Preparation, Spectroscopic and Biological Activity studies of the ligand 4,4'-Bis(4- amino- 2-hydroxy phenyl azo)- *O*-tolidine and some of its metal complexes

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Abstract

Fe(II), Mn(II) and Cr(III) metal complexes of a new aryl azo 4,4'-Bis(4-amino- 2-hydroxy phenyl azo)- *O*-tolidine [BAHPAT] the ligand was achieved preparation according to general procedure to prepare of azo ligand. Characterization of the ligand and its complexes were investigated by malting point , molar conductivity, FT-IR and electronic spectra which helped us to find the chemical structure of those complexes that showed the suggestion geometry around the Fe(II) , Mn(II) and Cr(III) ions are octahedral . Stoichiometry of ligand-metal were found to be (1:2) for the complexes. The work included the determination of stability constants of the complexes resulting from the reaction of that dye with the Lewis acid $FeCl_2$,$MnCl_2$ and $CrCl_3$ in ethanol by employing a spectrophotometric . The biological activity of complexes against four types of bacteria (*Enterococcus , Stap epidiermidis*) as example of gram positive, (*Salmanella , Proteus*) as example of gram negative bacteria was studied.

Introduction

Azo compounds are a very important class of chemical compound receiving attention scientific research. They are highly colored and have been used as dyes and pigments for along time (1) .Furthermore , they have been studied widely because of their excellent thermal and optical properties in applications such as optical recording medium(2,3) . toner (4) ink-jet printing(5) and oil-soluble lightfast dyes(6).Also the azo compounds constitute one of the largest classes of industrially synthesized organic compounds. They are important in drugs and cosmetics (7) and show a variety of interesting biological activities including antibacterial (8).

Recently, azo metal chalets have also attracted increasing attention due to their interesting electronic and geometrical features in connection with their application for molecular memory storage, nonlinear optical elements, and printing systems(9). 3,3'-dimethyl-4,4'-biphenyldiamine is toxic and carcinogenic in rodents; human data are not available. 3,3'-dimethyl-4,4'-biphenyldiamine is used as an intermediate in the manufacture of dyes and in the chemical laboratory in various analytical procedures, including determination of free chlorine in water(10).

The present study reports the synthesis ,characterization ,analytical and biological activity study of new azo imidazole ligand (BAHPAT), and some of it's metal complexes.

Experimental

Apparatus and materials

All the reagents and solvents were of reagent-grade quality and purchased from commercial suppliers .The Melting points were determined on a Electrothermal melting point, GOWLLANDS, England. IR spectra were recorded using KBr discs 4000-400 cm-^{1} on FTIR Test scan Shimadzu model 8400S.while the UV-Vis. Spectra were recorded in ethanol on Shimadzu model 1800 . Molar conductance measurements were determined in DMSO by using a Alpha Digital conductivity meter model 800 and the pH measurements were carried out using WTW pH meter model 720.

Preparation of ligand

(0.53 gm, 0.025 mole) of 3,3'-Dimethylbenzidine 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 3-amino phenol (0.47g, 5mmole) dissolved in 50ml alkaline ethanol cooled below 0°C. After leaving in the refrigerator for 30minutes. A solution of one molar sodium hydroxide was used to precipitate the product the pH of the solution should be maintain at (9–10),A dark brown crystals were precipitated, and a recrystallization from ethanol was done twice before using, m.p.280 °C The percentage yield 68%. Ligand was prepared according to general procedure (11).The procedure was seen in Fig.(1).

