

Synthesis, Spectral and Antibacterial study of Azo compound derived from (3,3'-Dimethyl-biphenyl-4,4'-diamine) and its Metal complexes

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Abstract

The present study includes synthesis of azo compound 4,4'-Bis(resorcinol-azo)-3,3'-Dimethyl-biphenyl [BRADB], the azo compound ligand was prepared from reaction 3,3'-Dimethyl-biphenyl-4,4'-diamine with conc.HCl and NaNO₂ then with resorcinol. The metal complexes were prepared by reaction of some transition metals with the above ligand in ethanol. The prepared compounds were characterized by (infrared and electronic spectral measurements) and conductivity measurement. From the molar ratio method, the stoichiometry of the prepared complexes was found to be 2:1 (metal:ligand). The molar conductance data revealed that all the metal chelates were non-electrolytes. The spectral studies showed the geometry around Fe ion is distorted octahedral, while the geometry around Zr and V ions are square-pyramidal.

The azo compound ligand and the metal chelates were studied for antibacterial activities against (*Enterococcus* and *Staphylococcus epidermidis*) are Gram positive and (*Salmonella* and *Proteus*) are Gram negative. The azo compound ligand and its chelates were exhibited a variable activity of inhibition on the growth of the bacteria.

4,4'-Bis(resorcinol azo)-3,3'-Dimethyl-biphenyl

3,3'-Dimethyl-biphenyl-4,4'-diamine

[BRADB]

.resorcinol

)

(-

(1:2) :

()

(*Enterococcus* and *Staphylococcus epidermidis*)

(*Salmonella* and *Proteus*)

Introduction

Azo compounds, with two phenyl rings separated by an azo (-N=N-) bond, are versatile molecules and have received much attention in research areas both fundamental and application⁽¹⁾. The strong electronic absorption maximum can be tailored by ring substitution to fall anywhere from the ultraviolet to red-visible regions, allowing chemical fine-tuning of color⁽¹⁾. This, combined with the fact that these azo groups are relatively robust and chemically stable, has prompted extensive study of azobenzene-based structures as dyes and colorants. Furthermore, the light-induced interconversion allows systems incorporating azo group to be used as photoswitches, effecting rapid and reversible control over a variety of chemical, mechanical, electronic and optical properties⁽²⁾.

Azo compounds have a great biological activity as well as industrial importance^(3,4). The interaction of metal complexes with biological systems, which is the field of biocoordination chemistry, is receiving an increasing interest⁽⁵⁾.

The present reports a synthetic, spectral and antibacterial study of azo compound (ligand) [BRADB] and its metal complexes.

Experimental

- All reagents and solvents were obtained from commercial sources and used as received.
- Melting points were determined on a Gallen kamp capillary melting point apparatus.
- Infrared spectra (in KBr pellets) were recorded on FT-IR testscan shimadzu model 8400s at college of science in Karbala University.
- The electronic spectra of the ligand and complexes were recorded on a UV-1800 Shimadzu spectrophotometer in DMSO.
- Molar conductance measurements were determined in DMSO by using WTW-Terminal 740 digital conductivity.

Synthesis of 4,4'-Bis(resorcinol azo)-3,3'-Dimethyl-biphenyl [BRADB]⁽⁶⁾

To a mixture of 3,3'-Dimethyl-biphenyl-4,4'-diamine (3.1g, 0.0146mol) and distilled water (15ml) contained in a beaker, concentrated hydrochloric acid (4ml) was added with cooling and the mixture was cold at (0°C) in an ice bath, a solution of sodium nitrite (1.3g, 0.0188mol) in (10ml) of distilled water was added dropwise to the mixture with stirring, the temperature of the ice bath was controlled between (0-5°C). A solution of (1.6g, 0.0146mol) of resorcinol in (15ml) of [10%] sodium hydroxide solution in (150ml) beaker was prepared and cold to (5°C) by immersion in an ice bath. The resorcinol solution was stirred vigorously and then the cold diazonium salt solution was added very slowly to the resorcinol solution, when all the diazonium salt solution was added, the mixture was allowed to stand in an ice bath for thirty minutes with occasional stirring. The solution was filtered, washed well with distilled water and dried upon filter paper.

