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08 October 2017

Dear Author (s)

Dr./Mr. Khalid J .AL-Adilee / University of Al-Qadisiyah, Iraq

Dr./Mr. Sajjad H. Jawad / University of Al-Qadisiyah, Iraq

Greetings

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Synthesis, spectral and biological studies of metal complexes with heterocyclic ligand derived from thiazolylazo dye

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Abstract

Metal(II) complexes have been synthesized with new azo dye ligand 2-[2'-(4,5-dimethyl thiazolyl) azo]-4-benzyl phenol (DMeTABP=HL) derived from coupling reaction between 4,5-di methyl thiazolylazonium chloride and 4 – benzyl phenol in alkaline alcoholic solution. Azo dye ligand and metal complexes have been characterized by elemental analysis (C.H.N.S), ¹H-NMR , mass, Electronic, IR , XRD spectral data , SEM ,thermal analysis (TGA,DSC and DTG) , magnetic susceptibility , metal contents and molar conductance .The elemental analysis of the metal chelate complexes confirms the stoichiometry of the type [M(L)₂] , where M =Ni(II) , Cu(II) , Zn(II) and [M(L)₂]Cl and where M = Co (III) and L = azo dye ligand. The spectra and analytical data indicated that the ligand (DMeTABP) behaves as a tridentate chelating agent with 1:2 [M:L] complexes and octahedral geometry.

Conductance measurement suggests the 1:1 electrolytic nature of Co(III) complexes but non-electrolytic nature of the rest metal complexes. The biological activity of azo dye ligand and its metal complexes against the pathogenic activities was tested against the sensitive organisms *Escherichia coli* (gram negative), *streptococcus* ,*staphylococcus* (gram positive) as antibacterial and *Alternaria spp* as antifungal. The activity data show that the metal complexes were of higher antibacterial and antifungals activity than the free ligand. The results are discussed. The biological activity also conducted cells viability and cytotoxicity assay on Cu(II) and Zn(II) _complex by using the lines of cancerous liver cells of the type HePG2 and compared with line of the ordinary cells

Key words : Thiazolyl azo ligand , metal complexes. characterization ,antibacterial , antifungal and anticancerous.

Introduction

Thiazolylazo dyes derivatives are an important class of organic complex compounds. The main application of thiazolylazo dyes in spectrophotometry is based on the coloured compounds resulting from their reaction with most metal ions, especially transition metals in spectral determination field to determined metal ions in nanogram levels various fields because of high sensitivity and selectivity [1]. The applications of theiazolylazo dye derivatives in chemical operations in clude solid phase extraction, liquid chromatography and clod point extraction [2]. Thiazolyl azo dye compounds and its metal complexes are very important in catalytic, industrial and their applications in wide variety of pharmacological activity in antitumor [3] , anticancer [4] , antifungal and antibacterial [5] substance. In the field of industry thiazolylazo dyes are having applications in different field such as leather, polymer, paper, paint, textiles and coating industries as a dyeing agent [6]. The dyeing susceptibility of the azo dye compounds depends on the functional groups present in the dyes, such as nitro, methyl, halogens, hydroxyl, carboxylic acids, amines and esters [7]. Also metal complexes of the azo dyes are having extensive application in electro – photographic tones as charged controlling agents, developers in powder coating materials, electric materials and in electrostatic separation processes in inkjets and in colour filters [8].The application of thiazolylazo compounds in proceeding involving liquid – liquid and extraction is based on the large solubility of these reagents in organic solvent [9]. Thiazolylazo reagents have been used as masking agents or in procedures involving electro analytical methods.

In the present study we report the synthesis, spectral characterization of thiazolylazo dye ligand (DMeTABP= HL) and its metal complexes. The azo dye and its metal complexes were studied by various spectral analysis and screened for their biological activities. Thiazolyl azo dye ligand(DMeTABP), Cu(II)

and Zn(II) complexes were studied for the prescription drug anticancer by using the lines of cancerous liver cell of the type HePG2 and comparing them with line of the ordinary cells.

Experimental

Chemicals and Methods

2-Amino-4,5-dimethyl thiazole and 4-benzyl phenol were purchased from Aldrich chemical company and all analytical grade chemical were used without further purification. All other organic chemicals solvents and metal chlorids were available from multiple companies, Fluka, B.D.H, Sigma and Aldrich.

The Thin layer chromatography (TLC) performed in-order to check the preliminary purity of the thiazolylazo ligand using Merck silica gel 60 F254 coated aluminium plates as the stationary phase and methanol as developing solvent. The melting points of the azo dye ligand and its metal complexes were taken in an open capillary tube which was uncorrected by using electro thermal 9300. The $^1\text{H-NMR}$ (400MHz) spectra were taken in DMSO- d_6 solvent using Bruker 400 spectrometer and TMS was used as internal reference standard. FTIR spectra were taken on shimadzu 8400 δ FTIR spectrometer in KBr pellets (cm^{-1}).

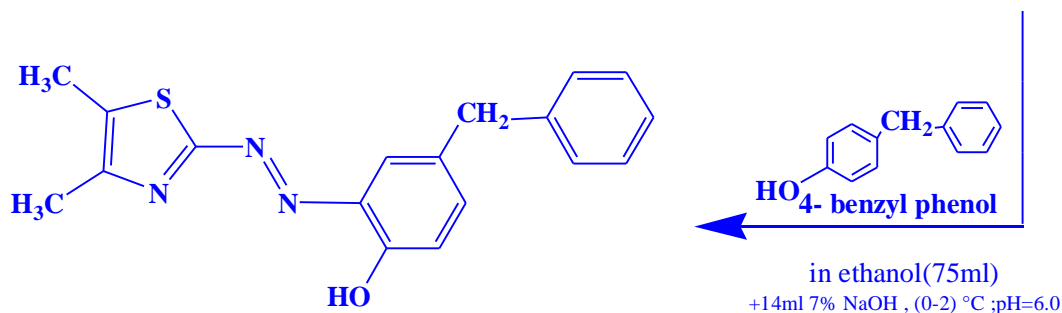
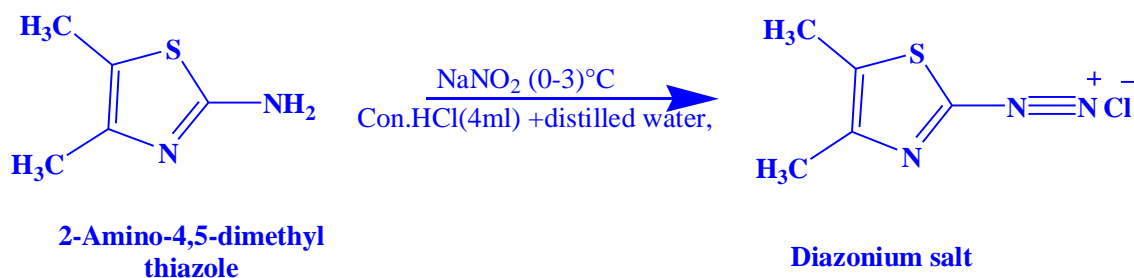
Mass spectra were taken on a shimadzu Agilent Technologies 5973series at 70°e and MSD energy using a direct insertion probe (Acq method 10 W energy) at $90\text{-}110^\circ\text{C}$. Electronic spectra were recorded using T 80-PG UV- visible spectrophotometer in absolute ethanol (10^{-3}M) in the range (200-1100)nm. Micro analytical data (C.H.N.S) were collected on EA 300 C.H.N.S Elemental analyzer. The metal content was determined by using atomic absorption technique by shimadzu AA-6300. X-ray diffraction was taken on Bestec Germany Aluminium anode model X-pertpro, wavelength of X-ray beam ($\text{Cu } k\alpha$) 1.54\AA , Anod material=Cu, the Voltage = 40KV and current = 30mA. SEM images were taken on micrograph kyky 3200. TGA, DSC and DTG analysis were measured with England PL-TG using Rheumatic scientific TGA-1000. Magnetic susceptibility for the prepared metal complexes was measured on a Burker Magnet (B.M) and the diamagnetic correction was made by Pascal's contents at room temperature by using faraday method. Molar conductivity measurements were recorded on conductivity bridge model in dry DMF (10^{-3}M) solution at room temperature. The pH solutions were measured on a Philips pw 9421 pH meter (± 0.001). The chloride ion contents in Co(III) complex was determined by more method [10] and AgNO_3 solution.

Synthesis of Heterocyclic Azo Dye Ligand (DMeTABP):-

The new heterocyclic thiazolylazo dye ligand (Scheme1) has been synthesized by the diazotization coupling reaction using Al-Adilee *et al* method with some modification for synthesis. This kind of azo dye compounds [11]. 2-Amino-4,5-dimethyl thiazole (1.28g, 0.01 mole) was dissolved in mixture of 30 ml distilled water and 4 ml of concentrated hydrochloric acid with continuously cooling and stirring until reach the temperature at (0-3) $^\circ\text{C}$. To this mixture a solution of (0.83 g, 0.012 mole) of sodium nitrite (NaNO_2) in 25 ml distilled water was added drop wise at (0-3) $^\circ\text{C}$ and the mixture stirred for 20 min, then leaving the solution to stable for a period of 15 minutes to complete the reaction.