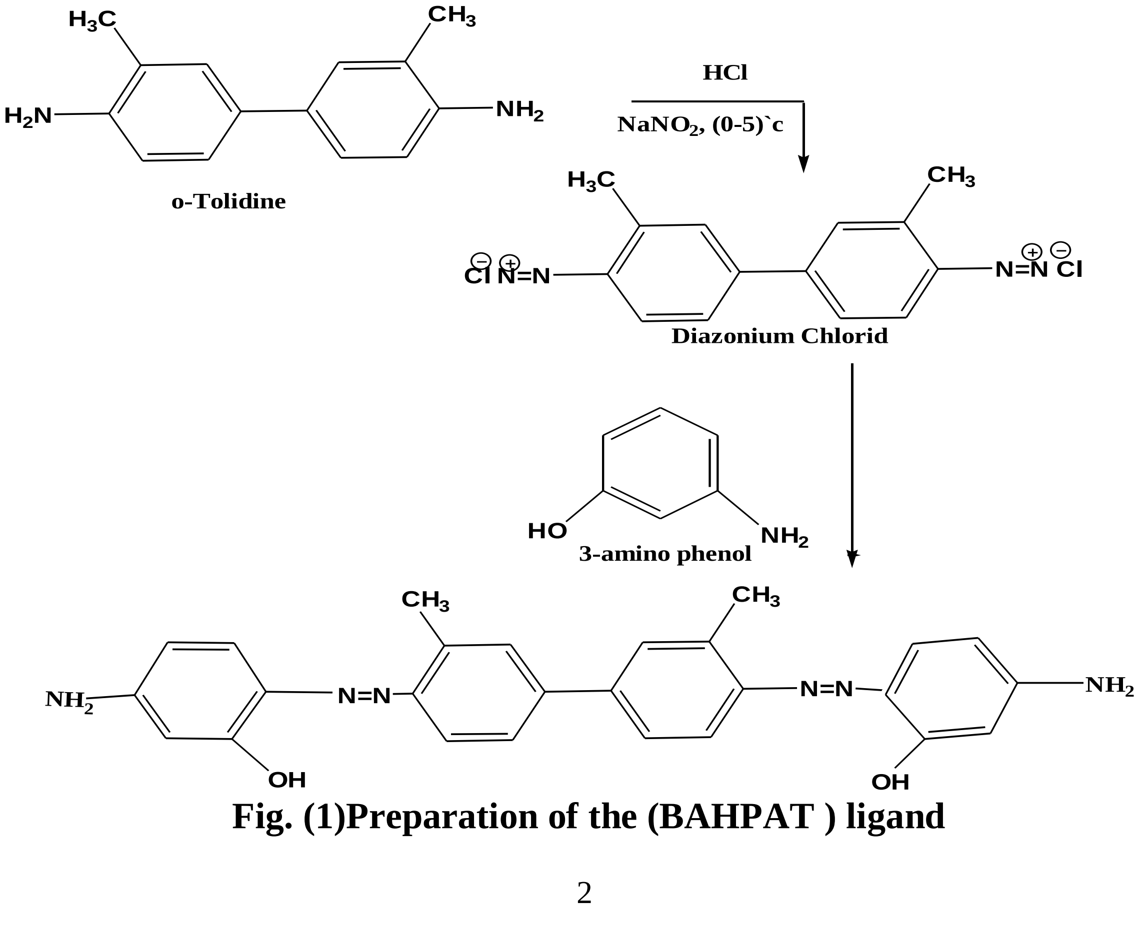

Fig. (1)Preparation of the (BAHPAT) ligand

Preparation of the solid complexes

To a ligand solution (5mmole in 10 ml ethanol), (10 mmole) of $MCl_2$and MCl_3 for the salts [M= Fe(II) ,Mn(II),Cr(III)] dissolved in (15 ml) ethanol was added drop wise with stirring. The pH of the reaction mixture was adjusted to ≈ (9) by using the sodium hydroxide .The mixture was refluxed for one hour then left over night. A solid complex was obtained, collected by filtration ,washed by ethanol several times and dried .

Characterization of ligand and its complexes

The complexes were insoluble in water but soluble in DMF and DMSO solvents. The ligand was Dark brown crystals, but the complexes of this ligand from red to brown color. Table (1) appears some physical properties of the prepared complexes.

Table (1) Physical properties of the ligand and it's complexes

Comp.	Formula	Color	Yield(%)	M.P(°C)
BAHPAT	$C_{28}H_{32}N_4O_2$	Dark brown	68	280
Fe^{II}- BAHPAT	$[Fe_2LCl_4(H_2O)_4]$	red	73	>300 dec.
Mn^{II}- BAHPAT	$[Mn_2LCl_4(H_2O)_4]$	Dark brown	61	>300 dec.
Cr^{III}- BAHPAT	$[Cr_2LCl_6(H_2O)_2]$	red	74	>300 dec.

Results and discussion

Molar Conductivity

The molar conductance of the complexes as shown in Table (2) were carried out in DMSO at room temperature , the values indicate that the complexes are non- electrolytes nature (12),where the values suggest that no anions present outside the coordination spheres .

Stiocheiometry of the complexes

Composition of the complexes were determined by Job's method and molar ratio method at fixed concentration and pH at maximum wavelengths of absorption. Both methods indicated that the ratio of metal ion to ligand molecules was (2:1). The results are given in Table 2.

Table (2) Metal : Ligand ratios ,Stability constant values and Molar Conductivity of the complexes.

Comp.	pH	λ_{max} nm	Metal :Ligand	β	Log β	Conductivity $S.mol^{-1}.cm^2$
Fe^{II}- BAHPAT	9.0	501	2:1	1.377×10^9	9.139	12.7
Mn^{II}- BAHPAT	8.0	486	2:1	9.68×10^8	8.985	11.4
Cr^{III}- BAHPAT	8.0	498	2:1	7.75×10^8	8.889	19.7

Stability constants are obtained spectrophotometricaly by measuring the absorbance of solutions of ligand and metal mixture at fixed wavelength λmax and pH values. The degree of formation of the complexes is obtained according

to the relationship $\beta = (1 - \alpha) / (4\alpha^3 c^2)$, and α = (Am -As)/Am, where As and Am are the absorbance of the partially and fully formed complex respectively at optimum concentration(13). The calculated β and Log β values for the prepared complexes are recorded in Table 2, the stability follows the sequence; Cr(III) < Mn (II)<Fe(II) the sequence of metal ions of the first row transition metal agree with Irving-Williams series of stability constant(13).

IR spectra

The infrared band assignments of the ligand complexes of Fe(II),Mn(II) Cr(III) ions (Table3). The ν(N═N) stretching vibration appears at 1383 cm^{-1} in the free ligand spectra, this band appearing at (1350-1320) cm^{-1} with different in shape and reduced in intensity in the spectra of complexes , band shifted and reduced intensity due to complex formation .(14) The broad band at (3600)cm^{-1} in the free ligand spectrum Fig.(6) which assigned to υ(-OH) stretching.(15)There is also a shifting noticed in the υ (C-O) group of phenol group from 1269cm^{-1} toward (1285-1300)cm^{-1} producing another evidence about involvement of phenol group in coordination with metal ions via oxygen group . (16) New bands in the region (499-515) cm^{-1} and (620-580) cm^{-1} were assigned in the spectra of metal complexes. These bands were not present in the spectrum of ligand, and they due to ν(M─N)and υ(M-O) vibrations respectively .(17) The band in the range of (3400-3415)cm$^{- 1}$ and (3390-3395)cm^{-1} indicates that the NH_2 group is not participate in coordination with the metal ions under investigation.(18) The appearance of these bonds support the involvement of azomethine and hydroxyl groups via nitrogen and oxygen atoms in complexation.