Synthesis of metal complexes

The various complexes were prepared by addition of (0.01mole) of FeCl₃ (1.62g), ZrOCl₂.8H₂O (3.22g) and VOCl₂.H₂O (1.55g) dissolved in (20ml) of ethanol to a stirring (0.005mole) of the azo compound ligand [BRADB] (2.27g) in (20ml) of ethanol. The pH of the reaction mixture was adjusted to ≈9 by using the sodium hydroxide. The resulted mixture was left at room temperature for thirty minutes; the precipitated solids were filtered, washed with ethanol and dried over anhydrous calcium chloride.

Antibacterial Activity

The agar-well diffusion method according to Perez *et. al.*⁽⁷⁾ was used to screen the antibacterial activity of each extract. Aliquots of (20mL) of Mueller-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)hours nutrient broth cultures of the following

bacteria (Gram positive: *Enterococcus* and *Staphylococcus epidermidis*) and (Gram negative: *Salmonella* and *Proteus*). 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 thirty 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 thirty minutes to allow for diffusion before incubating at (37°C) for (24hours). The produced zones of inhibition were measured for each organisms screened.

Results and discussion

The molar conductivity, percentage yields and melting points are presented in Table (I). The molar conductances of the compounds that dissolved in DMSO were below $15.00 \text{ ohm}^{-1} \cdot \text{cm}^2 \cdot \text{mol}^{-1}$ indicating that they were non-electrolytes ⁽⁸⁾. The three values suggest that no anions present outside the coordination spheres. In order to obtain the type of the complexation process (number of metals ion that bind with the ligand), a mole ratio method was used to study the ratio between the ligand and Fe(III), Zr(IV) and V(IV) ions. In this method, a series of different volumes solutions of the ligand were mixed with different volumes of Fe(III), Zr(IV) and V(IV) ions solution to obtain a constant volume each time (10ml). The results showed that the molar ratio of metal:ligand was (2:1). These chelates are powders stable in atmospheric conditions and insoluble in most common organic solvents (Table (II)).

Table (I): Some physical properties of azo compound ligand and the prepared complexes.

Compound	M.Wt. g.mol ⁻¹	M.P. (°C)	Color	Yield (%)	Conductivity in DMSO ohm ⁻¹ .cm ² .mol ⁻¹
[BRADB]	454.26	275-277	Brawn	80	-
Fe(III)-complex	623.21	>300	Reddish brawn	70	13.12
Zr(IV)-complex	810.48	>300	Green	63	11.00
V(IV)-complex	729.92	>300	Dark green	58	14.00

Table (II): Solubility of azo compound ligand and the prepared complexes.

Solvent Comp.	H ₂ O	Me-OH	Et-OH	CHCl ₃	DMSO
[BRADB]	+	+	+	+	+
Fe(III)-complex	-	-	-	÷	+
Zr(IV)-complex	-	-	-	÷	+
V(IV)-complex	-	-	-	÷	+

Soluble = + , Insoluble = - , Slightly soluble = ÷ .

Infrared spectra of the azo compound ligand and its chelates

The synthesis of the azo compound was accomplished in accordance with the sequence of reaction in scheme (1). The infrared spectral data is shown in Table (III) the assignment of infrared bands the azo compound and its chelates with Fe(III), Zr(IV) and V(IV) ions are in agreement with the expectation Figures (1,2,3). These data are in agreement with those previously reported for similar compound⁽⁹⁾.

The broad band at (3418cm⁻¹) in the free azo compound ligand spectrum Fig.(1) which assigned to $\nu(-OH)$ stretching. The low frequency and the broadening of this band suggest that this ligand has a strong hydrogen bonding ($-O-H \cdots N$) in the solid state^(10,11). This band

remains in the same region in free ligand and in complexation. Thus, the remaining of another hydroxyl group intact in solid complexes indicates its non-involvement in coordination of the ligand to the metal ions.

