



Synthesis, Spectral and Antibacterial Studies of New 1-(4-Antipyrine azo)-2-hydroxy-3, 6-Disulphonic Acid Sodium Salt with Group IIB Metal ions

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Abstract

A series of IIB group metal complexes have been synthesized from new azo ligand derived from 4-aminoantipyrine and 2-hydroxy-3, 6-disulphonic acid sodium salt. The structural features have been done on the basis of metal content C.H.N analysis, molar conductance measurement, FT-IR, UV-Vis and HNMR spectral studies. The natures of the prepared complexes were studied according to the Jobs method and Mole ratio method after fixing the optimum condition of pH and concentration. Beers law obeyed over a concentration range (1×10^{-4} - 3×10^{-4}) M. The stability constant of the complexes and Gibbs free energy have been confirmed. The antibacterial studies were also studied.

Keywords: azo dye, 4-aminoantipyrine, Spectral studies, Antibacterial studies.

Introduction

The heterocyclic compounds have excessive deals in pharmaceuticals and widely in organic synthesis [1]. Transition metal complexes show good thermal, optical and chemical properties. Therefore, they are widely used in applications such as optical data storage, photo switching, nonlinear optics and photochromic materials, dyes, chemical analysis, and pharmaceuticals [2-4]. The synthesis and properties of new transition metal complexes have been widely investigated owing to their possible applications in a variety of fields. The major characteristics of azo dyes is the presence of one or more azo moiety (-N=N-) [5, 6]. In our research we were able to prepare new ligand (AHS) and its complexes with [Zn (II), Cd (II) and Hg (II)] taking into account a spectral study in order to obtain optimum condition (concentration and pH) and metal to ligand ratio for preparation the complexes. Antibacterial activity for the ligand (AHS) and their complexes were tested.

Experimental

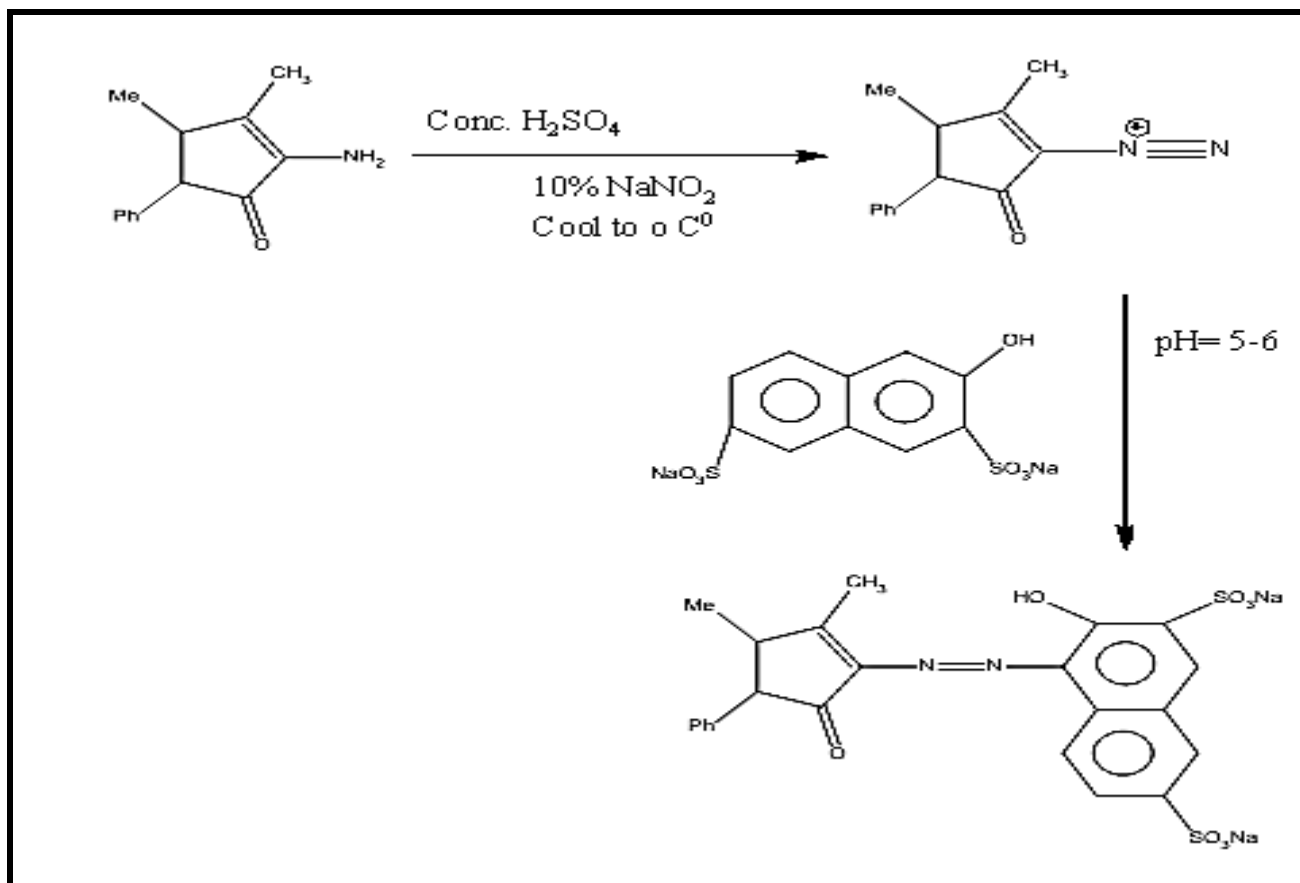
All substances and solvent are of top purity and used as found from the productions.