The resulting diazonium chloride solution was added drop by drop with constant stirring to solution contain of (1.84, 0.01 mole) 4-benzyl phenol was dissolved in 75 ml alkaline ethanol and 14 ml 7% sodium hydroxide and cooled to 0°C , the mixture was stirred for a further two hour in an ice-bath at (0-2) $^\circ\text{C}$.

The crude product was separated by filtration, washed with distilled water and dried. The purity of the azo dye was determined by thin layer chromatography (TLC). The yield of the reaction was 76% and its colour was dark brown crystals and m.p = 141°C . The molecular structure of azo dye ligand (DMeTABP) was elucidated by C.H.N.S, $^1\text{H-NMR}$, Mass, X-ray, FTIR and UV-Visible spectra.



2-[2-(4,5-Dimethyl thiazolyl) azo]-4-Benzyl phenol (DMeTABP)=HL

Scheme (1):- Synthetic pathway of heterocyclic azo dye ligand (DMeTABP)

Synthesis of Metal Complexes

The metal complexes of azo dye ligand (DMeTABP) was prepared by dissolving (0.647 g, 0.002 mole) from ligand in hot ethanol then added drop wise with vigorous to a stoichiometric amount of 1:2 [M:L] molar ratio for (0.001mol) Co(II), Ni(II), Cu(II) and Zn(II) chloride salt dissolved in 30 ml hot buffer solution (ammonium acetate) at pH=7.0. The mixture was heated to 70 °C for 30 min., then left over night. The solid product thus formed was filtered off, washed with 10 ml ethanol to remove any traces of unreacted materials and washed with distilled water. The metal complexes obtained were finally dried in oven at 70°C to several hours and keep in a desiccator under desiccators over anhydrous CaCl₂. The m.p , yield, molecular formal, M.wt , color and element analysis data (C.H.N.S) of thiazolylazo dye ligand(DMeTABP) and its metal complexes are collected in Table (1).

Table (1):- Analytical and Physical data of thiazolylazo dye ligand (DMeTABP) and its metal complexes

Compound	Color	m.p °C	Yield %	Molecular formula (Mole .W.t)	Found (Calc.)%				
					C	H	N	S	M
DMeTABP= HL	Dark brown	141	76	C ₁₈ H ₁₇ N ₃ OS (323.41)	(66.85) 66.09	(5.30) 5.36	(12.99) 12.88	(9.91) 10.08	-----
[Co(L) ₂] Cl	Bluish purple	163	81	C ₃₆ H ₃₂ N ₆ O ₂ S ₂ CoCl (739.64)	(58.50) 58.64	(4.36) 4.43	(11.37) 11.15	(8.67) 8.91	(7.97) 8.14
[Ni(L) ₂]	Blue	179	78	C ₃₆ H ₃₂ N ₆ O ₂ S ₂ Ni (703.51)	(61.46) 60.86	(4.58) 4.63	(11.95) 12.07	(9.11) 8.96	(8.34) 8.53
[Cu(L) ₂]	Dark purple	168	86	C ₃₆ H ₃₂ N ₆ O ₂ S ₂ Cu (708.35)	(61.04) 61.22	(4.55) 4.67	(11.86) 12.09	(9.05) 8.86	(8.97) 9.18
[Zn(L) ₂]	Greenish blue	176	65	C ₃₆ H ₃₂ N ₆ O ₂ S ₂ Zn (710.18)	(60.88) 60.71	(4.54) 4.61	(11.83) 11.94	(9.03) 8.84	(9.21) 9.43

Ligand= (DMeTABP)= HL

Result and Discussion

Characterization of Thiazolylazo Dye Ligand (DMeTABP) and Its Metal Complexes

The heterocyclic azo dye ligand (DMeTABP) was dark brown crystals but the isolated metal complexes were found to be of different colour crystals depending on the metal ion to interact with user azo

dye ligand . The thiazolylazo ligand and its solid metal complexes were stable in air, insoluble in water, completely soluble in common organic solvents. The experimental result of the elemental analysis of the prepared azo dye ligand (DMeTABP) and its metal complexes are in good agreement with theoretical expectations

Metal: Ligand Ratio

The composition of metal complexes were determined by the method of molar ratio at pH =7.0 and optimum concentration at wavelength of maximum absorption (λ_{max}). The solution of prepared metal complexes increase the intensity of the colors as approach my point of intersection ratio and the color continues constant at passing this point which indicates the metal complex formation [12]. The azo dye ligand (DMeTABP) was found to form 1:2 [M:L] chelates

Molar Conductivity Measurements

All metal complexes except of Co(III) complex show the conductivity measurement values ranging between 11.54-13.07 S.mol⁻¹.cm², Table (2), in DMF (10⁻³) at room temperature, these values indicating that there is no conductivity existence (non-electrolyte) and no chloride ions outside the coordination spheres indicating nonionic structure of this metal complex [13]. But the high value of molar conductivity of the Co (III) complex is 67.93 S.mol⁻¹.cm² respectively indicates that the metal complex is 1:1 electrolyte with ionic structure [14] because of the presence of chloride ion outside the coordination sphere [14]. The purpose of making sure the presence of chloride ions outside coordination sphere used More method determination of chloride ion was titration against standardized 0-10 M silver nitrate (AgNO₃) solution using few drops of 5% potassium chromate (K₂CrO₄) as indicator. The More method uses chromate ions as an indicator in the titration of chloride ions with a silver nitrate standard solution. After all the chloride has been precipitated as a white silver chromate precipitate, which signals the end point [10]

Table (2):- Optimal concentration, molar conductivity, stability constants values (β and Log β), maximum wavelength (λ_{max}) and molar absorptivity (ϵ) of metal complexes

Ligand (DMeTABP)	Metal ion	Optimal Conc. $\times 10^{-4}M$	Maximum Wavelength (λ_{max})nm	Molar Absorptivity (ϵ) $\times 10^3$ L.mol ⁻¹ .cm ⁻¹	Molar Conductivity S.Cm ² .Mol ⁻¹	Stability Constant (β) L ² .mol ⁻²	Log β
Ligand=HL (DMeTABP) $\lambda_{max} = 468$ nm $\epsilon = 7.61 \times 10^3$ L.mol ⁻¹ .cm ⁻¹ Conc.= $1.5 \times 10^{-4}M$	Co(III)	1.50	588	1.73	67.93	78.90×10^8	9.89
	Ni(II)	1.75	642	1.53	13.07	54.38×10^9	9.73
	Cu(II)	1.50	543	1.66	11.54	46.20×10^8	9.66
	Zn(II)	2.00	638	1.17	12.18	75.22×10^9	10.87

¹H-NMR spectra

The ¹H-NMR spectra of new thiazolylazo dye ligand (DMeTABP) and Ni(II)-complex were only because of signals from OH group disappeared indicating deprotonation of the OH group due to M–O bond formation therefore similar to other metal complexes [14] figures.1 and 2, was measured in DMSO-d₆ as solvent with TMS as an internal reference (400_{MHZ}). The ¹H-NMR spectrum of thiazolylazo free ligand showed a triplet signal at $\delta=2.49-2.56$ ppm (t, 6H, 2CH₃) was due to the presence of two methyl groups in both loctations 4 and 5 in the thiazol ring. A signal at $\delta= 4.01-4.08$ ppm (t, 2H –CH₂) was due to methylen group in benzyl phenol. The doublet peak at $\delta =6.67-6.69$ ppm (d,3H, Ar –H) was attributed to phenol protons . The signal at $\delta=7.01 -7.32$ ppm (m, 5H , Ar –H) was due to the presence of aromatic protons . A signal at $\delta= 9.61$ ppm (S ,H , OH) was due to the presence of – OH group ,While a signal at $\delta = 2.22-2.31$ ppm(q) was due to solvent protons [11]. The ¹H-NMR spectrum of Ni(II)– complex shows a signal at $\delta = 2.86-3.36$ ppm (t.6H.2CH₃) attributed to the methyl groups sbstuted in thiazol ring , while the signal at $\delta=4.17-4.32$ pmm (t,2H,-CH₂) was due to methylen group in benzyl phenol. The doublet peaks at $\delta= 6.37-679$ pmm (d,2H,(H₃ and H₅)- Ar-H) and triplet peaks at $\delta= 6.88-6.90$ ppm(t ,H (H₆) – Ar-H) were attributed to phenol protons . The multiple peaks at $\delta= 7.37-7.80$ ppm (m,5H,Ar-H) was due to the presence of aromatic protons (phenyl).The signal at $\delta= 2.48-2.86$ pmm was due to the existence DMSO-d₆ solvent. The signal of proton hydroxyl group disappearance spectrum of Ni(II)– complex indicates hydrogen atom of – OH group replacement by Ni (II) ion during coordination with thiazolylazo ligand [14]