The $\nu(-N=N-)$ stretching vibration appears at (1485cm⁻¹) in the free ligand spectra. This band appearing at (1400-1475)cm⁻¹ in the IR spectra of all the metal chelates. The movement of $\nu(-N=N-)$ stretch of the complexes to relatively lower energy compound to that of free ligand indicates coordination via the $-N=N-$ group⁽⁹⁾.

The spectrum of the ligand shows a absorption band at (1269cm⁻¹) due to $\nu(C-O)$. It is observed with a change in shape and shifted to higher frequencies at (1300-1365)cm⁻¹ in the metal chelates.

These differences suggest the linkage of metal ion with oxygen⁽¹²⁾.

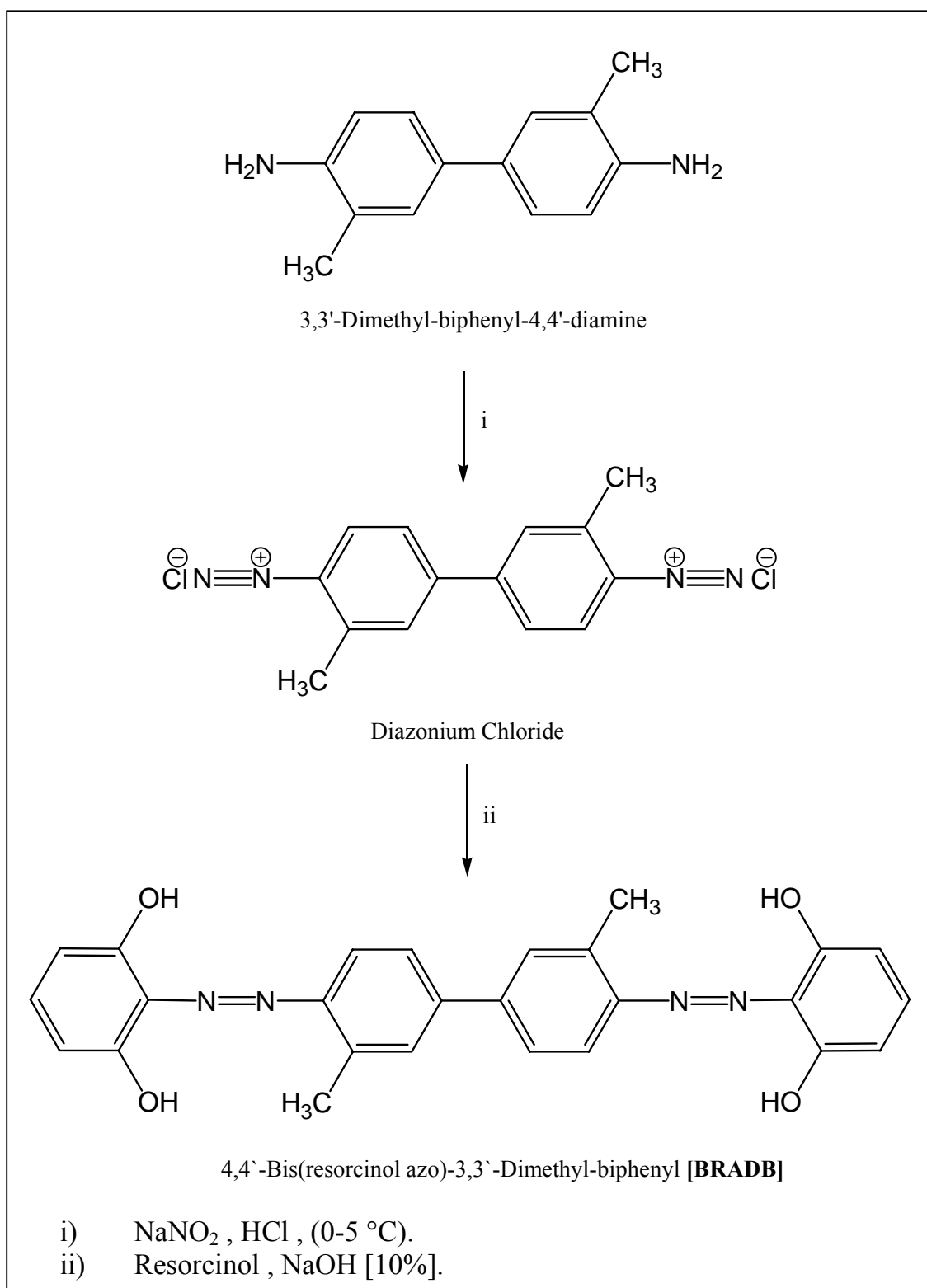
New bands which are not present in the spectrum of free ligand appeared at $(515-588)\text{cm}^{-1}$ and $(460-470)\text{cm}^{-1}$ are attributed to $\nu(\text{M-O})$ and $\nu(\text{M-N})$ vibrations, respectively. The appearance of $\nu(\text{M-O})$ and $\nu(\text{M-N})$ vibrations support the involvement of oxygen and nitrogen atoms