Table (3) Characteristic IR absorption bands of the ligand and it's complexes in cm^{-1} units

Comp.	υ (-N=N-)	υ (-OH)	υ (NH_2) asym.-sym.	υ (C-H)Ar.	υ (C-O)	υ (M-O)	υ (M-N)
BAHPAT	1383 m	3200 m	3468-5450 m	3080 w	1269 s	---	---
Fe^{II}-BAHPAT	1350 w	----	3400-3390 m	3100 w	1300 w	620 m	499 m
Mn^{II}-BAHPAT	1320 w	----	3408-3390 m	3020 w	1290 w	615 m	493 m
Cr^{III}-BAHPAT	1350 w	---	3415-3395 m	3000 w	1285 w	580 w	517 w

s=strong , w=weak, m=medium ,

Electronic spectra

The electronic spectral data of the ligand and its complexes were recorded in DMSO and their assignments are listed in(table 4). The UV-Visible spectrum of the ligand, Fig.(4) shows absorption peak at (36363)cm^{-1} and (21929)cm^{-1} assigned to ($n \rightarrow \pi^*$) and ($\pi \rightarrow \pi^*$) transitions respectively (19). While the electronic spectra of the complexes Figs.(6-8) exhibited that The electronic

spectra o the Fe(II) complex spectrum of this complex show bands at 27397 and 21186 cm-1, which are suggesting the existence of ${}^6A_1g \rightarrow {}^4T_2g(F)$ and ${}^6A_1g \rightarrow {}^4A_1g$ transitions with an octahedral spatial configuration (20).The Mn(II) complex obvious peaks at 21186, 2702and 20408 cm-1 , the first peak are attributed to charge transfer while the the second and third peak is assigned d-d transitions type${}^6A_1g \rightarrow {}^4Eg(D)$ and ${}^6A_1g \rightarrow {}^4T_2g(G)$ in octahedral structure about Mn(II) complex (21). The Cr(III) complex have electronic spectrum shows a band centered at 27027and 20366cm-1 which may assigned to${}^4A_2g \rightarrow {}^4T_1g$ (F) and ${}^4A_2g \rightarrow {}^4T_2g$ transition respectively in octahedral environment (22).

Table (4) Electronic spectra of the ligand and it's complexes

Compound	λ nm	νcm^{-1}	Assignment
BAHPAT	275	36363	$n \rightarrow \pi^*$
	456	21929	$\pi \rightarrow \pi^*$
Fe^{II}- BAHPAT	272	36764	Ligand field
	365	27397	${}^6A_1g \rightarrow {}^4T_2g(F)$
	501	21186	${}^6A_1g \rightarrow {}^4A_1g$
Mn^{II}- BAHPAT	281	21186	Ligand field
	370	27027	${}^6A_1g \rightarrow {}^4Eg(D)$
	486	20408	${}^6A_1g \rightarrow {}^4T_2g(G)$
Cr^{III}- BAHPAT	244	40983	Ligand field
	370	27027	${}^4A_2g \rightarrow {}^4T_1g$ (F)
	491	20366	${}^4A_2g \rightarrow {}^4T_2g$

Suggested chemical Structure formula for the complexes

According to these results the structural formula of prepared complexes may be proposed in Fig. (2)and Fig.(3)