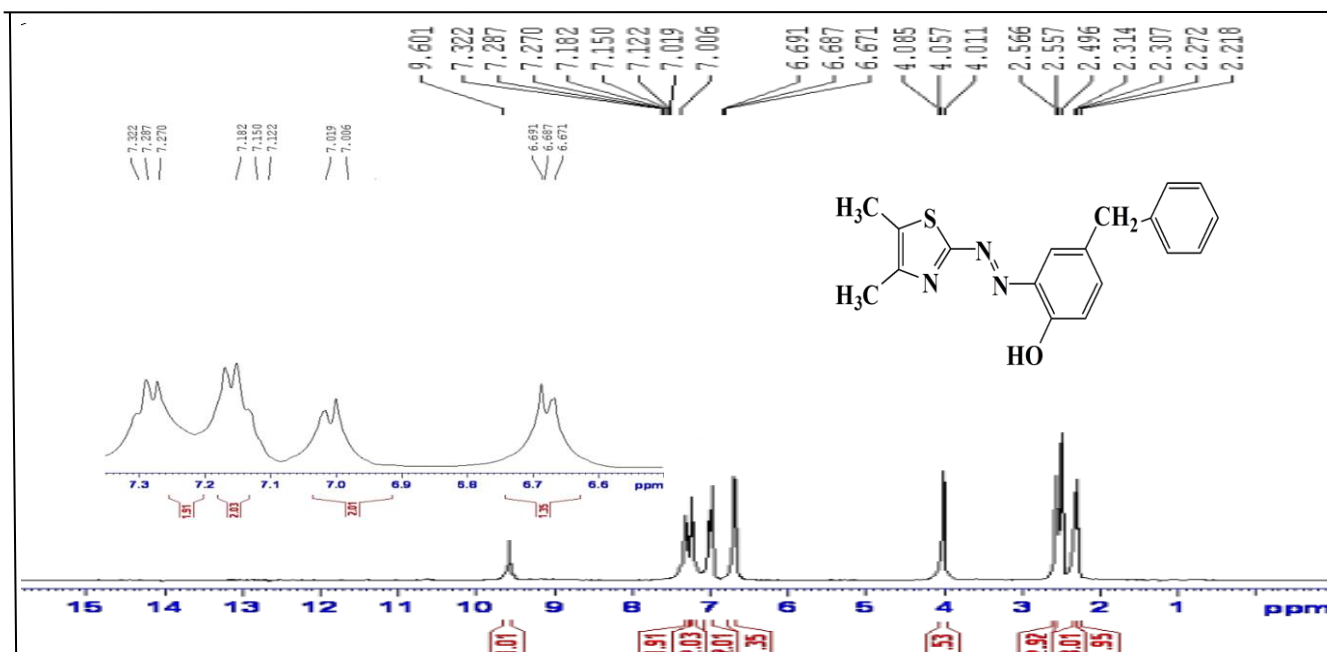


Figure (1):-¹H-NMR spectrum of thiazolylazo dye ligand (DMeTABP)

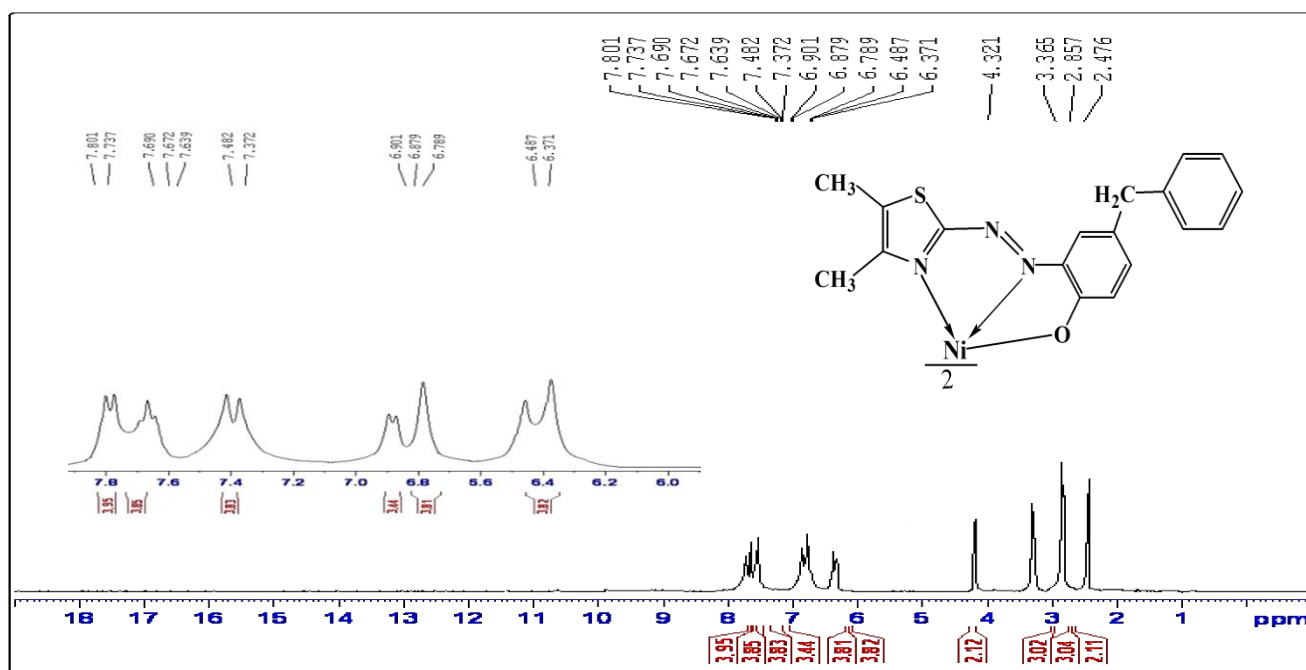


Figure (2):-¹H-NMR spectrum of Ni(II)- complex ;[Ni(L)₂]

Mass Spectra of New Thiazolylazo Dye Ligand

The mass spectral data fragmentation of new thiazolylazo dye ligand (DMeTABP) the mass spectrum of free ligand (Fig.3) showed a molecular ion peak M^+ at $m/z^+ = 323.4$ attributed to the original molecular weight of azo dye ligand (323.41), $[C_{18}H_{17}N_3OS]$ with relative abundance (100%) is an assurance on the accuracy of the molecular structure of the prepared azo dye ligand. Other peaks like (m/z^+) 294.4, 269.3, 253.2, 176.2, 85.2, 77.2, and 51.1 were due to various fragments ions $[C_{18}H_{17}NOS]$, $[C_{16}H_{14}NOS]$, $[C_{15}H_{11}NOS]$, $[C_9H_6NOS]^+$, $[C_3H_3NS]$, $[C_6H_5]^+$, and $[C_4H_3]^+$ respectively. This ligand takes the route to the mass fragmentation [11].

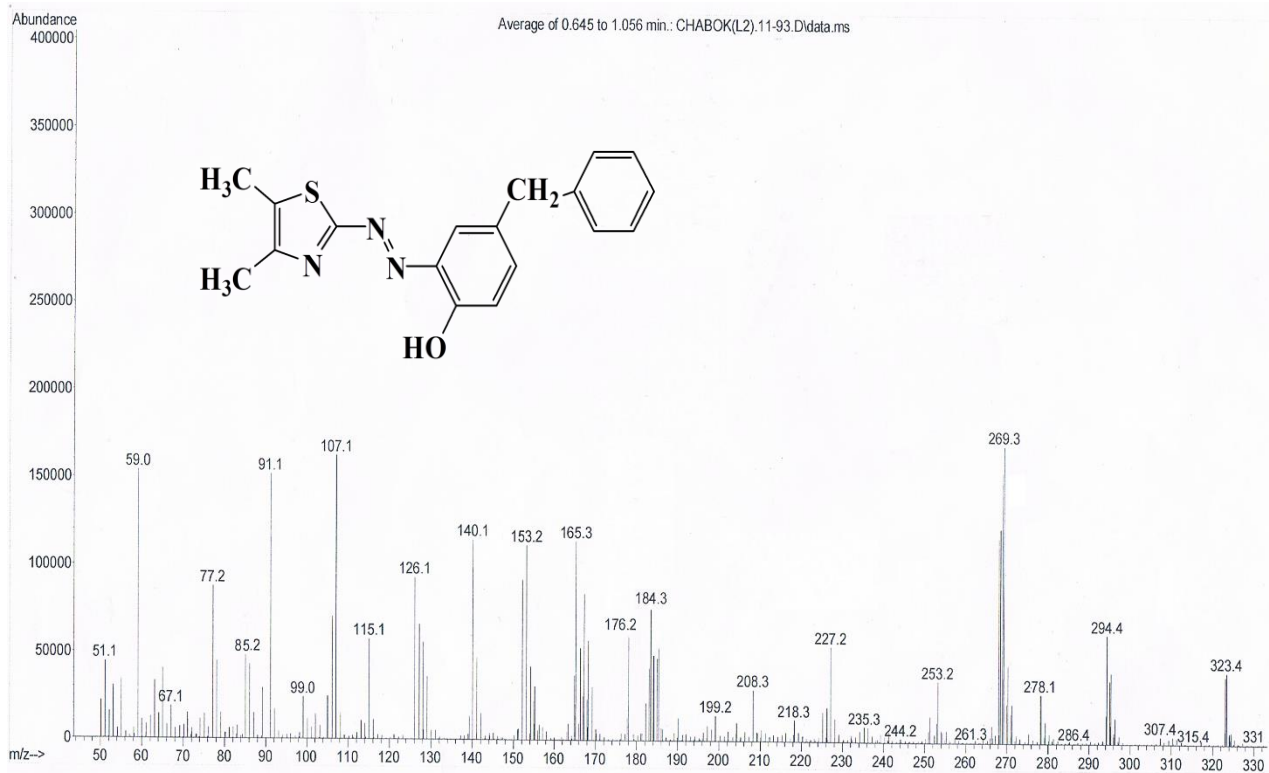


Figure (3):- Mass spectrum of thiazolylazo dye ligand (DMeTABP)

Infrared Spectra

The infrared spectra of thiazolylazo ligand (DMeTABP) and its metal complexes have been studied by using KBr disc in range $(4000 - 400) \text{ cm}^{-1}$. The shifts in the positions or change in shape of the metal complexes bands compared with those absorption bands due to thiazolylazo ligand suggest the probable modes of bonding in the metal complexes .