in chelation with the metal ions under investigation⁽¹³⁾.

The most important conclusion drawn from this discussion is that the azo compound ligand is coordinated to the central metal ion as NO bidentate ligand. The free bonding sites are the central azo nitrogen atom and the oxygen of the hydroxyl group.

Table (III): Characteristic IR (cm^{-1}) absorption bands of azo compound ligand and the prepared complexes.

Compound	ν (-OH)	ν (C-H) arom.	ν (C-H) aliph.	ν (-C=C-) arom.	ν (-N=N-)	ν (C-O)	ν (M-O)	ν (M-N)	$\delta(\text{C-H})$ arom. o.o.p.
[BRADB]	3418	3020	2850	1593	1485	1269	-	-	819
Fe(III)-complex	3392	3080	2980	1520	1475	1331	588	460	830
Zr(IV)-complex	3385	3020	2941	1630 1556	1400	1365	520	470	837
V(IV)-complex	3390	3100	2949	1643 1560	1400	1300	515	465	831



Scheme (1): Preparation of the [BRADB].

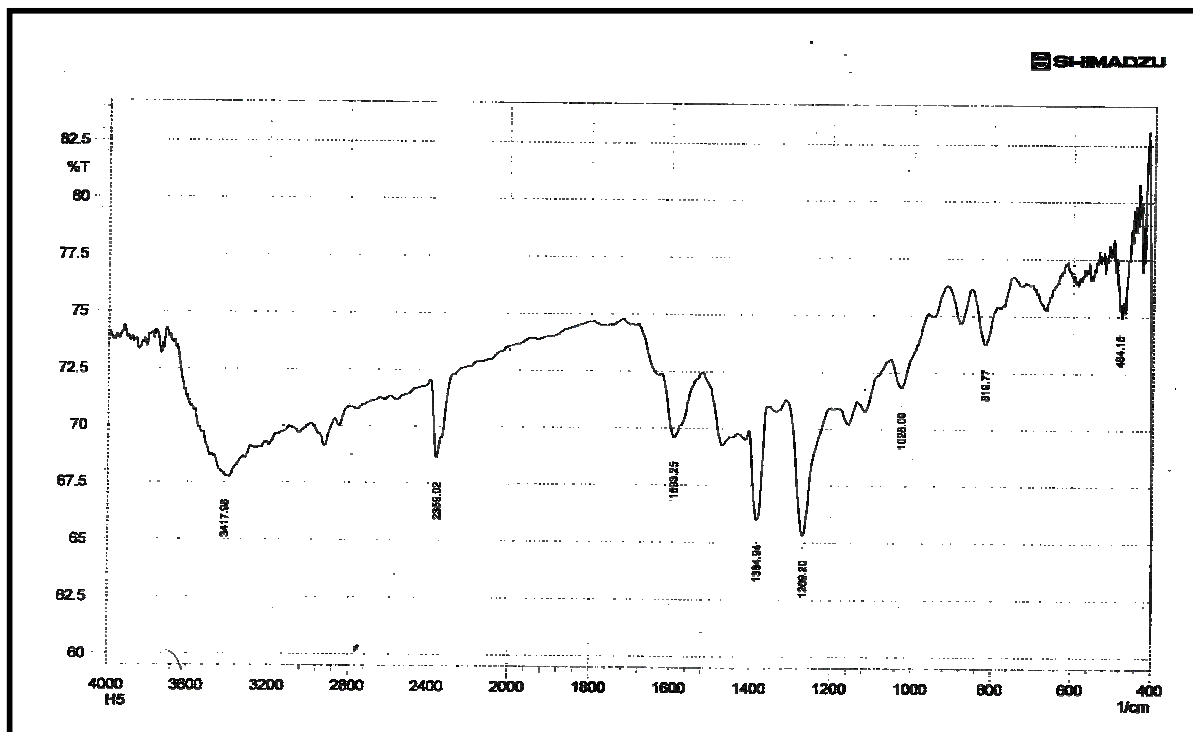


Fig.(1): FT-IR spectrum of (BRADB)