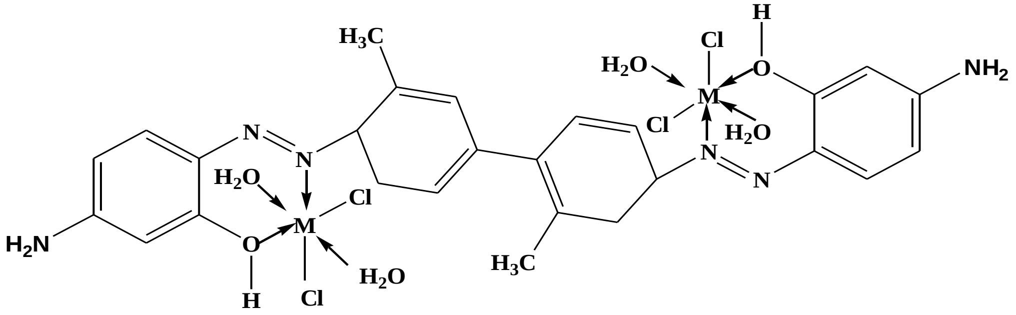

Fig.(2) The suggested chemical Structure formula for Fe(II), Mn(II) complexes

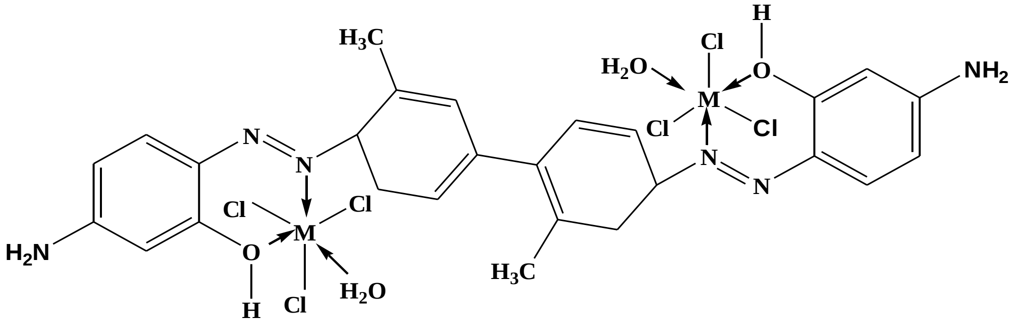

Fig.(3) Suggested chemical Structure formula for Cr(III) complex

Antibacterial Activity

The antibacterial test was done according to well – plate method(23).By using Muller Hinton agear by inoculating 50 µl of fresh culture broth (18 hrs) of the tested bacteria [gram positive bacteria : *Enterococcus* and *Staph epidermidis*] and [gram negative bacteria : *Salmonella* and *Proteus*_] for 24hrs at 37 °c. As shown in table (5) the ligand and its complexes the results demonstrate that *Enterococcus* was sensitive to ligand and its complexes while the *Proteus* showed a reverse activity which was resistance to ligand and its all complexes . the *Staph epidermidis* was sensitive to ligand and Cr(III) complex while resistance to Fe(II) and Mn (II) complexes . where *Salmonella* was sensitive to Cr(III) and Mn(II) complexes while resistance to ligand and Fe(II) complex .From other side when compared the ligand with its complexes , it was shown that the ligand and its complexes showed an activity against *Enterococcus* and *Staph epidermidis* while lost its activity towards *Salmonella* and *proteus* . The Cr(III) complex showed an activity against all bacteria except *Proteus* which lost its activity towards all tasted bacteria except the *Enterococcus* which exhibit a moderate activity . Finally Mn(II) complex showed an activity against to *Enterococcus* and *Salmonella* but no effect on *Staph epidermidis* and *proteus* .

Table(5) Biological activity of the 10ppm of the complexes Cr^{III}, Fe^{III} and Mn^{II} expressed as zone of inhibition (mm) using agar well diffusion method.

Comp.	Zone of Inhibition			
	Enterococcus	*Staph epidermidis*	*Salmonella*	*Proteus*
BAHPAT	++	+++	-	-
Fe^{II}- BAHPAT	++	-	-	-
Mn^{II}- BAHPAT	++	-	+++	-
Cr^{III}- BAHPAT	+++	+	+++	-

(+++) high active –Inhibition zone > 12 mm
(++) moderate active –Inhibition zone > 9-12 mm
(+) slightly active –Inhibition zone > 6-9 mm ,(-) inactive < 6 mm

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تحضير ودراسة طيفية وفعالية حيوية للمركب 4,4'-Bis(4- amino- 2-hydroxy phenyl azo)- *O*-tolidine وبعض معقداته

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الخلاصة:

حضر مركب الازو 4,4'-Bis(4- amino- 2-hydroxy phenyl azo)- *O*-tolidine [BAHPAT] حيث حضر بالطرق العامة لتحضير مركبات الازو كما حضر معقدات مع العناصر Fe(II), Mn(II) and Cr(III) . كما شخص اللكند والمعقدات بقياس درجة الانصهار و التوصيلية المولارية و تقنية مطيافية الاشعة تحت الحمراء ومطيافية المرئية و ما فوق البنفسجية وهذا ساعد في اقتراح التركيب الكيميائي للمعقدات (ثماني السطوح). ومن دراسة النسب المولية وجد ان نسبة الفلز الى اللكند هي 1:2 . كما تم حساب ثابت الاستقرارية لهذه المعقدات الناتجة من تفاعل هذه الصبغة مع حوامض لويس على هيئة كلوريدات باستخدام المطيافية الضوئية. و تضمن دراسة جوانب التأثير البايلوجي للمعقدات المحضرة في نمو اربع اجناس بكتيرية ممرضة الاولى والثانية منها موجبان لصبغة كرام وهما: (*Enterococcus , Stap epidiermidis*) والاثنان الاخران سالبة لصبغة كرام وهما: (*Salmanella , Proteus*) وباستخدام طريقة الحفر بالأكار وجد ان لهذه المركبات فعالية متباينة في تثبيط نمو البكتريا المدروسة.