The spectrum of free ligand (DMeTABP) shows a medium absorption band at 3367 cm^{-1} due to δ (-OH) group, the disappearance of this band in the metal complexes is indicative of the loss of a hydrogen from the hydroxyl group because it is linked to the metal ion [15]. Three weak bands had been observed at 3082 cm^{-1} , 2958 cm^{-1} and 2920 cm^{-1} in the free ligand spectrum which are due to $\nu(\text{C-H})$ aromatic, $\nu(-\text{CH}_2)$ of benzyl phenol and $\nu(-\text{CH}_3)$ group located in the thiazole ring respectively, these bands are stable in position in both free ligand and metal complexes . Ligand spectrum show another medium band at 1612 cm^{-1} which is attributed to $\nu(\text{C}=\text{N})$ of thiazole ring [16]. This band has a small change in intensity and shape and has been shifted to lower frequencies in prepared metal complexes spectra [16] . These differences are due to the linkage of metal ion with nitrogen thiazole ring, a medium intensity absorption band is observed at 1439 cm^{-1} in the ligand spectrum which is due to the azo group $\nu(-\text{N}=\text{N}-)$, while the spectra of the metal complexes show shifting of this band towards low frequencies indicating the participation of the nitrogen of azo group nearest phenyl ring in the coordination with metal ions to formation of the metal complexes [17]

A band at 1249 cm^{-1} has also been appeared in the free ligand spectrum due to $\nu(\text{C}-\text{S})$ of thiazole ring [7] .The fixed of this band in its position and its nonchange in the metal complexes means that the sulfur atom of thiazole ring does not participate in coordination [12]

In the far infrared spectra of all metal complexes , there are new absorption bands observed in the region of $(520-565) \text{ cm}^{-1}$ and $(451-455) \text{ cm}^{-1}$ which have never been observed in the free ligand spectrum , that may be attributed to $\nu(\text{M}-\text{O})$ and $\nu(\text{M}-\text{N})$ [18]. So, the above infrared spectral data lead to suggest that thiazolylazo ligand (DMeTABP) behaves as a tridentate chelating agent coordinating through the positions of nitrogen of azo group which nearest phenyl ring , phenolic oxygen and thiazole ring nitrogen to give two five membered chelate ring . The IR spectra of azo dye ligand and some metal complexes are shown in figures 4

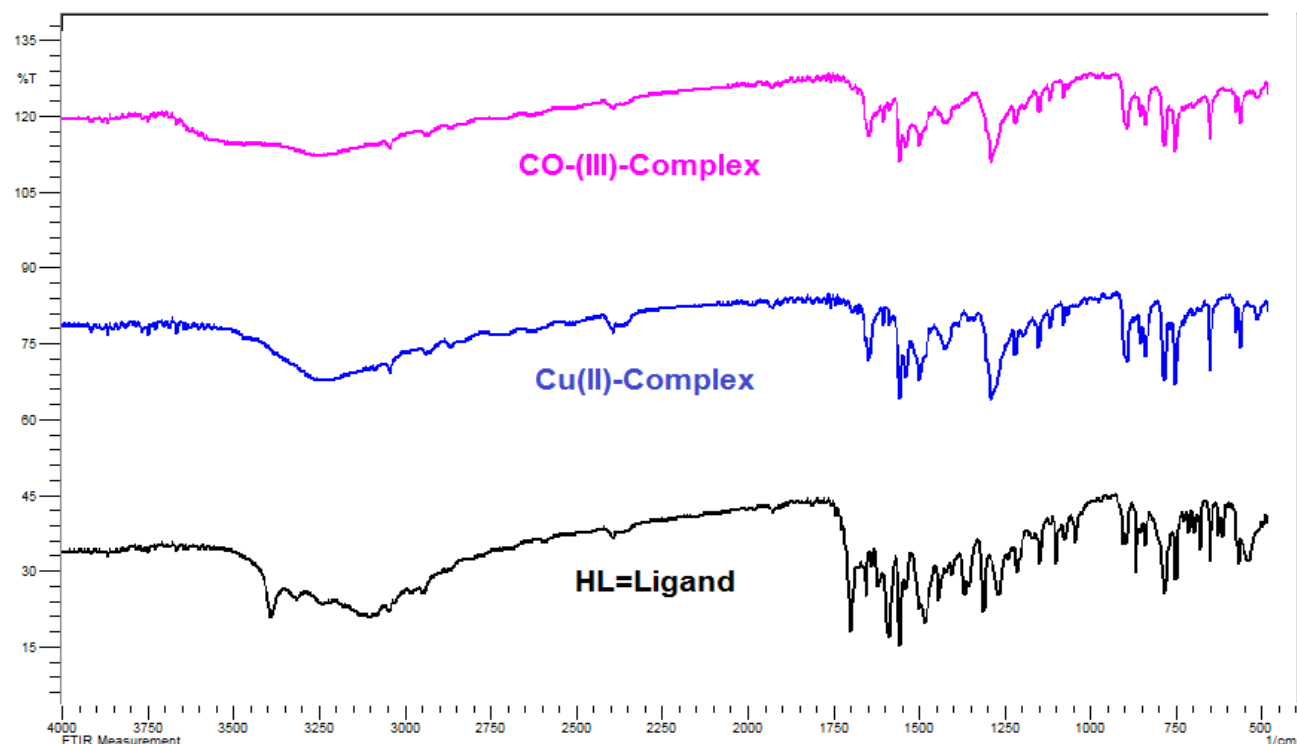


Figure (4) :- IR Spectrum of thiazalylazo dye ligand (DMeTABP) and its metal complexes

Electronic Spectral Studies

The electronic absorption spectra of thiazolylazo ligand (DMeTABP) and its metal complexes were measured in freshly prepared absolute ethanol (10^{-3} M) in the UV-vis. region (200-1100)nm at room temperature. The spectral data are presented in Table 3. The Electronic spectral of free azo dye ligand show two absorption bands at 242nm(41322cm^{-1}) and 438nm (22831cm^{-1}). The first band can be due to a $\pi \rightarrow \pi^*$ transition in thiazole ring [19], while returning the second band to the $n \rightarrow \pi^*$ transition resulted from the presence of group containing double bond, in addition to the presence of hetero atom carrying a ion pair of electrons such as (C=N-) in thiazole ring and intermolecular charge transfer taken place from phenolic ring to the thiazole ring through the azo group (-N=N-). This band was shown at a red shift on coordination with a metal ions [20].

The electronic spectrum data of Co(III)-complex exhibited four absorption bands at 972nm(10288cm^{-1}), 588nm(17007cm^{-1}), 362nm (27624cm^{-1}) and 328nm(30488cm^{-1}) these three bands were assignable to ${}^1A_{1g} \rightarrow {}^1T_{2g(F)}(\nu_1)$, ${}^1A_{1g} \rightarrow {}^1T_{1g(F)}(\nu_2)$ and ${}^1A_{1g} \rightarrow {}^1T_{1g(p)}(\nu_3)$ transitions respectively, While the fourth band was at 328 nm due to center ligand transitions [20].

In the electronic spectrum of Ni(II) – complex, three absorption bands were observed, the first band was medium and broad at 976nm (10246cm^{-1}) attributed to ${}^3A_{2g} \rightarrow {}^3T_{2g(F)}(\nu_1)$ transitions and the second band was strong and broad at 642 nm (15576cm^{-1}) due to ${}^3A_{2g} \rightarrow {}^3T_{1g(F)}(\nu_2)$ transitions, While the third band detected at 362nm (27624cm^{-1}) may be assignable to ${}^3A_{2g} \rightarrow {}^3T_{1g(p)}(\nu_3)$ transitions, and the band that appears when 321nm (31153cm^{-1}) was due to center ligand.