Micro elemental analysis (C.H.N) was gained on a (Eure EA 3000 Elemental analyzer). UV-Vis Spectra were performed in ethanol on a (Shimadzu UV-160A) ultra violet-visible spectrophotometer. FTIR-spectra were recorded on a (Shimadzu FTIR-8400s Fourier Transform Infrared) spectrophotometer (200-4000) cm^{-1} using CsI discs.

The ¹HNMR spectra were gained on a ¹HNMR Spectrometer 400 MHz, Advance III 400 Bruker, Germany" using DMSO as a solvent. PH measurements were performed using (HANNA instruments pH Tester / Pocket pH Tester). Conductivities were determined for (10^{-3} M) of complexes in DMSO at 25 C⁰ using HANNA instrument/ conductivity tester. PH measurements were performed by using HANNA instrument pH tester. Melting points have been gained by using sturat melting point apparatus.

Synthesis of Ligand (AHS)

Was synthesized according to the method reported in the literature [7] with some modifications as shown in Scheme (1).



Scheme 1: Preparation of AHS

Synthesis of Complexes

The complexes were prepared via adding gradually with stirring hot ethanolic solution of (2mmole) ligand to stoichiometry quantity of (M: L) mole ratio of (1:1) (2mmole) of [Zn (II), Cd (II) and Hg (II)] which were dissolved in the prepared buffer solution at optimum pH. The mixture was heated to (60°C) with stirring for (4 hour), then left to cool at room temperature. The colored precipitate was filtered, washed and desiccated in a vacuum desiccator.

Buffer Solution

Ammonium acetate (0.01 M, 0.771gm) was dissolved in one litter of doubly deionized distilled water. For adjusted pH range (5-8) was used acetic acid and ammonia solution.

Standard Solution

A series of ethanolic solution of ligand (AHS) were prepared in the range of concentrations (10^{-3} – 10^{-5} M). And also a series of standard solutions of metal chlorides of [Zn (II), Cd (II) and Hg (II)] were prepared within the range of concentration (10^{-3} - 10^{-5}).

Discussion

Azo compound was prepared by two steps, the first step was called diazotization of

primary amine after that the second step a coupling the diazonium salt to nucleophile like substituted aromatic ring or heterocyclic ring [7]. The presence of an azo group with lone pair of electron on the nitrogen atoms provides a binding site for coordination with metal ion. In addition another coordination site may also be providing by the presence of other donor atoms such as nitrogen, oxygen and Sulphur on aromatic rings on both sides of the azo group [8].

For this reason the azo compounds are considered to be one the most common compound used as ligands. This kind of ligands have high tendency towards many metal ions, since stable chelated complexes of five or six-membered ring are usually formed[9]. According to it, the structure feature of the ligand (AHS) and their complexes with group IIB were established from FT-IR, UV-Vis spectroscopy, HNMR, elemental analysis and conductivity measurements.

The complex has been prepared by direct reaction of alcoholic solutions of the ligand (AHS) with aqueous solution of zinc chloride at optimum pH (6.5) and in a mole ratio (1:1) metal to ligand molar ratio. The elemental analysis result are in good convention with

calculated data from expected formula for each synthesized compounds as was tabulated in Table (1). The red color of solid prepared (M (AHS)] complexes are quite stable to air and moisture, insoluble in water

but highly soluble in ethanol, DMSO, DMF and acetone. The physical properties and elemental analysis for the ligand and their complexes were tabulated in Table (1).