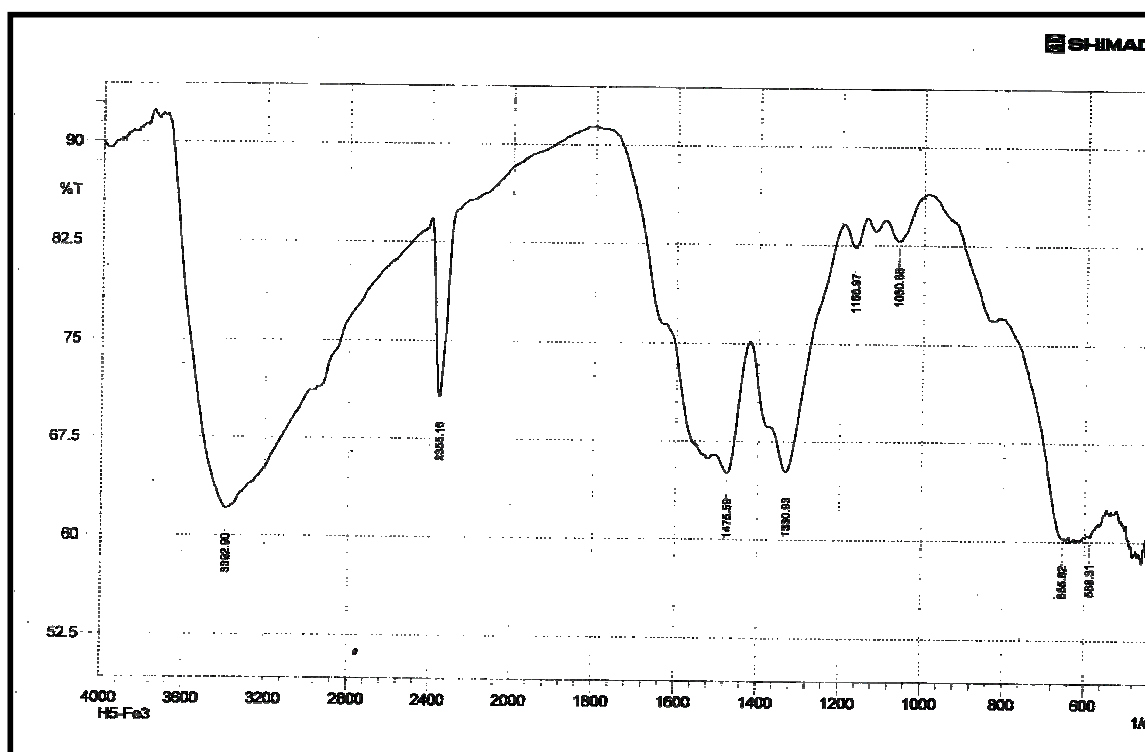


Fig.(2): FT-IR spectrum of Fe(III)-complex

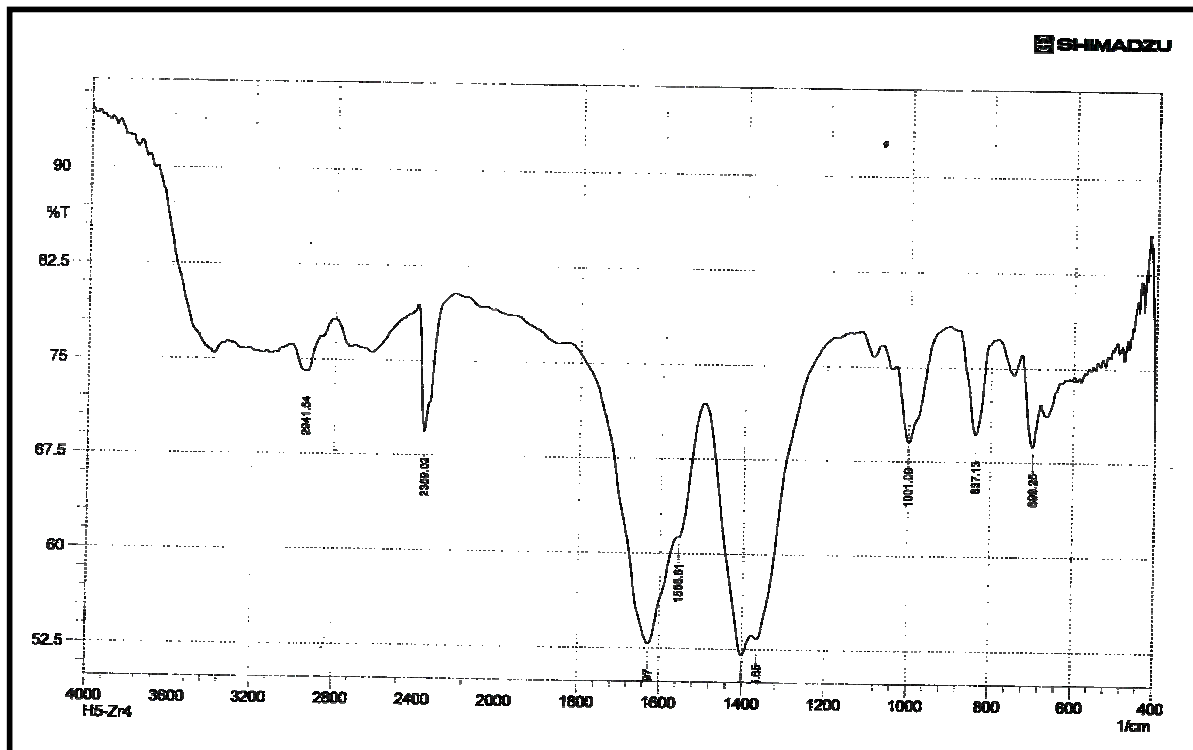


Fig.(3): FT-IR spectrum of Zr(IV)-complex

Electronic absorption spectra of the azo compound ligand and its chelates

The electronic absorption spectra of the ligand, Fig.(4) showed two absorption peaks at exhibits (257nm) (38910cm^{-1}) which is assigned to ($\pi \rightarrow \pi^*$) transition. Another peak at (374nm) (26737cm^{-1}) assigned to ($n \rightarrow \pi^*$) transition⁽¹⁴⁾.