The dark purple colored of Cu(II)-complex showed a single broad band around at 543 nm (18416cm^{-1}). The broadness asymmetric band of the three transition ${}^2B_{1g} \rightarrow {}^2A_{1g} (dx^2-y^2 \rightarrow dz^2)_{(\nu_1)}$, ${}^2B_{1g} \rightarrow {}^2B_{2g} (dx^2-y^2 \rightarrow dyz^2)_{(\nu_2)}$ and ${}^2B_{1g} \rightarrow {}^2B_{2g} (\text{charge transfer})_{(\nu_3)}$, which were of similar energy and gave rise to only one broad absorption band(${}^2B_{1g} \rightarrow {}^2E_g$). The broadness of the band may be due to dynamic Jahn-Teller distortion [19].

The absorption electronic spectrum of the Zn(II)-complex did not show any d-d transition because of saturation with electrons (d^{10}). The absorption band at 638 nm (15674cm^{-1}). This band may be due to a charge transfer (M \rightarrow L,CT) transition to this metal complex. The transition may refer to the combination of MLCT [$d\pi(\text{Zn}^{+2}) \rightarrow \pi^*(\text{L})$] transition where $\pi^*(\text{L})$ was believed to be primarily dominated by the LUMO of the azo imine chromophore [21]. The UV-visb spectra of azo dye ligand (DMeTABP) and some metal complexes are shown in figures 5 and 6

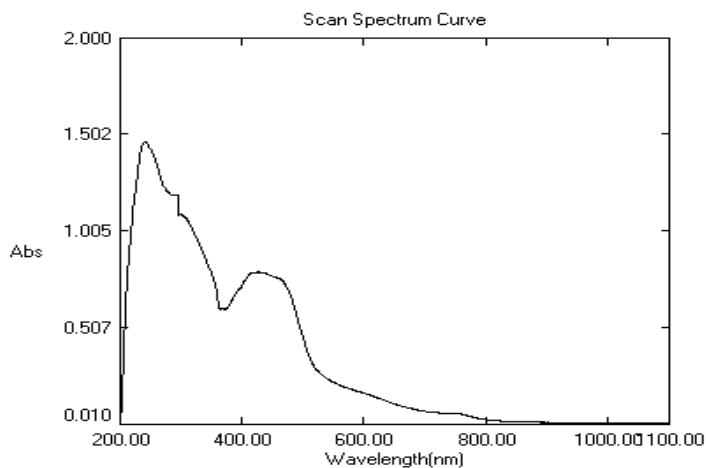


Figure (5):- UV. Visib. spectrum of thiazolylazo Dye ligand (DMeTABP)

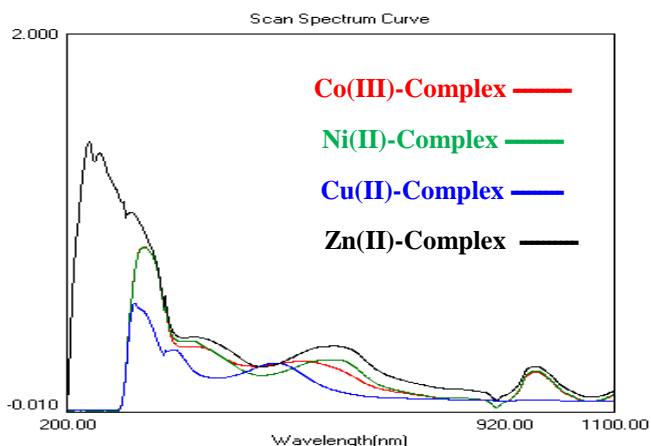


Figure (6):- UV. Visib. spectra of Co(III), Ni(II), Cu(II), Zn(II) complexes

Magnetic Studies

The magnetic moments obtained at room temperature for Co(III), Ni(II) and Cu(II) complexes and are listed in Table 3. At room temperature the Co(III)-complex show magnetic moments WHICH has been found to be diamagnetic. The low spin behavior and this was proved by calculating the magnetic susceptibility as value of this complex is zero, this complex indicates that Co(II) ion which is oxidized to Co(III) ion during complexation in aqueous solution with presence of strong ligand such as thiazolylazo ligand which may suggest a regular geometry ($t_2g^6 e_g^0$) and d^2sp^3 hybridization [22].

The Ni(II)-complex show a paramagnetic behavior and the magnetic moments value of 2.86 B.M. indicating the octahedral environment because of the presence of two unpaired electrons ($t_2g^6 e_g^2$, high spin) and sp^3d^2 hybridization [23].

The Cu(II)-complex exhibited the magnetic moments value 1.72 B.M. which indicates an distorted octahedral geometry (Z-in or Z-out) corresponding to one unpaired electron ($t_2g^6 e_g^3$) and sp^3d^2 hybridization [24].

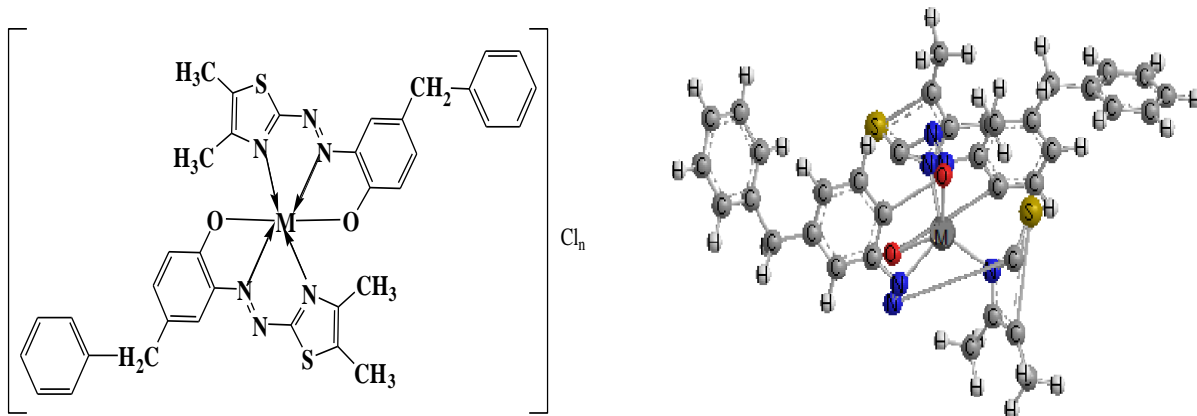
The magnetic susceptibility measurement for Zn(II) complex show a diamagnetic behavior as it diamagnetic complex with d^{10} configuration ($t_2g^6 e_g^4$) and sp^3d^2 hybridization for this metal complex indicates the octahedral environment around the Zn(II), ion. The metal complex has an arrangement d^{10} ($t_2g^6 e_g^4$) and sp^3d^2 hybridization [25].

Table(3):- Electronic spectra (nm, cm^{-1}), magnetic moments, proposed geometry and hybridization of prepared metal complexes

Compounds	λ_{max} (nm)	Absorption bands (cm^{-1})	Transitions	μ_{eff} (B.M)	Geometry	Hybridization
Ligand=HL (DMeTABP)	438	22831	$n \rightarrow \pi^*$	-----	-----	-----
	242	41322	$\pi \rightarrow \pi^*$			
	504	19841	${}^2A_{2g} \rightarrow {}^4E_g, {}^4A_{1g} ({}_{(G)}U_2)$			
	332	30120	${}^2A_{2g} \rightarrow {}^4T_{1g(P)}(U_3)$			
[Co(L) ₂] Cl	972	10288	${}^1A_{1g} \rightarrow {}^1T_{2g(F)}(U_1)$	Dia	Octahedral (Regular)	d^2sp^3 (Low spin)
	588	17007	${}^1A_{1g} \rightarrow {}^1T_{1g(F)}(U_2)$			
	362	27624	${}^1A_{1g} \rightarrow {}^1T_{1g(P)}(U_3)$			
	328	30488	Center ligand			
[Ni(L) ₂]	976	10246	${}^3A_{2g} \rightarrow {}^3T_{2g(F)}(U_1)$	2.86	Octahedral (Regular)	Sp^3d^2 (high spin)
	642	15576	${}^3A_{2g} \rightarrow {}^3T_{1g(F)}(U_2)$			
	362	27624	${}^3A_{2g} \rightarrow {}^3T_{1g(P)}(U_3)$			
[Cu(L) ₂]	543	18416	${}^2B_{1g} \rightarrow {}^2E_g$	1.72	Octahedral (distorted) (Z-in or Z-out)	Sp^3d^2
[Zn(L) ₂]	638	15674	$d\pi(Zn)^{+2} \rightarrow \pi^*(L)$	Dia	Octahedral (Regular)	Sp^3d^2

B.M= Bohr magneton

According to these results the structure of these metal complexes may be proposed in figure 7, shown below:

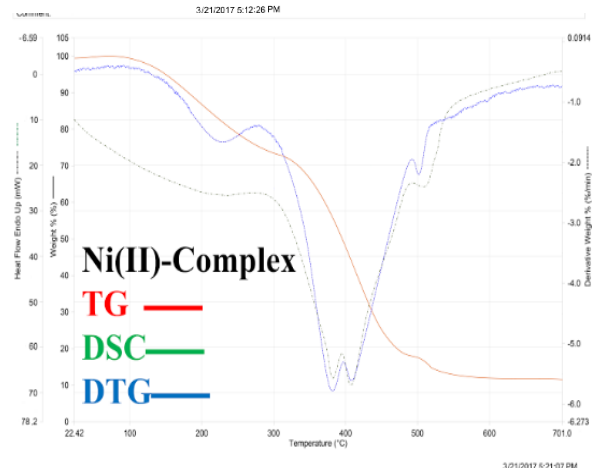
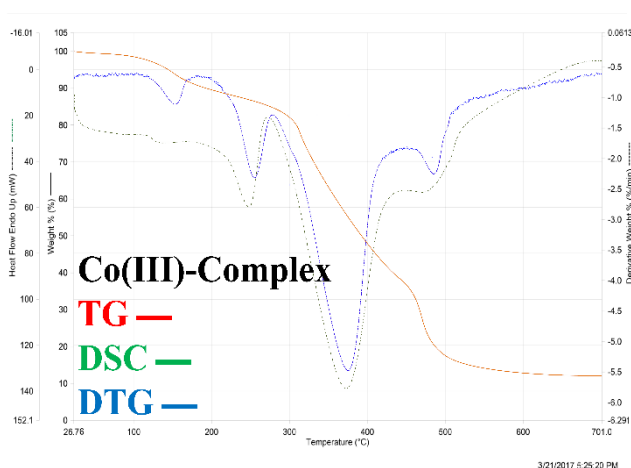
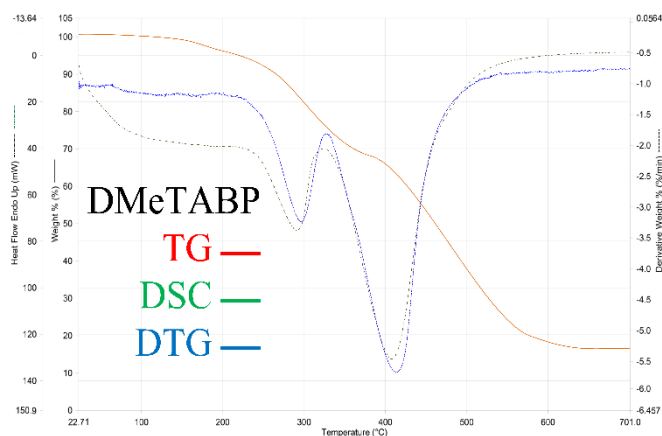


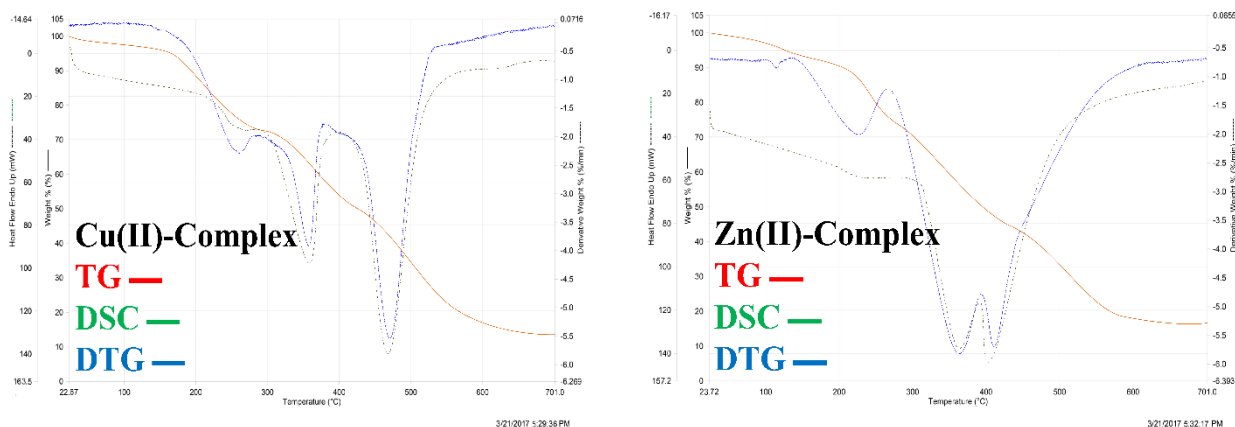
M= Co(III) ; n=1 M= Ni(II) ; Cu(II) and Zn(II) n=0

Figure(7):-The suggest structural formula of chelate complexes.

Thermal Analyses

The thermal stability of the ligand and its metal complexes investigated. They have thermal properties and are examined from ambient in the temperature range 22–800 °C by some thermo gravimetric assays like (TGA) , (DTG) and (DSC) that mean thermo gravimetric analysis, differential thermo gravimetric and differential scanning calorimetric respectively in nitrogen atmosphere. The thiazolylazo ligand (DMeTABP) and its metal complexes are presented. That in this range (22-100) loss occurs CO₂ and moisture, there is no water molecules inside and outside the coordination in ligand and all complexes . shown in [figure\(8\)](#).

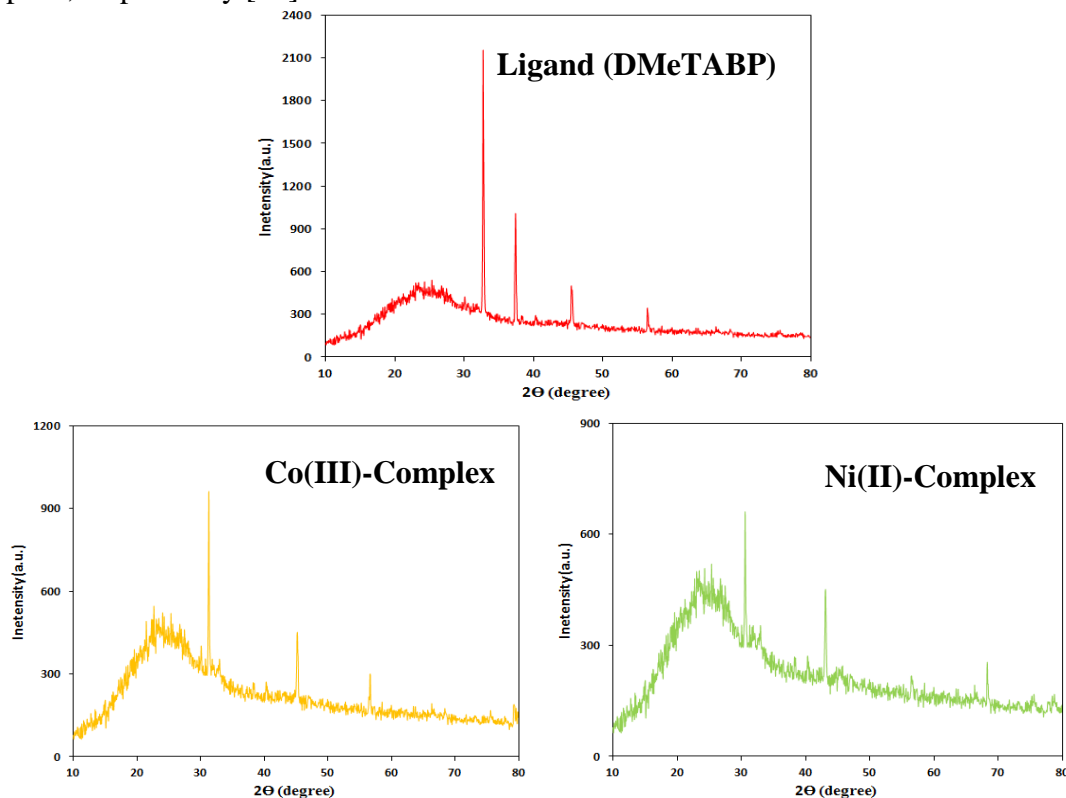




Figure(8):- TG-DTG-DSC curves of prepared ligand (DMeTABP) and its metal complex

X-Ray Diffraction Study (XRD)

It has been studied that the crystalline structure of prepared ligand (DMeTABP) and its metal complexes in solid state by using x-rays diffract meter in the range of $2\theta = 0-80^\circ$ value, to know some properties like crystalline structure, crystalline volume and assessing the purity and crystallization. The (XRD) patterns are shown in figure 9, in this figure, we observed that the spectrum of ligand (DMeTABP) of The high granular crystallization rate is higher than the low granular crystallization rate. This means that the ligand is more homogeneous than the X-ray diffraction peaks, while we can observe that the remaining complexes such as Co(III), Ni(II), Cu(II) and Zn(II) are similar in the term of crystalline nature. The height of these peaks depends on the crystalline arrangement, the properties of the crystalline network, and crystalline levels. The appearance of the high peaks in the ligand indicates that the presence of crystalline level is a homogenous crystal formation, while structures are less homogeneous. To calculate d-spacing or 'd' values of reflections were obtained using Bragg's equation $n\lambda = 2d\sin\theta$, where 'd' is the spacing between the crystalline levels, n is an integer (1,2,3 ...), λ is the wavelength of X-ray $\text{Cu K}\alpha = 1.540598 \text{ \AA}$, θ is the diffraction angle, the XRD peak shifts to lower angle in metal complexes because of the increase of d-spacing in the complexes and the average size of the particles and their size distribution were evaluated by the Scherer equation, $D = k\lambda / \beta \cos\theta$, where D is the average grain size, k is Blank's constant (0.891), λ is the X-ray wavelength (0.15405 nm), and θ and β are the diffraction angle and full width at half maximum of an observed peak, respectively [26].