Table 1: The physical properties and elemental analysis for the ligand and their complexes

Compound M. wt (gm/mol)	Yelid (m.p) ^o	Color λ_{max} (nm)	Elemental analysis%				
			(cal.)				
			C	H	N	M	Cl
AHS $C_{21}H_{16}N_4S_2O_8Na$ (562)	78 (161)	Orange (487)	44.51 (44.60)	2.77 (2.83)	9.64 (9.91)	—	—
[Zn($C_{21}H_{16}N_4S_2O_8Na$)Cl] (661.89)	71 (273)	Reddish-violet (550) (514)	38.00 (38.07)	2.21 (2.26)	8.38 (8.46)	9.81 (9.87)	5.36 (5.23)
[Cd($C_{21}H_{16}N_4S_2O_8Na$)Cl] (709.9)	65 (267)	Violet (554)	35.49 (35.40)	2.25 (2.23)	7.88 (7.84)	15.83 (15.80)	5.00 (4.92)
[Hg($C_{21}H_{16}N_4S_2O_8Na$)Cl] (798.19)	68 (261)	Red (510)	31.57 (31.49)	2.00 (1.97)	7.01 (6.98)	25.14 (25.10)	44.47 (44.39)

Effect of PH and Concentration on [(M (II)-(AHS)] Solution

At first it was studied the electronic spectra for mixing solutions for the ligand and metal ions to reach to optimum pH and

concentration at fixed maximum wave length (λ_{max}). The optimum concentration was selected for complex solution depended on which solution gives the highest absorbance at constant (λ_{max}) within different pH Figure (1).

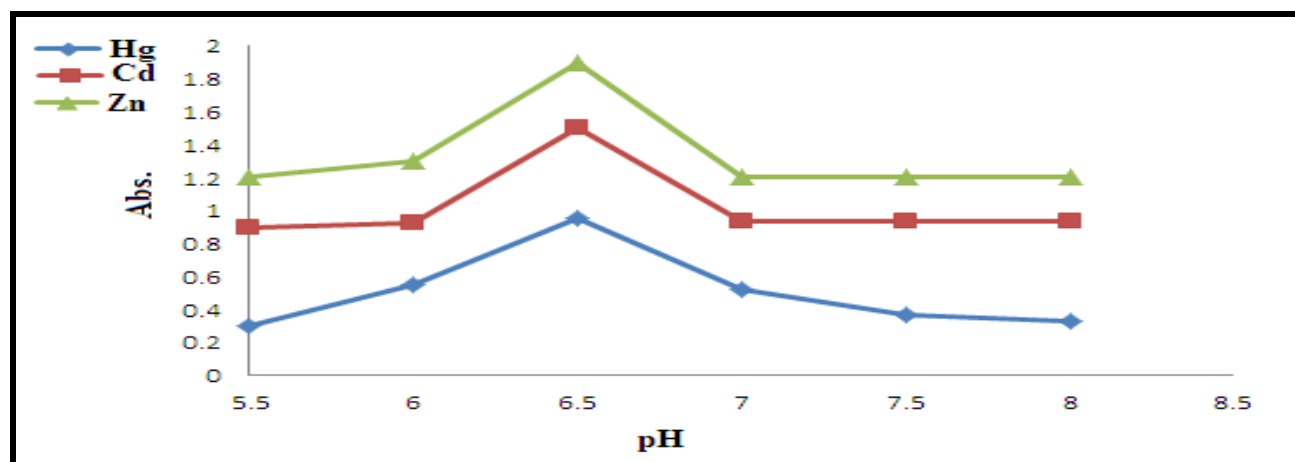


Fig.1: Effect of pH on absorbance at λ_{max} for metal ion -AHS complex solution at different molar concentration

The experimental results proved that prepared complexes had optimum performance at pH (6.5) while the concentration of (2×10^{-4} M) obeyed the lambert beers law and showed a clear intense

color. A straight line, with the correlation factor ($r=0.99$) was obtained when the absorbance plotted against the molar concentration (Figure 2). The data were listed in Table (2).

Table (2): Absorbance for molar conc. of metal ion-AHS complexes solution at optimum pH and λ_{max}

Molar Conc. $\times 10^4$	Absorbance [Zn(II)-AHS]	Absorbance [Hg(II)-AHS]	Absorbance [Cd(II)-AHS]
1	0.08	0.143	0.41
1.5	0.128	0.234	0.61
2	0.168	0.30	0.94
2.5	0.197	0.38	1.04
3	0.213	0.466	1.26