For Fe(III) chelate, Fig.(5) the spectral data show three bands at (243nm) (41152cm^{-1}), (407nm) (24570cm^{-1}) and (465nm) (21505cm^{-1}), the first band is due to the charge transfer, but the second band is attributed to ${}^6A_1 \rightarrow {}^4E(G)$ transitions and the third band is due to ${}^6A_1 \rightarrow {}^4T_2(G)$ transitions. The spectra suggest distorted octahedral geometry⁽¹⁵⁾. While the Zr(IV) and V(IV) chelates \rightarrow are diamagnetic with no ligand field transition. The electronic spectra of these complexes exhibit high intense charge transfer transition in the region \rightarrow (385-420)nm ($25974\text{-}23805\text{cm}^{-1}$) which are assigned to intraligand transitions⁽¹⁶⁾. The correlation of the experimental data

allows assigning an square-pyramidal for the Zr(IV) and V(IV) chelates. The proposed structural representations are presented in Figures (6 & 7).

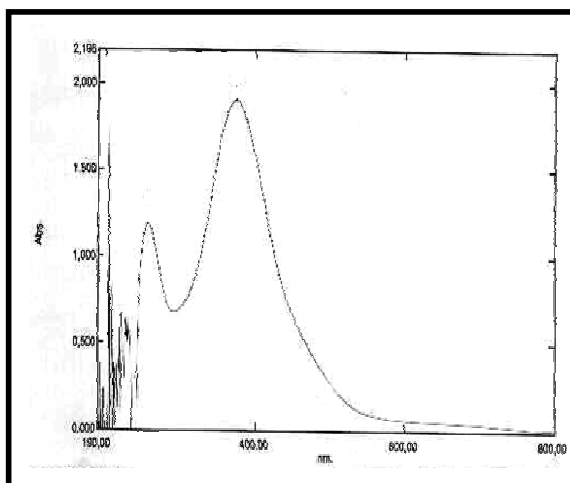


Fig.(4): UV-Vis. Spectrum of (BRADB) complex

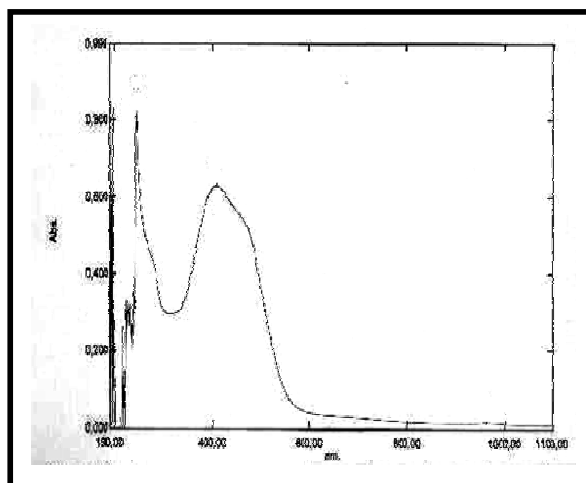


Fig.(5): UV-Vis. Spectrum of Fe(III)-complex

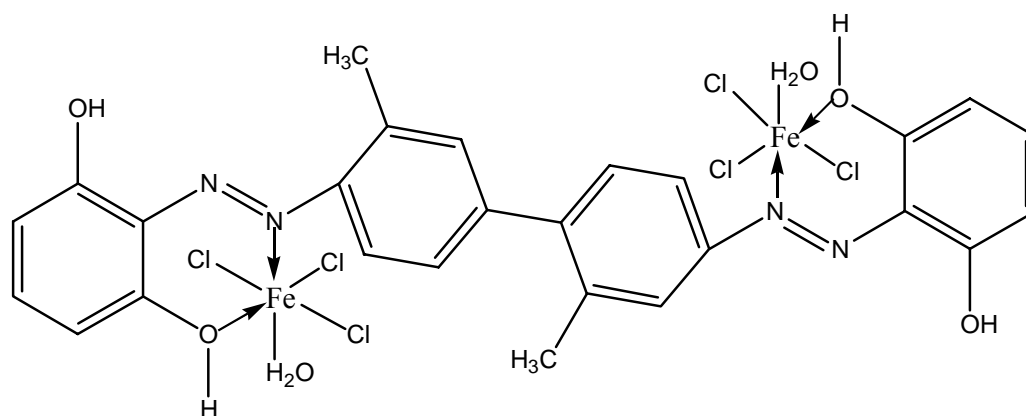
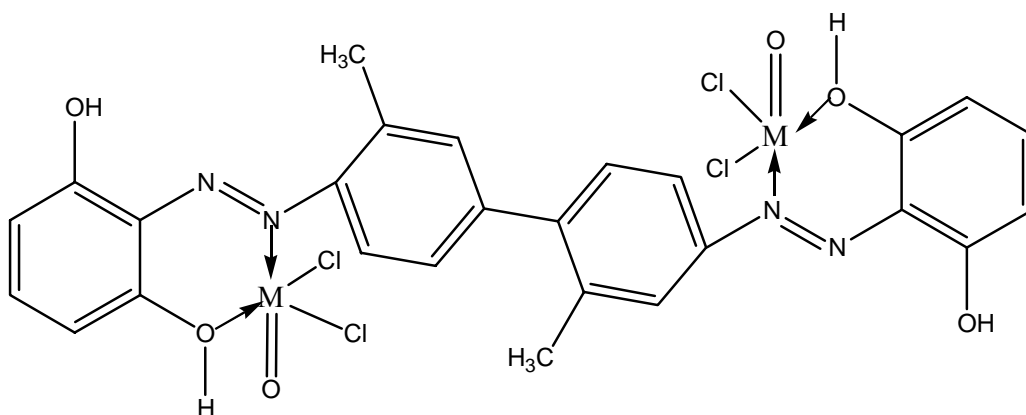


Fig.(6): The proposed structural formula of Fe(III)-complex.



Where M= Zr(IV) and V(IV)

Fig.(7): The proposed structural formula of Zr(IV)-complex and V(IV)-complex.

Antibacterial Activity

The antibacterial test was done according to well-plate method⁽⁷⁾, by using Muller-Hinton agar by inoculating (50 μ L) of fresh culture broth (18hours) of the tested bacteria [gram positive bacteria: *Enterococcus* and *Staphylococcus epidermidis*] and [gram negative bacteria: *Salmonella* and *Proteus*] for (24hours) at (37°C).