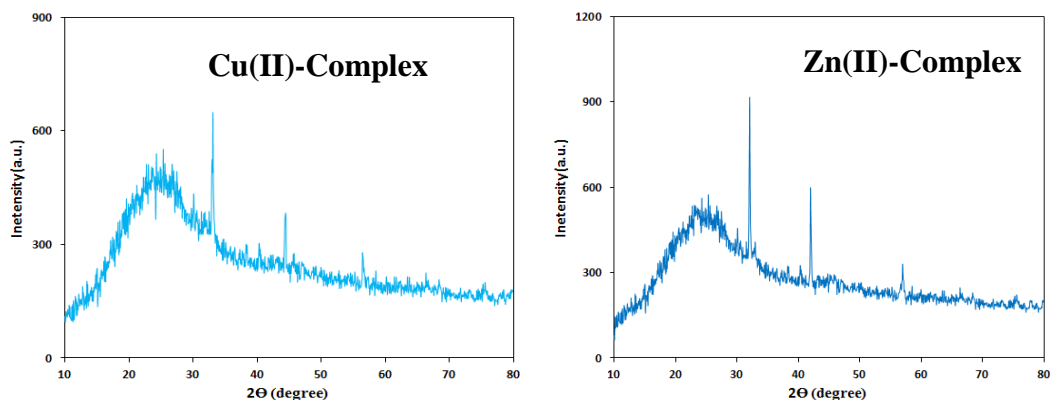


Figure (9):- XRD patterns of ligand (DMeTABP) and its metal complexes

SEM Analysis

Scanning electron microscopy (SEM) was employed to observe the morphology and particle size of the ligand (DMeTABP) and its metal complexes. The surface image is obtained with the SEM technique provides general information regarding the microstructure, surface morphology, particle size, chemical composition, and porous structures of the surfaces, in addition to the distribution of these particles. The SEM image of ligand and metal complexes have been illustrated in figure 10. SEM image shows the ligand (DMeTABP) that has a homogeneous particle shape surface with fewer aggregations and a particle size rate of 100 nm [27]. The calculations of particles size were performed by using MagniSci software. Co(III)-complex was shown in the form of less homogeneous crystalline particles with a particle size of 145 nm. While the analysis of SEM for Ni(II)-complex shows that its particles are homogeneous and the surface particle size is 115 nm with a lower proportion of aggregates, the analysis of SEM for Cu(II)-complex is shown as a heterogeneous particle surface and the particle size is 140 nanometers with a lower proportion of aggregates. The SEM analysis of Zn(II)-complex is of a spherical shape and the average size of the particles is 150 nm with a lower proportion of the aggregates

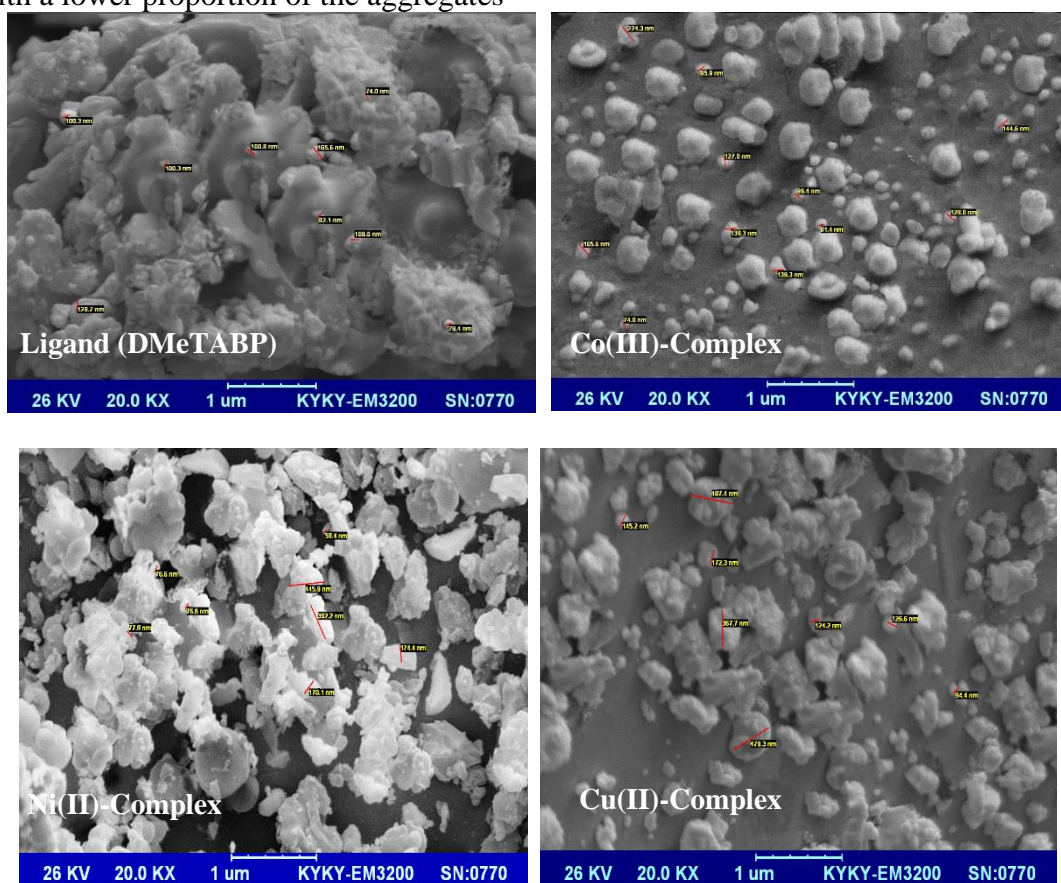


Figure (10):- SEM images of ligand (DMeTABP) and its prepared metal complexes

Pharmacology Results

1. Antibacterial Activity

The effect of the ligand and its metal complexes have been tested for in vitro against the sensitive organisms *Escherichia Coli*, *Streptococcus* and *Staphylococcus aureus* as antibacterial and *Alternaria spp* as antifungal. These compounds were also compared with antibiotic drugs Chloramphenicol (C) and Amikacin (AK) and it gave almost identical results to the ligand and its metal complexes by using spots diffusion method. All metal complexes have a high activity and sensitivity towards *Escherichia coli* bacteria except of ligand (DMTABP). The *staphylococcus aureus* bacteria was of a high activity and sensitivity towards ligand and all metal complexes. As for *streptococcus* bacteria We note that the prepared metal complexes have an effect except the ligand (DMeTABP) and Co(III)- complex. We note that the ligand (DMeTABP) and all its metal complexes has higher activity and sensitivity to *Alternaria* fungi than it does in bacteria. We also note that the metal complexes are more active against Gram-positive bacteria *staphylococcus aureus*, while Gram-negative bacteria are more resistant. This may be due to the presence of a double membrane surrounding each bacterial cell. Although all bacteria have an inner cell membrane, gram-negative bacteria have a unique outer membrane. This outer membrane excludes certain drugs and antibiotics from penetrating the cell [28]. Treatment of the results is clearly specified as shown in Table 4.

Table(4):-Antibacterial activity data(zone of inhibition in mm) of azo dye ligand (DMeTABP) and its metal complexes

Compounds.	Anti- bacterial Activity			Anti- fungActivity
	<i>E.coli</i>	<i>Staphylococcus</i>	<i>Streptococcus</i>	<i>Alternaria</i>
(DMTABP)	0 -	7 ++	0 -	21 +++
[Co(L) ₂]Cl	15 +++	17 +++	0 -	35 ++++
[Ni(L) ₂]	9 ++	21 +++	13 +++	36 ++++
[Cu(L) ₂]	8 ++	25 +++	9 ++	30 +++
[Zn(L) ₂]	21 +++	24 +++	14 +++	40 ++++
Chloramphenicol	17 +++	27 +++	9 ++	38 ++++
Amikacin (AK)	0 -	22 +++	17 +++	0 -
Control DMSO	0 -	0 -	0 -	0 -

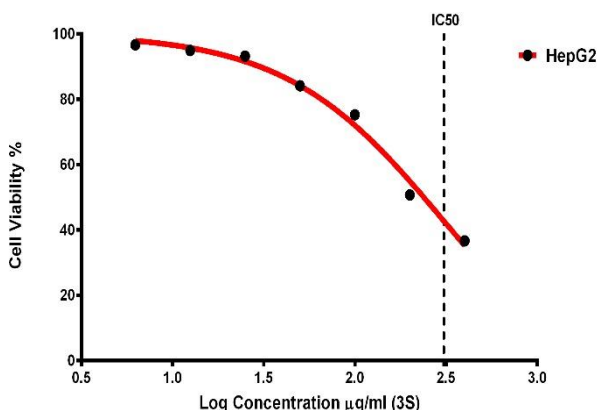
2. Cell Viability And Cytotoxicity Assays.

