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Recent Advances in Synthesis, Characterization and Biological Activity of Nano Sized Schiff Base Amino Acid M(II) Complexes

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Abstract: Azomethine amino ligands derived from the condensation of 3-methoxysalicylaldehyde (MS) or 4-diethylaminosalicylaldehyde (DS) with α-amino acids (L-phenylalanine (P) and DL-tryptophan (T)) were synthesized. All ligands were analyzed by IR, ^{1}H NMR, ^{13}C NMR and their melting points. Interaction of the obtained azomethine amino ligands with metal salts produced novel nano sized Fe(II) and Cu(II) complexes. The isolated complexes were characterized by elemental analysis, infrared spectra, ultraviolet-visible and thermal analysis (TGA) in dynamic air atmosphere. The Kinetic and thermal parameters were computed from the thermal data using Coast and Redfern method. The molar conductance values of complexes are relatively low, indicating the non-electrolytic nature of the complexes. Magnetic susceptibility measurements show that the investigated complexes are paramagnetic. Moreover, the stability constants of the prepared complexes were determined spectrophotometrically. The analytical results suggest that amino acid Schiff bases behave as dibasic tridentate ONO ligands, and coordinate with Fe(II) and Cu(II) ions in octahedral geometry according to the general formula $[M(HL)_2].nH_2O$. The particle size of the prepared complexes was determined using TEM and it was found to be in nano scale. Moreover, the antimicrobial effects of the ligands and their complexes were screened against some types of bacteria such as *Bacillus subtilis (+ve)*, *Escherichia coli (-ve)* and *Micrococcusluteus (+ve)* and other types of fungi such as *Asperagillus niger, Candida glabrata* and *Saccharomyces cerevisiae*. The results of these studies indicate that the metal complexes exhibit a stronger antibacterial and antifungal efficiency compared to their corresponding ligands. The interaction of the complexes with CT-DNA was monitored using spectral studies, viscosity measurements and gel electrophoreses. Furthermore, it was found that the prepared complexes could bind to DNA in an intercalating mode.

Keywords: Azomethine, Amino acid, TGA, TEM, Antimicrobial, DNA interaction.

1 Introduction

Schiff base complexes have undergone a phenomenal growth during the recent years because of the versatility offered by these complexes in the field of industries, catalysis and biological system, and so forth. The transition metal ions play significant roles in various enzymes functions. Certain drugs play a vital role as bio-ligands in the biological systems. Similarly, nitrogen containing bases such as derivatives of pyrrole, pyridine, pyrimidine, pyrazine and purine have a vital role as bio-ligands. Imine or azomethine groups are present in various natural, naturally derived and non-natural compounds. The imine group present in such compounds has been shown to be critical to their biological activities [1-3]. Due to the presence of the imine group, the electron cloud of the aromatic ring and electronegative nitrogen, oxygen and sulfur atoms in the Schiff bases molecules, these compounds effectively prevent corrosion of mild steel, copper, aluminum and zinc in acidic medium [4].

Schiff base amino acid complexes act as good chelating agents and behave as efficient biologically active and

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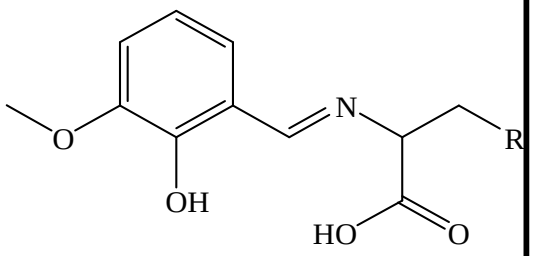
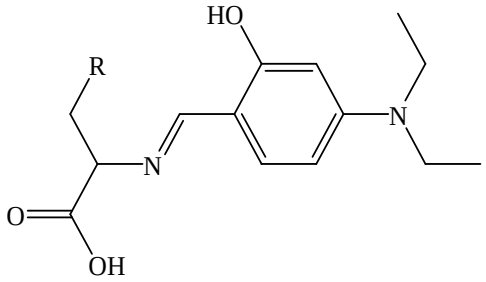
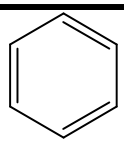
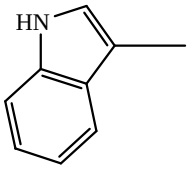
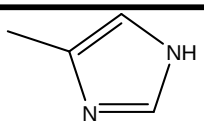
cytotoxic agents. Studying the interaction between transition metal complexes and DNA has attracted many interests due to their importance in cancer therapy, design of new types of pharmaceutical molecules and molecular biology [5, 6].The complexes of transition elements with Schiff bases have wide applications in food industry, dye industry, catalysis, fungicidal, agrochemical, anti-inflammable activity, antiradical activities and biological activities [7, 8].