As shown in Table (IV) the azo compound ligand [BRADB] and its chelates (Fe(III)-complex, Zr(IV)-complex and V(IV)-complex) exhibited a variable activity on which *Enterococcus* was sensitive to azo compound ligand [BRADB], Fe(III)-complex and Zr(IV)-complex and resistance to V(IV)-complex. The *Staphylococcus epidermidis* and *Proteus* were sensitive to V(IV)-complex and resistance azo compound ligand [BRADB], Fe(III)-complex and Zr(IV)-complex where the *Salmonella* was sensitive to azo compound ligand [BRADB] and resistance to all its chelates. From other side when compared the complexes with the main compound. It was shown that azo compound ligand [BRADB] exhibit a strong activity against *Enterococcus* and *Salmonella* and no effect against *Staphylococcus epidermidis* and *Proteus*. The V(IV)-complex lost its effect in *Enterococcus* and *Salmonella* while display a high activity towards *Staphylococcus epidermidis* and *Proteus*, the Zr(IV)-complex and Fe(III)-complex exhibite a moderate activity against *Enterococcus* and lost its activity towards *Staphylococcus epidermidis*, *Salmonella* and *Proteus*.

Table (IV): Antibacterial activity of the azo compound ligand and its chelates expressed as zone of inhibition (mm) using agar well diffusion method.

Compound	<u>Enterococcus</u>	<u>Staph epidermidis</u>	<u>Salmonella</u>	<u>Proteus</u>
[BRADB]	+++	-	+++	-
Fe(III)-complex	++	-	-	-
Zr(IV)-complex	++	-	-	-
V(IV)-complex	-	+++	-	++

(+++) high active - Inhibition zone > 20-30mm

(++) moderate active - Inhibition zone > 10-20mm

(-) Inactive < 6mm

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