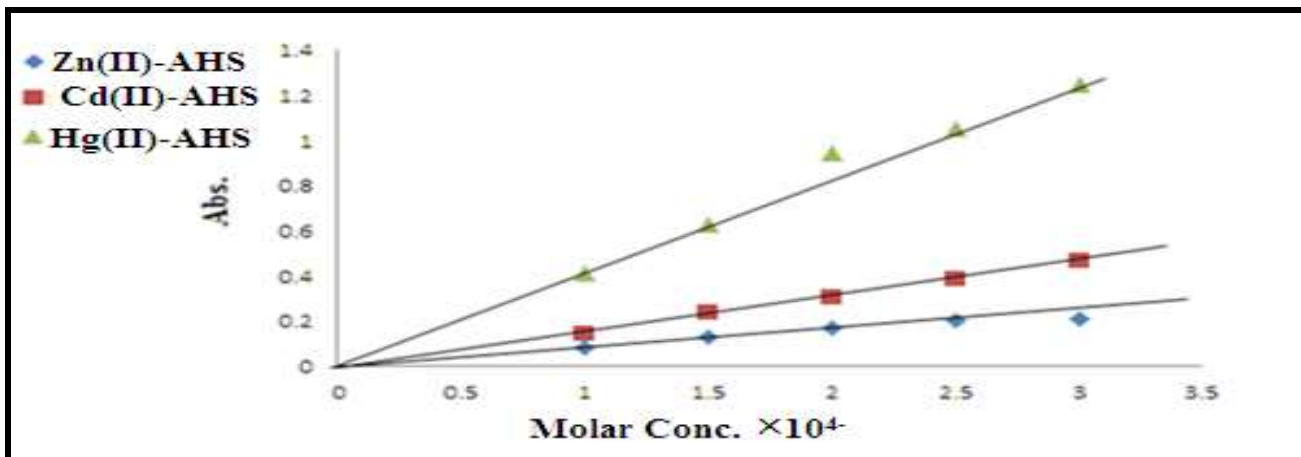


Fig.2 : Linear relationship between molar concentration and absorbance for metal ion-AHS complexes solution at optimum pH and λ_{max}.

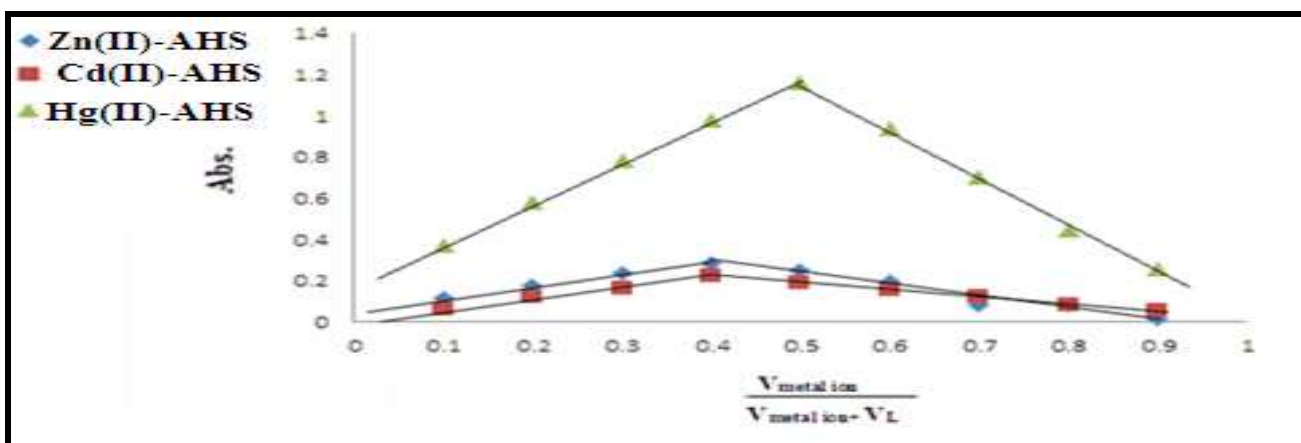


Fig. 3: Job Method plot of metal ion-AHS ligand complexes solution at optimum pH and λ_{max}.

The stoichiometry of the prepared complexes completed in solution by Job method at fixed pH and concentration at certain (λ_{max}). The ratio was (1:1) metal ion to ligand, the stoichiometry of the prepared complexes [AHS-Zn (II), AHS-Cd (II) and AHS-Hg (II)] was (1:1) metal to ligand (Figure 3).

Determination of Stability Constant and Gibbs free energy of Prepared Complexes

The stability constant (K) for M (II) complexes in a mole ratio (M: L) (1:1) was computed based on the equation [10]:

$$K = \frac{1-\alpha}{\alpha^2 C}$$

Where:

$$\alpha = \frac{A_m - A_s}{A_m}$$

α = degree of dissociation.

A_m = the absorption of solution containing the same volume of metal and excess of ligand.

A_s = the absorption of solution containing a stoichiometric volume of ligand and metal ion.

c = the concentration of the complex solution in mole/ L.