Among these, heterocyclic Schiff base ligands and their complexes possess great importance due to their pharmacological properties [9]. Deoxyribonucleic acid (DNA) is a most important target molecule in anticancer and antiviral therapies. Furthermore, the interaction of these

Table 1: The prepared Schiff base amino acids ligands

				**Side Chain**
Aldehyde	Ligand Abbreviation	Aldehyde	Ligand Abbrev-iation	**R**
3-methoxysalicyla-dehyde	MSP	4-diethylaminosalicylaldehyde	DSP	
	MST		DST	
			DSH	

complexes with DNA has gained much attention due to their possible applications as new therapeutic agents [5, 7, 10]. Some drugs show increased activity when administered as metal chalets and inhibit the growth of tumors [11]. The transition metal ions are responsible for the proper functioning of different enzymes. If their concentration exceeds a certain level then their toxic effects become evident. Certain drugs play a vital role as bio-ligands in the biological systems. Therefore, this paper shows synthesis of Fe(II) and Cu(II) Schiff base amino acid complexes and their characterization by various physicochemical methods. Moreover, antimicrobial studies of the investigated

complexes were tested against many types of bacteria and fungi. Moreover, the interaction between DNA and the synthesized complexes was examined using absorption spectra, viscosity measurements and gel electrophoresis.

2 Experimental

2.1 Chemicals

All chemicals were used for complex preparation such as 3-methoxysalicylaldehyde ($C_8H_8O_3$) (MS), 4-diethylamino salicylaldehyde ($C_{11}H_{15}O_2N$) (DS), amino acids (L-Phenylalanine (P) and DL-Tryptophane (T)), Calf thymus DNA (CT – DNA), and the metal salts $Cu(CH_3COO)_2$. H_2O and $(NH_4)_2$ $Fe(SO_4)_2.6H_2O$ were of the highest purity and obtained from (Sigma-Aldrich).

2.2 Instruments

Melting points for the isolated ligands and decomposition points for its complexes were monitored on a melting point apparatus, Cimarec 3 Thermolque. The carbon, hydrogen, and nitrogen contents were determined on a Perkin Elmer (2400) CHNS analyzer. IR spectra (4000–400 cm^{-1}) were recorded on Shimadzu FT-IR model 8101 spectrometer using KBr pellets. ^{1}HNMR and ^{13}CNMR spectral measurements were recorded on a BRUKER, using DMSO as an internal reference. The TG/DT analyses were recorded on a Shimdzu corporaion 60 H at 10 degrees min^{-1}. The UV-Vis spectra were recorded on a PG spectrophotometer model T+80 at 298k. Magnetic susceptibility measurements of the metal complexes were done on a Gouy balance at room temperature using $Hg[Co(SCN)_4]$ as a calibrant. Molar conductance was measured on an Elico CM-180 conductometer using 1 m mol L^{-1} solutions in DMSO. A HANNA 211 pH meter at 298 k equipped with a CL-51B combined electrode was used for pH measurements, calibrated against standard buffers (pH 4.02 and 9.18) before measurements. Antimicrobial screening was carried by using agar well diffusion. Viscosity measurements were performed by using viscometer immersed in a thermo stated water bath maintained at 25 ^{o} C. Gel electrophoresis was visualized under UV a transilluminator and photographed with a Panasonic DMC-LZ5 Lumix Digital Camera.