Cytotoxicity was measured by colorimetric assay based on tetrasolium salt MTT (3,5-4,5-Dimethyl thiazol-2-yl) 2,5-diphenyltetrazolium bromide. The MTT assay is based on the protocol first described by [29]. In our study the lines of cancerous cells of the type HePG2 were used and compared with natural cells line (WRL-68). The cells line (HepG2) and natural cells line (WRL-68) were shown for concentrations ranging from 400-25 µg / ml for each of the Cu(II) and Zn(II) complexes. The toxic effect was estimated by extracting the percentage of inhibition compared to control (100% growth). These lines have obtained from the University of Malaya \ Faculty of Medicine \ Department of Pharmacy \ Research Center for New Treaties in Malaysia. Fresney method is used for the development of the line of cancerous cell of the liver HePG2. The data are statistically analyzed by one way analysis of variance ANOVA (Duncan) which is performed to test whether group variance was significant or not. The data are expressed as mean ± standard error and statistical significances were carried out using SPSS program version 20 and drowned using Graph Pad Prism version 6. The figures(11-14) shows the relationship between the biological activity of the cancerous line cells of the liver HePG2 and natural cells (WRL-68). This assay is performed to measure the cells vitality and inhibition rate by applying different concentrations of metal complexes Cu(II) and Zn(II) on cell lines. Data analysis which is carried out in µg / ml and log values of µg / ml is being plotted in Graph pad Prism 6 using log (Inhibitor) versus response curve. It is showed that the effect of Cu(II) and Zn(II) complexes with ligand on growth of (hepG2) cells and normal cells (WRL-68), and it also showed that the

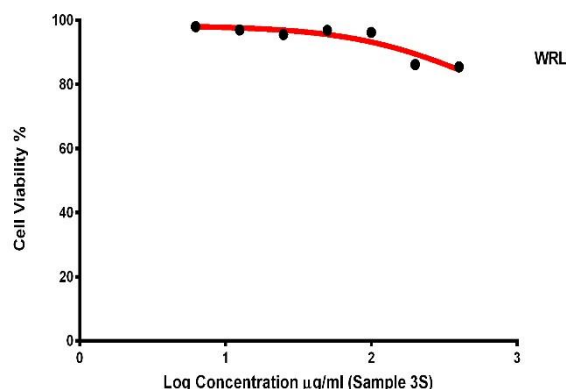
lowest inhibitory rate of cell growth at 25 µg / ml and the highest inhibition ratio at 400 µg/ml concentration for hepatocyte cell carcinoma cells (HepG2) and natural cells (WRL-68).

It was observed that the inhibition ratios of the Cu(II)-complex differs according to the type of cells line. The number of living cells remaining after reacting with the Cu(II)-complex is between (93.17-36.65)%, for the cancerous cell line of the liver HePG2 and (95.52 - 85.46)% for natural cell line (WRL-68). It has also observed that the highest ratio of the inhibition of the Cu(II)-complex for the cancerous cell line of the liver HePG2 is 36.65%, whereas the highest ratio of inhibition for cells line of natural cells most (WRL-68) is 95.52%. As shown in the Table 5. One of the important things that must be referred to and obtained through the tests of the lines of cancerous liver cells (HepG2) is the so called (inhibitor Concentration Fifty) , which symbolizes (IC50), and this concentration kills approximately half of the cells.

In the case of the interaction of the Cu(II) complex with the liver cancerous cell line (HepG2), the inhibitory of half is equal to 307.7 µg / ml, while we can note that the concentration of the inhibitory (IC50) is within the scope of concentrations from which it is taken. It is also observed that the inhibition ratios of the Zn(II) complex vary according to the type of cells line. The number of living cells remaining after reacting with the complex is between is 68.06% and 99.37% for hepG2 cells. The normal cells line (WRL-68) is between (85.23% - 99.47%). It has found that the highest rate of inhibition of the cancerous cells of the liver at the concentration of 400 µg \ ml, where the number of living cells left after the reactors with the Zn(II)-complex 68.06%, while it has observed that the highest rate of inhibition of the natural cell line with the same emphasis above, where the number of living cells left after the reactors with the with the Zn(II)-complex is 85.23%. As shown in the Table 6. We note that the concentration of half-inhibitory (IC50) of Zn(II)- complex is 349.5 µg \ ml for the cancerous cell line of the liver HePG2 . While (IC50) for natural cell line (WRL-68) is 2607 µg \ ml



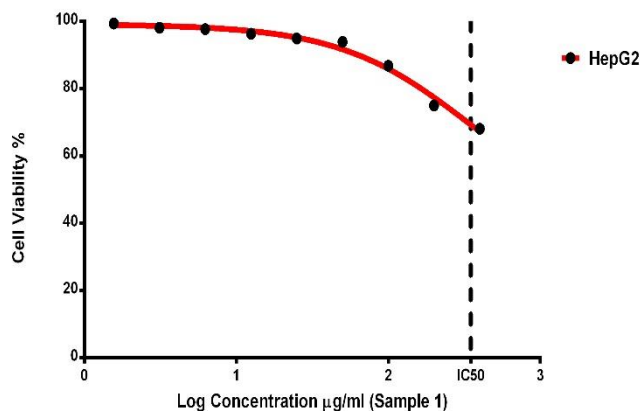
Figure(11):- Cytotoxicity effect of the Cu(II)-complex on HePG₂ cells after 24 hours incubation at 37°C



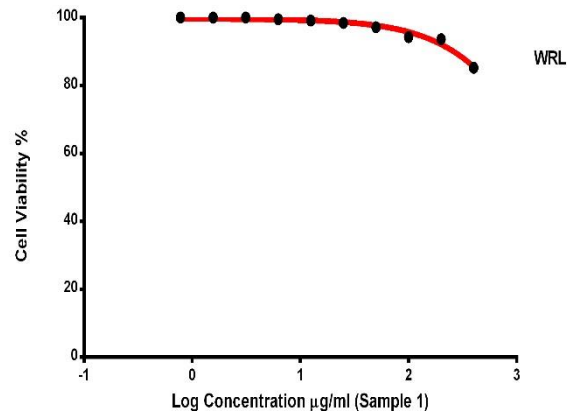
Figure(12):- Cytotoxicity effect of the Cu(II)-complex on (WRL68) cells after 24 hours incubation at 37°C

Table (5):- Effect of Cu(II)- complex on hepatocellular cancerous cells HepG2 and comparison with natural cells line (WRL68) for same concentration using 24-Hour MTT Test at 37 ° C

inhibition concentration (IC50)	Concentrations used (µg \ ml) \ percentage of live cell rate					Cellular lines
	(Average ± standard error)					
	25	50	100	200	400	
307.7	93.17 ± 0.9284	84.10 ± 2.319	75.26 ± 0.9690	50.72 ± 1.622	36.65 ± 1.345	Cytogenetic cell lines of hepG2 liver
506.6	95.52 ± 0.9920	96.84 ± 1.023	96.22 ± 1.138	86.19 ± 1.327	85.46 ± 2.899	natural cells line (WRL68)



Figure(13):-Cytotoxicity effect of the Zn(II)-complex on HePG₂ cells after 24 hours incubation at 37°C



Figure(14):-Cytotoxicity effect of the Zn(II)-complex on (WRL68) cells after 24 hours incubation at 37°C

Table (6):- Effect of Zn(II)-complex on hepatocellular cancerous cells HepG2 and comparison with natural cells line (WRL68) for same concentration using 24-Hour MTT Test at 37 ° C

inhibition concentration (IC50)	Concentrations used (µg \ ml) \ percentage of live cell rate (Average ± standard error)					Cellular lines
	25	50	100	200	400	
349.5	94.90 ±1.807	93.88 ±1.386	86.74 ±4.160	75.01 ±1.427	68.06 ±3.777	Cytogenetic cell lines of hepG2 liver
2607	98.45 ±0.4381	97.16 ±0.9823	94.28 ±0.7490	93.63 ±0.8357	85.23 ±1.581	Non - cancerous cell line(WRL68)

Conclusions

In this article we reported a synthetic and spectral identification of new thiazolylazo dye ligand (DMeTABP) and its metal complexes with Co(III), Ni(II), Cu(II) and Zn(II) ions. The structure of ligand and its metal complexes was confirmed by elemental analysis and spectroscopic techniques. The geometry proposed for all metal complexes is octahedral structure and is not effected by air, light and a moisture. It suggest high stability. The ligand and its metal complexes are found to have higher biological activities toward antibacterial and antifungal .

The biological activity also conducted cells viability and cytotoxicity assays on Cu(II) and Zn(II) complexes by using the lines of cancerous liver cells , of the type HePG2 and compared with line of the ordinary cells , through tests conducted to identify the possibility of using the Cu(II) and Zn(II) complexes as drugs to treat some cancerous diseases that affect humans

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