From Table (3) we calculated that M (II) complexes were stable. The thermodynamic parameters of Gibbs free energy (ΔG) were

also studied. The ΔG data have been calculated from the equation below:

$$\Delta G = -R T \ln k$$

Where R = gas constant = 8.3 J.mol⁻¹.K, T = absolute temperature (Kelvin).

Table (3) was shown that the formations of all prepared complexes are spontaneous.

Table 3: Stability constant (K) and Gibbs free energy (ΔG) for the prepared complexes

Complexes	Am	As	α	K	Log K	ΔG
Zn-AHS	0.304	0.155	0.4901	1.7325×10 ⁹	9.23869	-52969.47
Cd-AHS	0.194	0.100	0.4845	1.8130×10 ⁹	9.25842	-53082.57
Hg-AHS	0.303	0.154	0.4900	1.8028×10 ⁴	9.1658	-53604.44

The Electronic Spectra

The electronic spectra of the prepared ligands (AHS) and their complexes within the range (250-1100) nm in ethanol (10⁻⁴M) at room temperature .All data are listed in Table (4), Figure (4-7). The value of the molar extinction coefficient (ε) of the ligand (AHS) and their complexes in the range (1×10⁻⁴ - 3×10⁻⁴ L.Mole⁻¹.cm⁻¹) which leads to high sensitivity .The spectrum of the free ligand (AHS) has been displayed mainly three

peaks, the first and second peaks were appeared at (206nm, 48543 cm⁻¹; 236nm, 42372cm⁻¹) respectively which were attributed to (π→π*) transition of intra charge transfer of aromatic rings [11]. The third peak (λ_{max}) at (487nm, 20533cm⁻¹) were related to the (π→π*) for charge transfer then place through the azo group [12]. The electronic spectra for the prepared complexes have been showed high intense charge transfer (MLCT) transition as in Table (4).