2.3 Synthesis of Schiff base ligands

Three Schiff bases were obtained from L-Phenylalanine (P) or DL-Tryptophan (T) and 3-methoxysalicylaldehyde or 4-diethylaminosalicylaldehyde .The imine derivatives of L-Phenylalanine (P) or DL-Tryptophan (T) have been obtained according to the following method. Ethanolic solution (30 ml) of 5 mmol of 3-methoxysalicylaldehyde or 4-diethylaminosalicylaldehyde (0.76 g, 0.995 g respectively) was added to 5 mmol of L-Phenylalanine or DL-Tryptophan dissolved in hot ethanol. Then the mixture was stirred and refluxed for 2 hours and then it was cooled to room temperature. After 24 h the obtained precipitate was filtered out and washed by hot ethanol [5, 7, 12, 13]. The authenticity and purity of obtained compounds were examined by proton NMR measurements. The structures of the Schiff base amino acids prepared in this study are shown in Table 1.

2.4 Synthesis of the investigated complexes

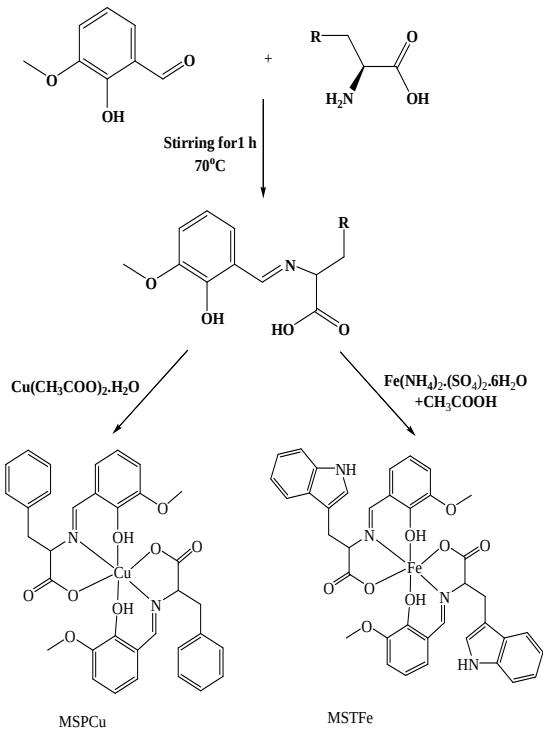
Three novel complexes were synthesized by mixing 40 ml aqueous solutions of the amino acids prepared by dissolving 5 mmole of (0.825g phenyl alanine or 1.045 g tryptophan) with 50 ml hot ethanolic solutions of 3-methoxysalicylaldehyde (5 mmole, 0.76g) or 4-diethylaminosalicylaldehyde (5 mmole, 0.995 g,). Then the mixture was stirred at 70^o C for 1 hour. Then a 40 ml aqueous-ethanol mixture of 2.5 mmole of $(Cu(CH_3COO)_2.H_2O$ (0.5 g) or 30 ml aqueous-ethanol of 2.5 mmole $(NH_4)_2Fe(SO_4)_2.6H_2O)$ (0.98 g). In order to avoid oxidation of Fe(II), a few drops of glacial acetic acid was added.

The mixture was stirred at room temperature for 3 hour. The color changed from yellow to green color for MSPCu, blue color for DSTCu and dark brown color in case of MSTFe. The obtained product was evaporated over night. The obtained solid product was filtered, washed with water, and dried in vacuo over anhydrous $CaCl_2$ [5, 7, 13, 14].

2.5 Thermogravimetric analysis

In the present investigation, heating rates were suitably controlled at 10^o C min^{-1} under oxygen atmosphere, and the weight loss was measured from the ambient temperature up to 750^{o}C. Thermogravimetric analyses of the Schiff base amino acid complexes were used to: (i) get information about the thermal stability of these new complexes, (ii) decide whether the water molecules (if present) are inside or outside the inner coordination sphere of the central metal ion, and (iii) suggest a general scheme for thermal

decomposition of these complexes [15]. TG can directly record the loss in weight with time or temperature due to dehydration or decomposition.