Figures

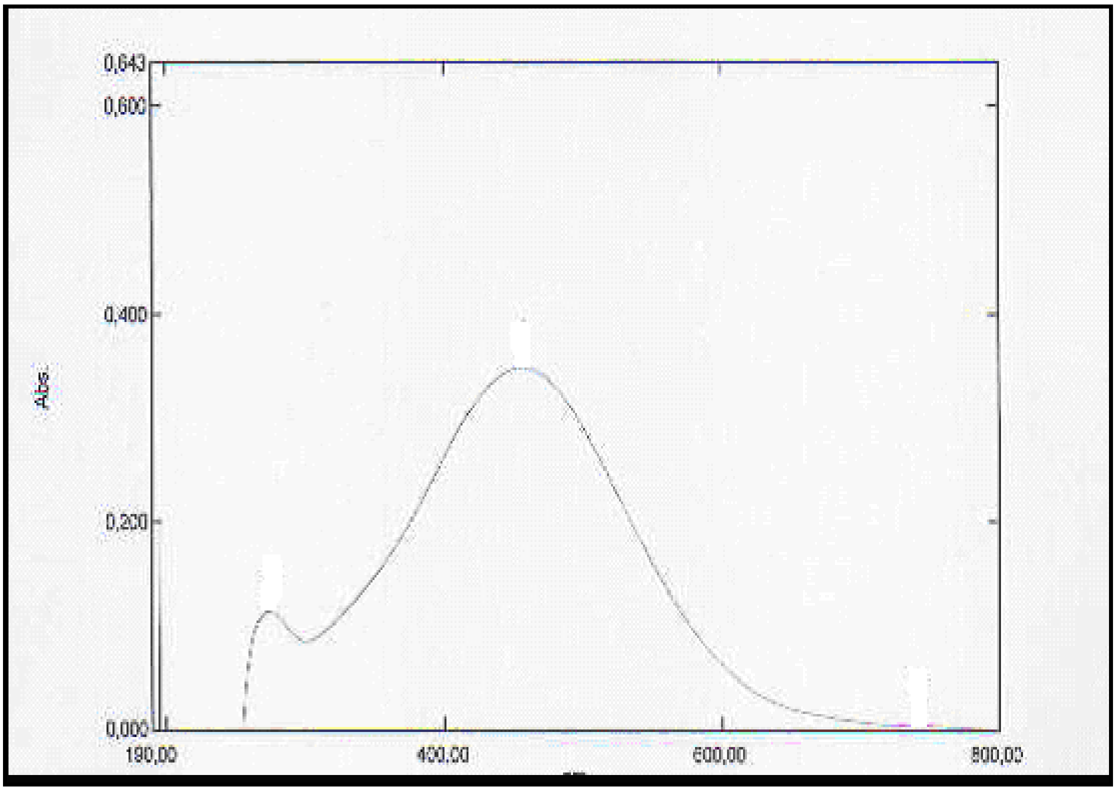

Fig.(4): ***UV-Vis. Spectrum of (*****BAHPAT)**

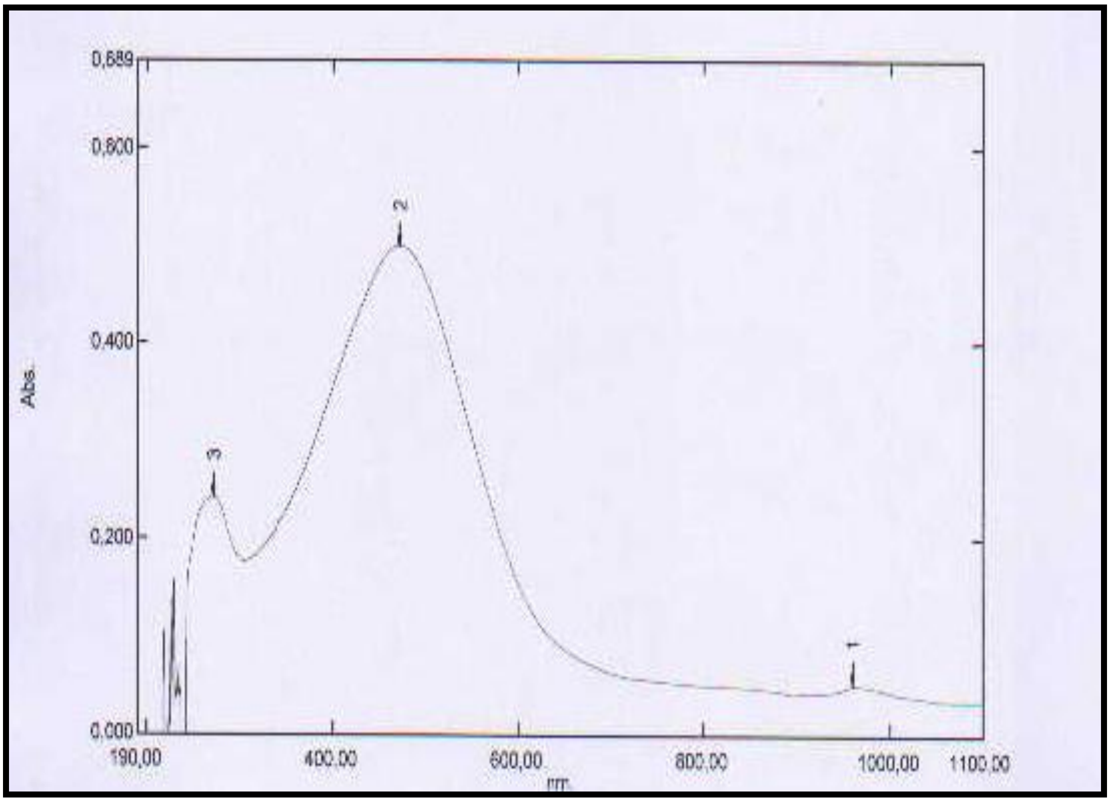

Fig.(5): ***UV-Vis. Spectrum of Fe(II) complex***