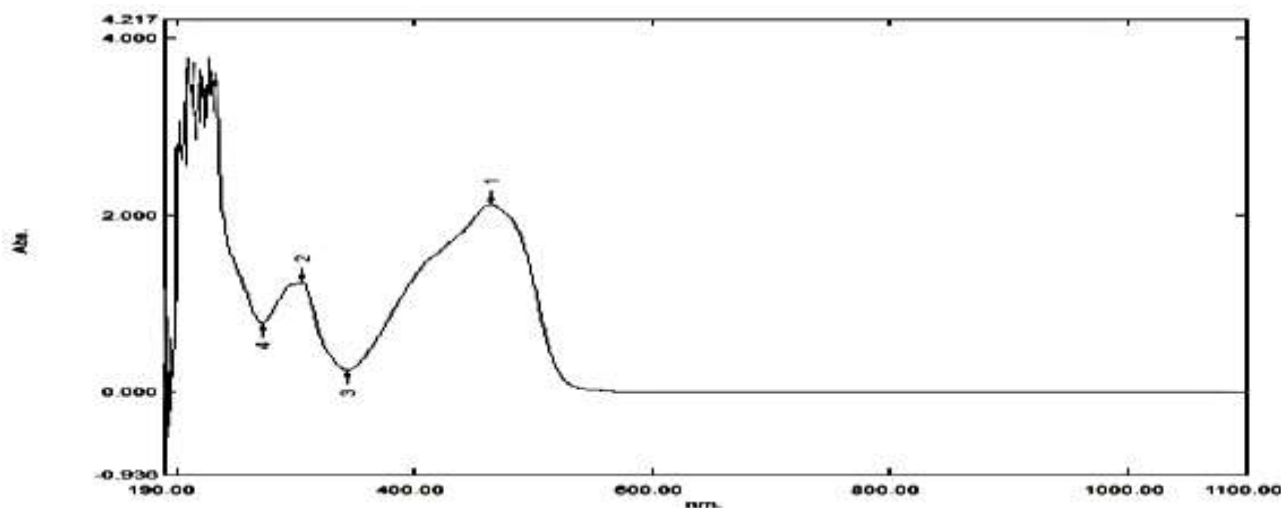


Fig.4: UV-Vis spectrum of the AHS complex

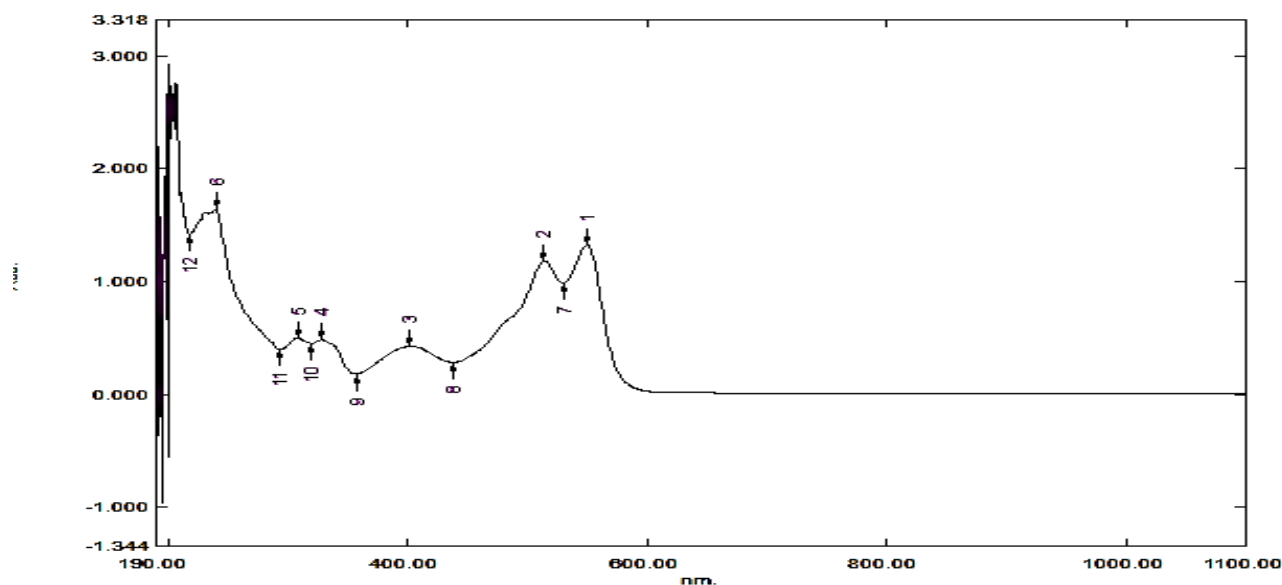


Fig.5: UV-Vis spectrum of the Zn-AHS complex

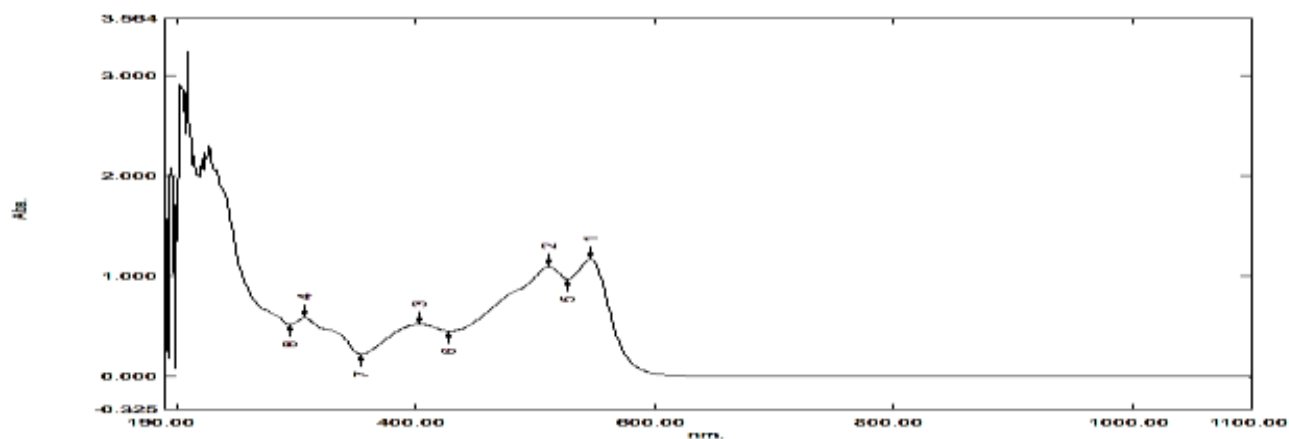


Fig.6: UV-Vis spectrum of the Cd-AHS complex

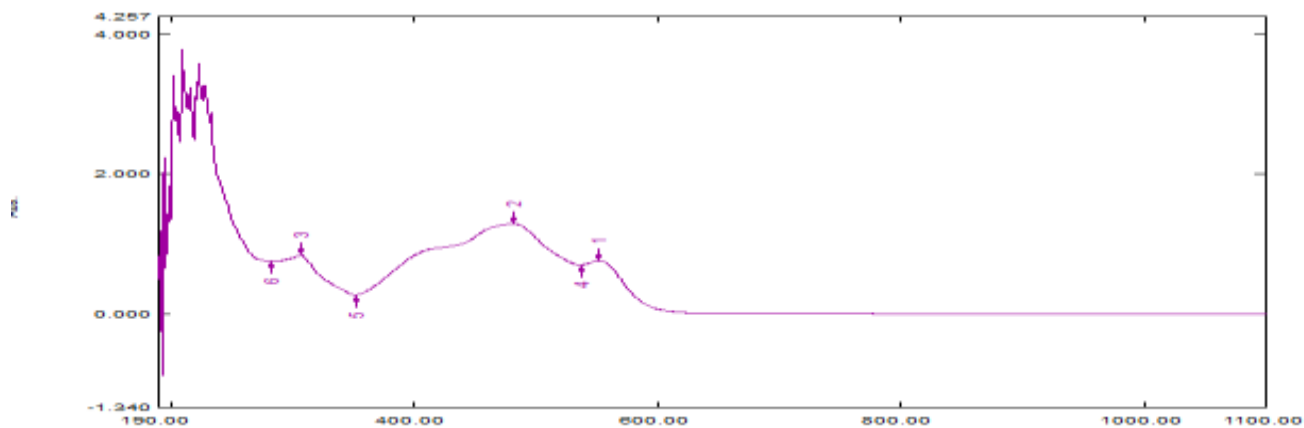


Fig.7: UV-Vis spectrum of the Hg-AHS complex

Table 4: The UV-Vis spectra for the prepared complexes at ($10^{-4}M$)

Compounds	λ nm	Wave Number cm^{-1}	$\epsilon \times 10^4$ $L.mol^{-1}.cm^{-1}$	Assignment
[Zn(C ₂₁ H ₁₆ N ₄ S ₂ O ₈ Na)Cl]	550	181818	1.323	MLCT
	514	194553	1.184	
[Cd(C ₂₁ H ₁₆ N ₄ S ₂ O ₈ Na)Cl]	546	183150	1.167	MLCT
	512	195312	1.096	
[Hg(C ₂₁ H ₁₆ N ₄ S ₂ O ₈ Na)Cl]	552	181159	0.760	MLCT

The FTIR- spectra of the Ligand and its Complex

For characterization, the FTIR spectra of the ligand (AHS) and its complexes in CsI were compared. The spectra of the prepared AHS ligand Figure (8), Table (5) showed the absorption bands characteristic of the spectrum of the ligand Figure (8) with some differences ascribed to the formation of respective ions associated. The most significant information on geometry the prepared complex comes from the analysis of antipyrine carbonyl group, azo group and hydroxyl group for naphthol.

- The FTIR spectrum of the ligand (AHS) was shown abroad and strong band at (3479) cm^{-1} corresponding to ν (OH). This band was shifted to (3450) cm^{-1} in the spectrum of the complexes with changed in shape .Which indicates consistency through hydroxyl group of naphthol [13].