Scheme 1

2.6 Kinetic data for TGA of the prepared complexes

The thermodynamic activation parameters of decomposition processes of complexes namely activation energy (E^*), enthalpy (H^*), entropy (S^*) and Gibbs free energy change of the decomposition (G^*) were evaluated graphically by employing the Coats- Redfern relation [16].

$$\log[\frac{\log[W_\infty/(W_\infty - W)]}{T^2}] = \log[\frac{AR}{\phi E^*}(1-\frac{2RT}{E^*})] - \frac{E^*}{2.303RT} \quad (1)$$

Where W_∞ is the mass loss at the completion the decomposition reaction, W is the mass loss up to temperature T, R is the gas constant and ϕ is the heating rate. Since $1\text{-}2RT/E^* = 1$, the plot of the left hand side of equation (1) against 1/T would give straight line. E^* was then calculated from the slope and the Arrhenius constant, A, was obtained from the intercept. The other kinetic parameters; the entropy of activation (S^*), enthalpy of activation (H^*) and the free energy change of activation (G^*) were calculated using the following equation:

$$S^* = 2.303\ R \log \quad (2)$$

$$H^* = E^* - RT \quad (3)$$

$$G^* = H^* - TS^* \quad (4)$$

Where, (K) and (h) are the Boltzman's and Planck's constant, respectively.

2.7 Antimicrobial evaluation

The newly synthesized ligands and its complexes were screened against two Gram-positive (*Bacillus subtilis* and *Micrococcusluteus*) and one Gram-negative (*Escherichia coli)* by well diffusion method [7, 13, 17] using agar nutrient. The antifungal activities were tested against fungus: *Asperagillus niger*, *Candida glabrata* and *Saccharomyces cerevisiae* by well diffusion method using potato dextrose agar as the medium. Ciprofloxacin and Amphotricine B are used as control for bacteria and fungi, respectively. The suspension of each microorganism was added to a sterile agar medium, then poured into sterile Petri plates and left to solidification. The well was dug in the agar media using sterile metallic borer in each plate. The test solution (15, 30 mg/ml) was prepared by dissolving the compounds in DMSO and the well was filled with the test solution using micropipette. The plates were incubated for 24 h in the case of bacteria and 72 h for fungi at 35° C. The extracts were subjected to further assay with a series on time basis (24, 48 and 72 h). During this period, the test solution was diffused and affected the growth of the inoculated microorganisms. Activity was determined by measuring the diameter of the zone showing complete inhibition (mm). Growth of inhibition was compared with the control. The zone of inhibition is given as the average of three independent determinations.

2.8 Interaction with Calf Thymus DNA

DNA binding studies are important for the rational design and construction of new and more efficient drugs targeted to DNA. A variety of small molecules interact reversibly with double stranded DNA, primarily through three modes. Electrostatic interactions with negatively charged nucleic sugar–phosphate structure, which are along the external

DNA double helix and do not poses selectivity. The second type is through binding interaction with two groves of DNA double helix. The third type is through intercalation between the stacked base pairs of native DNA [5, 7, 13, 18].

Different concentrations of metal complexes were titrated with incremental amounts of CT-DNA over the range (3 – 30 μ M). The equilibrium binding constant (K_b) values for the interaction of the complex with CT-DNA were obtained from absorption spectral titration data using the following equation [5, 7, 13, 19].

By using spectral studies, electronic absorption spectrum of the complex was recorded before and after addition of CT-DNA in the presence of Tris - HCl buffer (pH 7.5).

$$[DNA]/(\varepsilon_a - \varepsilon_f) = [DNA]/(\varepsilon_b - \varepsilon_f) + 1/K_b(\varepsilon_b - \varepsilon_f)$$

Where ε_a is the extinction coefficient observed for the charge transfer absorption at a given DNA concentration, ε_f the extinction coefficient at the complex free in solution, ε_b the extinction coefficient of the complex when fully bound to DNA, K_b the equilibrium binding constant, and [DNA] the concentration in nucleotides. A plot of [DNA]/ (ε_a - ε_f) versus [DNA] gives K_b as the ratio of the slope to the intercept. The standard Gibb´s free energy for DNA binding was calculated from the following relation [5, 7, 13].

$$\Delta G^{o}{}_{b} = -RT \ln K_b$$

Where R is the general gas constant, T is the absolute temperature and K_b is the binding constant.

