMR spectrum Figure (5) displayed a peak at δ (178.22) ppm which is due to pyrazolin carbonyl group, while the C=N pyrazolin moiety carbon signal is showed at δ (168.26) ppm. The multiplet peaks at δ (162.16 - 122.23) ppm are due to aromatic carbons of 2-naphthol and phenyl moieties. A peak at δ (167.13) ppm which is assigned to C=C-OH and a signal at (121.81) duo to C=C-Br carbons in 2-naphthol rings. The peak at δ (163.74) ppm is due to C=C-N site linked of 2-naphthol with azo group for pyrazolin ring. The signals at δ (47.34) ppm are assigned to the middle and terminal C-C carbon atoms of diaminobutane moiety in the ligand.

UV-Vis Spectral Studies

The electronic absorption bands along with the conductivity values have briefed in Table (5). The UV-Visible spectrum of azo-schiff ligand Figure ( ) in ethanol (5x10-4 M) within the range (270-420) nm appeared two absorptions at (270 and 340) nm (37037.03 and 29411.76) cm^{-1} , which is due to π–π* transition and a broad low intensity band at (420) nm (23809) cm^{-1},which was attributed to n → π*

transition of (C.T) intermolecular charge-transfer taken place through the azo group (-N=N-)

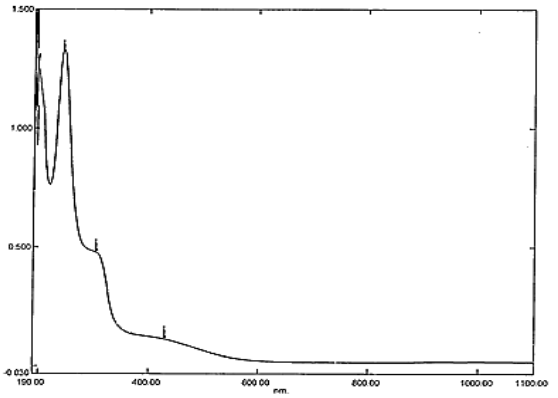


Fig. 6 UV-Vis of ligand

The spectra of the metal ions complexes within (5x10-4 M) at optimum pH showed bathochromic transferals of ligand band. The assigned bands to intraligand π→π* for the Co(II), Ni(II), and Cu(II) complexes were observed at (38500, 32172), (37811, 31523) and (37535, 31014) cm-1 respectively. The changing in a color of free ligand solutions and high shift in the (λmax) offers a worthy sign for coordinating and complex forming.

Antimicrobial Activity

The ligand(BHAP)derivatives and their complexes enjoy with a great importance in the biological field in the inhibition of biological activity, hybrid atoms such as oxygen, nitrogen and sulfur qualify for the bonding with the different elements, so we decided to study the biological activity to the new (BHAP) ligand and its synthetic complexes with some metal ions for two types of pathogenic bacteria *are (Staphylococcus aureus* - Gram Positive and *Escherichia coli* - Gram Negative), which it growth on the (Muller Hinton Agar) at temperature 37 °C. The zones of inhibition formed by the ligand(BHAP)and its complexes were recorded in (mm) for their antibacterial activity, against Staphylococcus aureus and Escherichia coli. The tests showed that the prepared complexes gave varying inhibition results for these types of bacteria, and the reason is due to the different groups attached to the compound. They are shown in the following Table.

Table (5): the data of antibacterial activity (zone of inhibition) (mm) of ligand (BHAP) and its complexes at (0.001M)

Compound / Bacteria	Gram(+) *St-aureus*	Gram(-) *E.coli*
Control (S) DMSO	6	6
Ligand	15	14

L-Cr	18	17
L-Co	17	16
L-Ni	18	17
L-Cu	18	18
L-Pt	20	19

Images of antimicrobial activity of the ligand (BHAP) and its complexes vs Staphylococcus aureusbacteria

Conclusion

Preliminary procedures were used to produce and characterize the Azo-Schiff base complexes of (Pt, Cr, Cu, Co, Ni). FTIR and NMR spectroscopy investigations revealed that the chemicals were successfully synthesized. The antibacterial activity of all produced (Pt, Cr, Cu, Co, Ni) complexes was evaluated against gram-positive *S. Aureus* and gram-negative *E. Coli* bacterial strains, and the complexes were found to be bacteriostatically stable. With increasing concentration, (Pt, Cr, Cu, Co, Ni) complexes showed a good scavenging trend.

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