- A strong and doublet band which was appeared at ($1652, 1616$) cm^{-1} was attributed to the combination of the $\nu(C=O)$ and $\nu(C=C)$. On complexation a negative shift with change in shape was observed. Where it appeared at (1610) cm^{-1} in the spectrum of the complexes. The significant change in this band may be result of coordination of Zn (II) through the oxygen of antipyrine (C=O) group [14].
- Band characteristic of the azo group vibrations has been attributed to strong and shoulder band at (1496) cm^{-1} in the spectrum of the ligand. When coordination the ν (N=N) was appeared as doublet medium bands at (1475 & 1440) cm^{-1} . These indicating the engagement at azo group in the chelating ring [15].
- New bands were observed for Zn-O, Zn-N and Zn-Cl at ($505, 441$ and 244) cm^{-1} respectively [16].

The above conclusion show that the ligand (AHS) acts as tridentate ligand coordinated through oxygen of naphthol, oxygen of

carbonyl group of antipyrine and nitrogen of azo group.

Table 5: Main band of FTIR for the ligand (AHS) and its Complexes

Compound	AHS	[Zn(AHS)Cl]	[Cd(AHS)Cl]	[Hg(AHS)Cl]
$\nu(\text{OH})$	3479 w	3450 w	3461w	
$\nu(\text{C=O})$	1652 m 1616	1610 s.	1612 s.d 1596	
$\nu(\text{N=N})$	1496 m	1475 m.d 1440	1475 m.d 1436	
$\nu(\text{M-N}_{\text{azo}})$	—	441 m.	437 m.	430 m.
$\nu(\text{M-Cl})$	—	244 sh.	275 sh.	260 sh.
$\nu(\text{M-O})$	—	505 b.	501 s.	510 s.