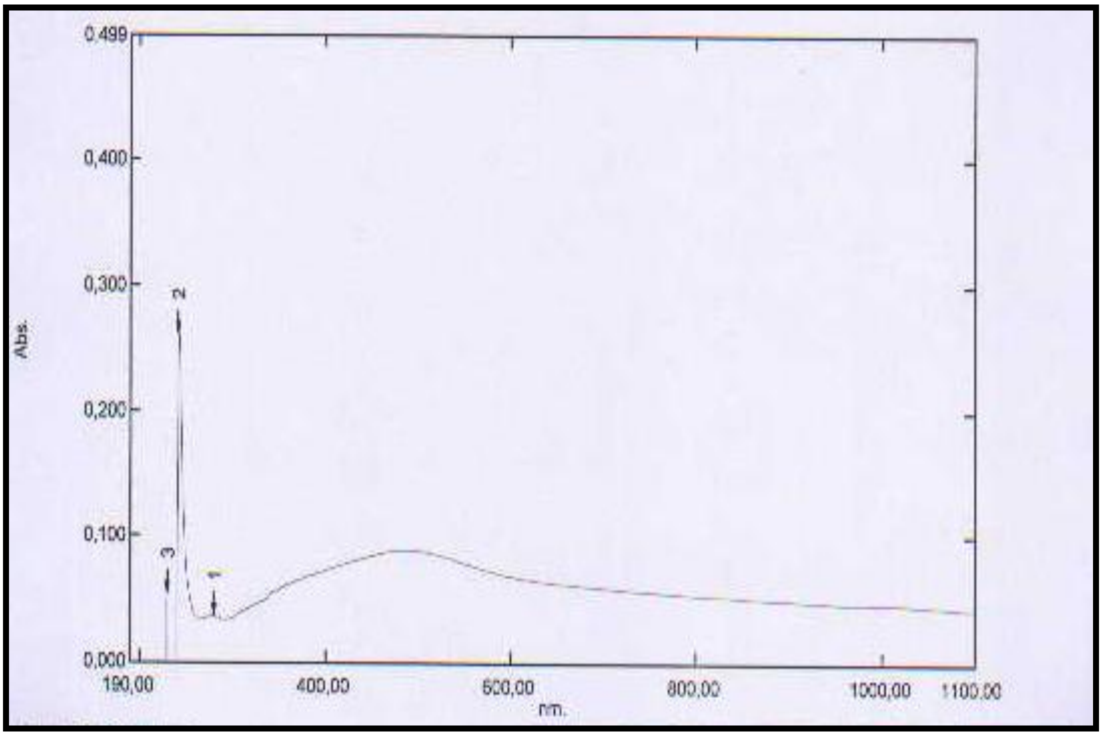

Fig.(6): ***UV-Vis. Spectrum of Mn(II) complex***

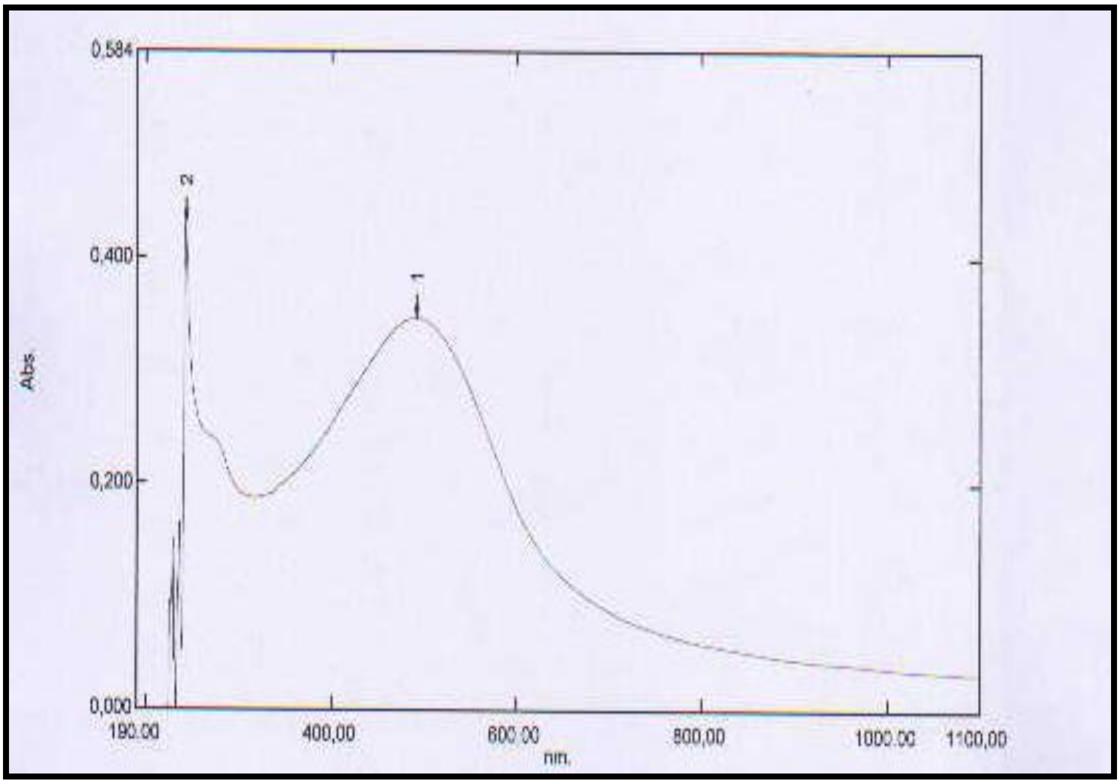

Fig.(7): ***UV-Vis. Spectrum of Cr(III) complex***