By using viscosity measurements, viscosity experiments were carried out in an Ostwald viscometer maintained at 25° C in a thermostatic water-bath, to minimize complexities arising from DNA flexibility. The flow times of the samples were recorded with a digital stopwatch operated timer for different concentrations of the complex (10 – 250 μ M), maintaining the concentration of DNA constant (250 μ M). The control sample was carried out on EB by using the same method. The buffer flow time was recorded as t^o. Data obtained were presented as $(\eta/\eta^\circ)^{1/3}$ Vs 1/R (R = [DNA] / [Complex]), where η is the viscosity of DNA in presence of complexes and η° is the viscosity of DNA alone. Viscosity values were calculated from the observed flow time of DNA-containing solution corrected for flow time of buffer alone (t^o), $\eta = t - t^o / t^o$ [5, 7, 13, 20].

By using gel electrophoresis, solutions of DNA were freshly prepared before each experiment using doubly distilled water containing 0.1 M Tris - HCl buffer, pH = 7.2. The concentration of DNA solutions was determined by UV-Vis spectrophotometer at 260 nm using a value of 6600 $M^{-1}cm^{-1}$ for the absorption coefficient. The purity (freedom from bound protein) was assessed from the ratio of the absorbencies at 260 nm and 280 nm. In general, the commercial DNA preparation was found to be free of protein (A_{260nm}/A_{280nm}= 1.9) according to this criterion and no further purification was attempted. Cleavage reactions were run between the metal complexes and DNA, and the prepared solutions were subjected to electrophoresis on 1% agarose gel prepared in TBE buffer (45μM Tris, 45μM boric acid and 1μM EDTA, pH 7.3). Then (20 micron) of ethidium bromide was added to the above solution and mixed well. The gel was mounted into electrophoretic tank; enough electrophoretic buffers were added to cover the gel. DNA sample (10 micron) and metal complex (20 micron) were mixed with loading dye (bromophenol blue) and incubated for 30 min at 25° C, then loaded into the well of the submerged gel using a micropipette.

Table 2: ^{1}HNMR spectral data of the investigated Schiff base amino acids ligands

Comp.	^{1}H NMR (δ, ppm), in DMSO
MSP	13.6 (s, 1H, COOH), 8.4(s, 1H, CH=N), 7.4 – 6.6(m, 8H - Ar), 4.3(s, 1H, OH), 3.9(m, 1H, CH), 3. 8(S, 3H, OCH_3), 3.2 (d, 2H, CH_2).
MST	13.8-13.6 (s,1H,COOH),10.9 (s,1H,NH), 8.2 (s, 1H, CH=N), 7.5 – 6.5(m, 8H - Ar), 4.2(s, 1H, OH), 3.0(d, 2H, CH_2), 3.8 (S, 3H, OCH_3), 3.7(m, 1H, CH),
DST	11.5 (s, 1H, COOH), 9.5(s, 1H,NH), 8.3(s, 1H, CH=N), 6.0 - 7.5(m, 8H – Ar), 4.3(s, 1H, OH), 3.3 (q, 4H, $2CH_2$-CH_3), 2.7 (d, 2H, CH_2 - Ar), 0.9 - 1.2 (t, 6H, $2CH_3$-CH_2), 3.5 (s, 1H, CH).

Table 3: ^{13}C NMR spectral data of the investigated Schiff base amino acids ligands.