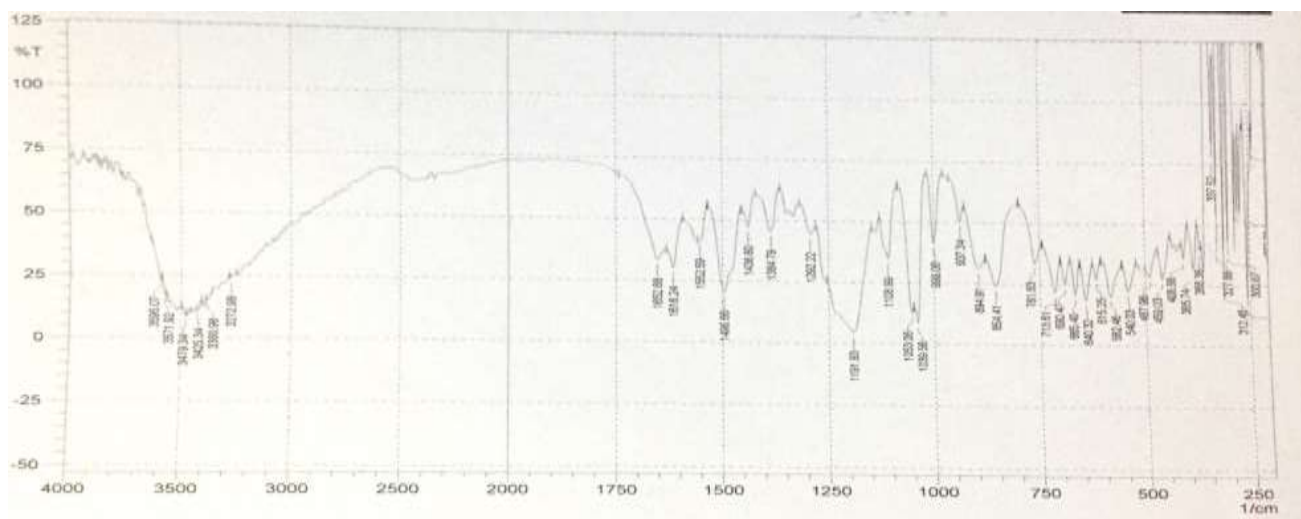


Fig.8: FTIR spectrum of AHS ligand

The HNMR Spectra

The (AHS) ligand and the diamagnetic [Cd (II)-AHS] complex were recorded HNMR spectra in DMSO solution with TMS as an internal standard. In the HNMR spectrum for the free ligand noticed a signal of one proton at ($\delta=5.85$ ppm) that approved the presence of hydroxyl moiety in naphthol ring.

This signal was disappeared in the HNMR spectrum of the complex which was indicated that hydroxyl moiety is involved in the coordination ring after the proton loss. As well as a broad singlet signal at ($\delta=3.88-3.92$ ppm, 5H) attributed to (Ph-N) in antipyrine moiety. At the same time the multiple signal at ($\delta=7.33-8.55$ ppm, 4H) in the spectrum for ligand which was referred to aromatic protons of naphthol ring. A slightly chemical shift was noticed in the spectrum of [Cd (II)-AHS] due to complex formation. Aliphatic proton for methyl moiety were

appeared at (1.80 ppm, 6H) as a singlet in the spectrum of ligand this signal was appeared in the spectrum of the complex in the same place with change in shape [16,17].

Antibacterial Activity of Prepared Ligands and its Complexes

Two types of bacteria were used in this experiment, Gram Negative Bacteria, *Escherichia Coli (E-Coli)* and Gram Positive Bacteria, *Staphylococcus aureus (Staph)* and Ciprofloxacin used as reference .By using disc diffusion method [18]. All data are recorded in Table (6). The results explain that the ligand and its complexes have b more toxicity against *Staphylococcus aureus* what it is for *E-coli* except [Hg (II)-AHS] which were having high toxicity.

The inhibition activity for the ligand and its complexes can be explained on the basis of tweedy’s chelation theory [2].

Table 7: The Diameters (mm) of deactivation of two bacteria of the prepared ligand (AHS) and its complexes

Compound	G+(Staph)	G-(E-Coli)
AHS	24	10
Zn-AHS	19	12
Cd-AHS	17	10
Hg-AHS	19	22
Ciprofloxacin	18.15	18.5
Control (C) (DMSO)	0	0

Conclusion

Zn (II), Cd (II) and Hg (II) complexes with azo ligands (AHS) derived from 4-amino-antipyrine and 2-hydroxy-3, 6-disulphonic acid sodium salt. The prepared complexes were synthesized and characterized *via*

various physio-chemical methods. The analysis confirmed the structures and composition of the gained complex together. All synthesized compounds showed a powerful deactivating ability beside the thoughtful bacteria.

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