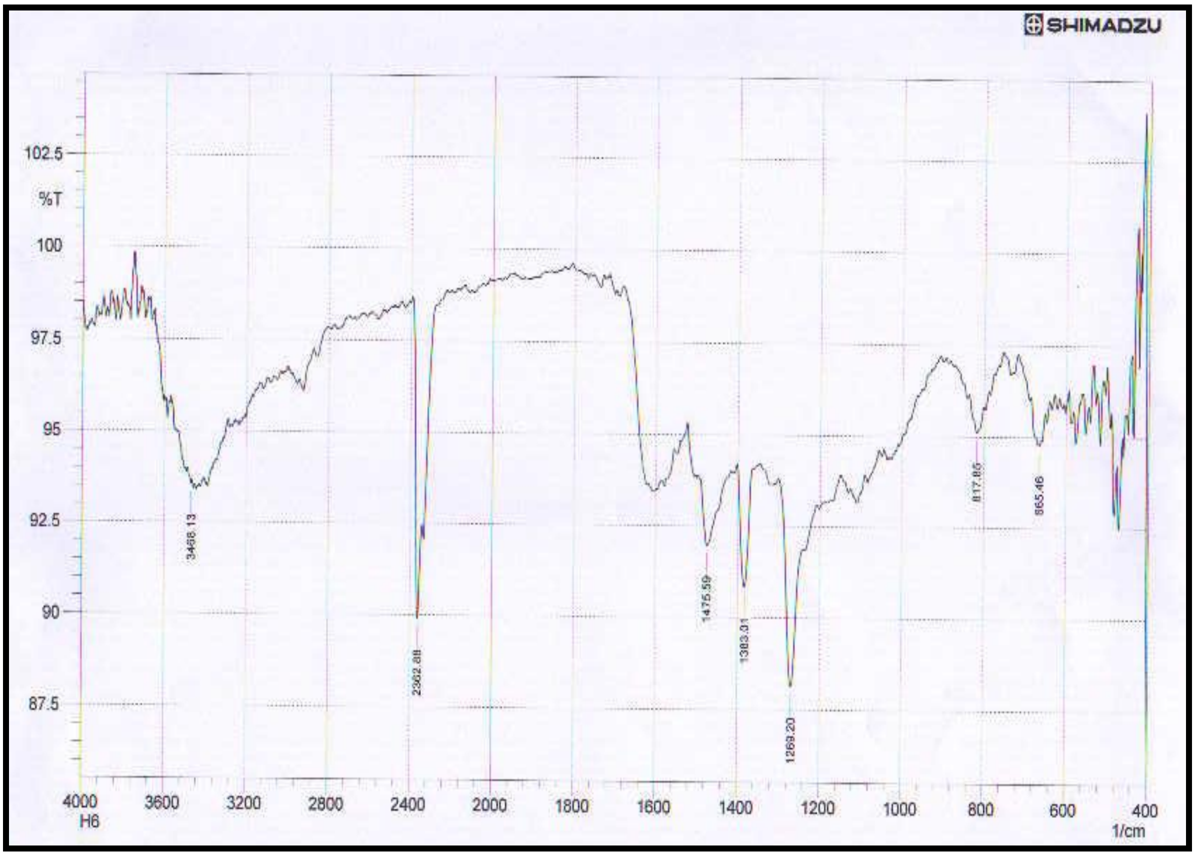

Fig.(8): ***FT-IR spectrum of (BAHPAT)***

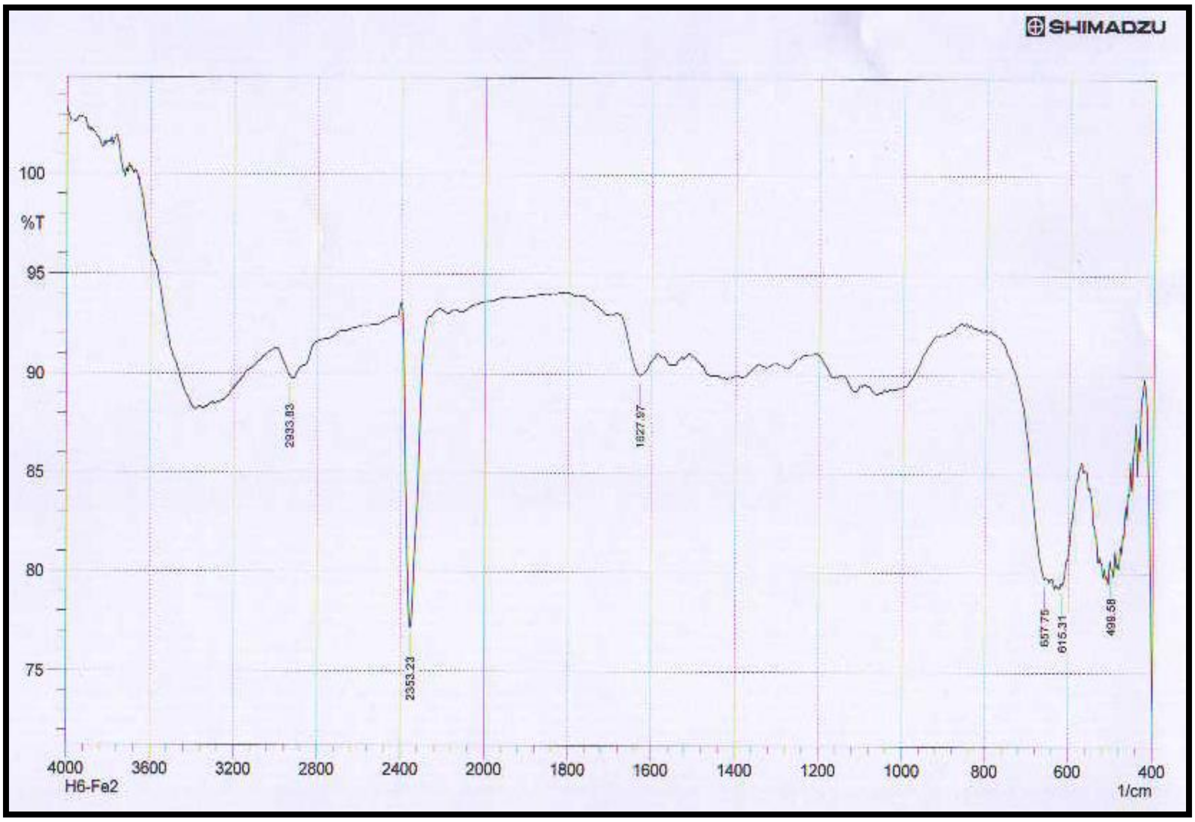

Fig.(9): ***FT-IR spectrum of Fe(II) complex***

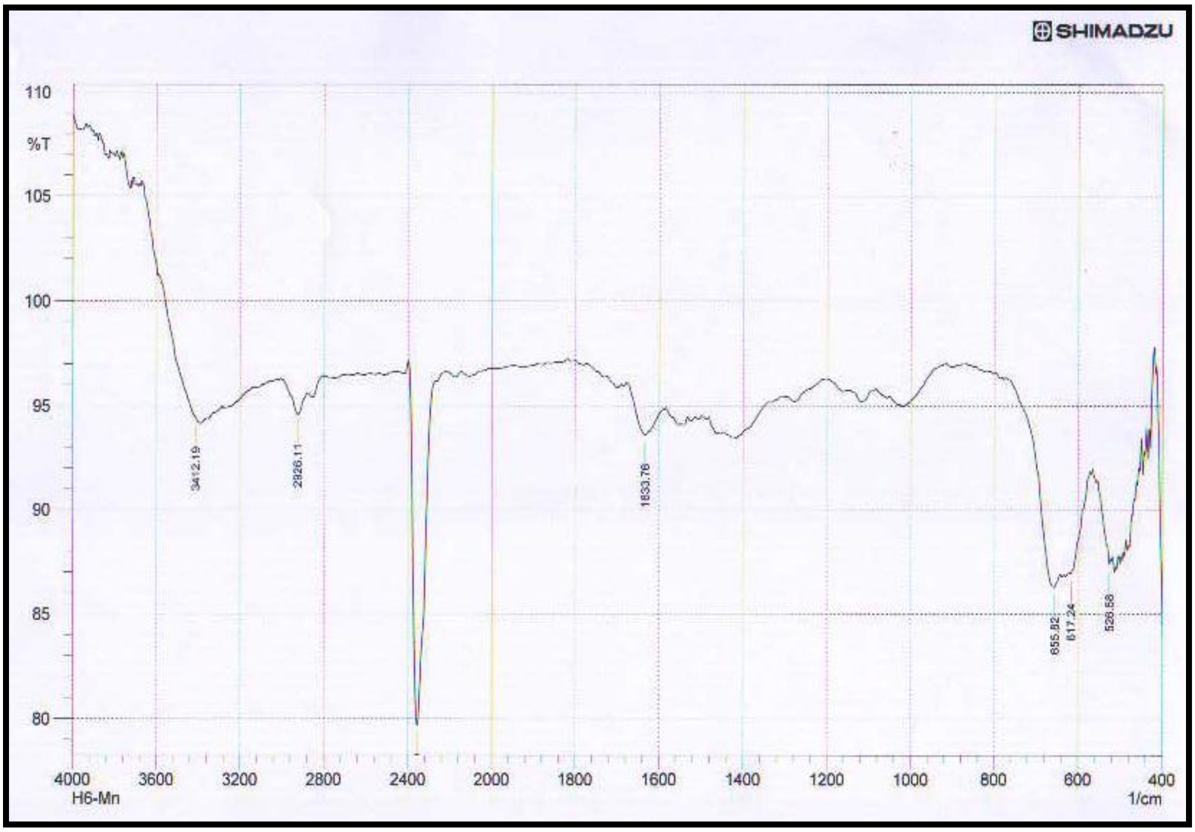

Fig.(10): ***FT-IR spectrum of Mn(II) complex***

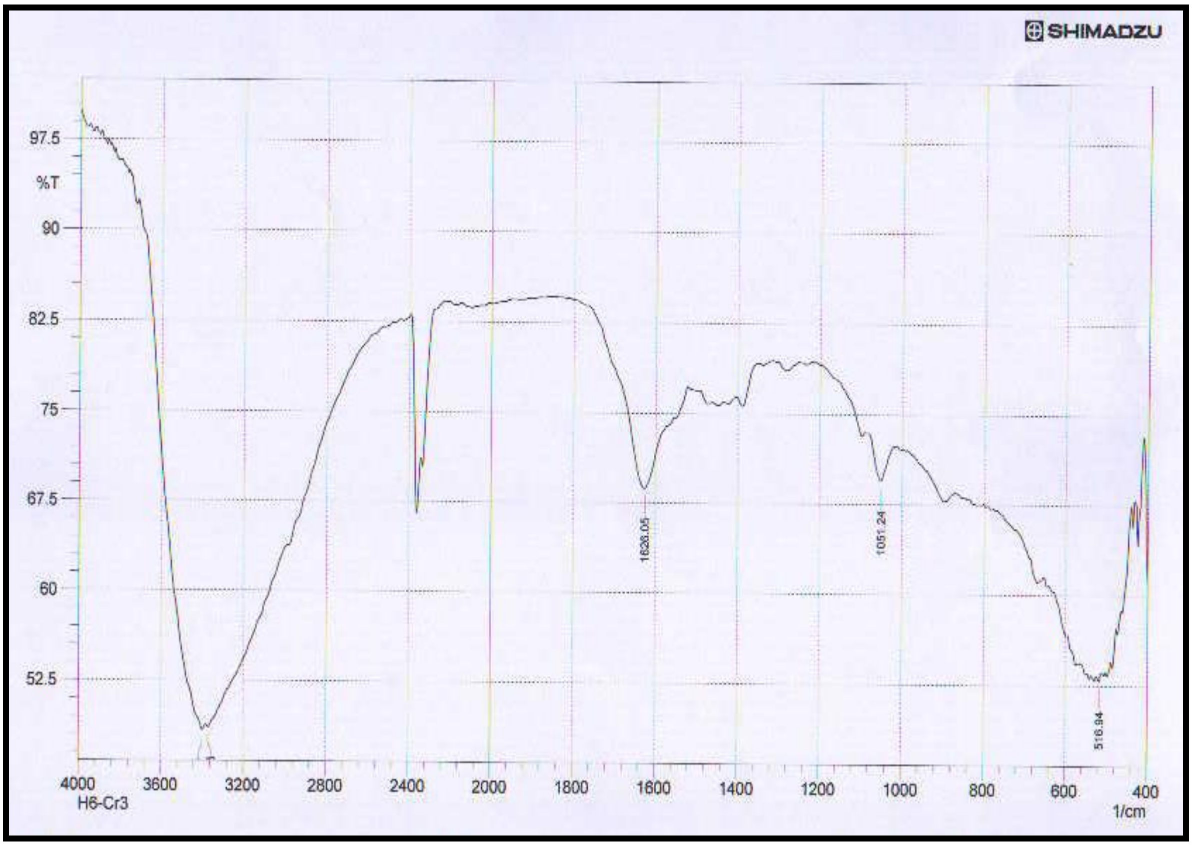

Fig.(11): ***FT-IR spectrum of Cr(III) complex***