Compound	^{13}C NMR (δ, ppm), in DMSO
MSP	193.0 (COOH), 171.0(CH=N), 149.3, 143, 138.8, 128.6, 128.6, 128.3, 128.3, 125.8, 122.2, 122.7, 117.8, 119.4 (12CH - Ar), 71.0(OCH_3), 55.0(CH), 41.0(CH_2).
MST	171.0(COOH), 168.0 (CH=N), 165.0, 155.0, 145.0, 138.0, 136.0, 125.0, 122.0, 121.0, 120.0, 114.0, 117.0, 112.0, 110.0, 70.0 (14CH - Ar), 58.0(OCH_3), 52.0(CH), 29.0(CH_2).
DST	194.0(COOH), 163.0 (CH=N), 148.0, 144.0, 139.0, 133.0, 131.0, 130.0, 128.0, 126.0, 123.0, 113.0, 109.0, 104.0, 103.0, 100.0 (14CH - Ar), 53.0(CH), 38.0 (CH_2-N), 36.0 (CH_2-Ar), 13.0 (CH_3).

Table 4: Analytical and physical data of Schiff base amino acids ligands and their complexes

Compound	Empirical formula (Molecular weight)	μ_{eff} (BM)	Λ_m, ohm^{-1} cm^2 mol^{-1}	M.p Dec. (^oC)	Elemental Analysis found (calc.)		
					C%	H%	N%
MSP	$C_{17}H_{17}NO_4$ (299.0)			220	67.7 (68.2)	4.9 (5.7)	4.5 (4.7)
MSPCu	$C_{34}H_{36}O_{10}N_2Cu$ (695.5)	2.3	8.4	280	58.5 (58.7)	5.3 (5.2)	3.87 (4.07)
MST	$C_{19}H_{18}N_2O_4$ (338.0)			240	67.3 (67.5)	5.1 (5.3)	8.07 (8.3)
MSTFe	$C_{38}H_{38}O_{10}N_4Fe$ (766)	4.9	32.5	>300	56.8 (57.0)	4.9 (4.9)	6.9 (7.3)
DST	$C_{22}H_{25}N_3O_3$ (379.0)			200	69.6 (69.7)	6.5 (6.6)	10.9 (11.1)
DSTCu	$C_{44}H_{53}O_{8.5}N_6Cu$ (864.5)	2.1	9.8	280	57.3 (57.4)	5.7 (5.8)	9.7 (9.9)

The electrophoresis was performed at a constant voltage (100 V) for about 1-2 h (until bromophenol blue had passed through 50% of the gel) in TBE buffer.The gel was visualized under UV a transilluminator and photographed with a Panasonic DMC-LZ5 Lumix Digital Camera [13, 21].

3 Results and discussion

Results of ^{1}HNMR and ^{13}CNMR for ligands are tabulated in Tables 2, 3.

3.1 Microanalysis measurements

The analytical data of the ligands and their metal complexes are given in table 4. The elemental analyses show 1: 2 (metal: ligand) stoichiometry for the solid complexes. The conductivity values in DMF with different concentrations in the range from (0.0025 to 0.0100) Mol dm^{-3} are (8.4, 32.5 and 9.8 for MSPCu, MSTFe and DSTCu respectively) given in Table 4. All complexes were relatively low, indicating the non-electrolytic nature of these complexes [5, 7, 13, 22]. Th microanalysis results suggest that 3-methoxysalicylaldehyde or 4-diethylaminosalicylaldehyde amino acid Schiff bases behave as dibasic tridentate ONO ligands and coordinate to M(II) in octahedral geometry according to the general formula$[M(HL)_2].nH_2O$. The magnetic susceptibilities of all complexes at room temperature are shown Table 4. The values are (4.85, 2.29 and 2.07 BM) for MSTFe, MSPCu and DSTCu respectively, which were consistent with octahedral geometry of the complexes.

3.2 IR spectra

Results of IR spectra are showed in Table 5. The IR spectra provide valuable information regarding the nature of the functional group attached to the metal atom. In order to study the bonding mode of Schiff base to the metal complexes. The IR spectrum of the free ligand is compared with the spectra of the complexes. The broad band at 3443 -3389 cm^{-1} which assigned to ・(OH) vibration of water molecules associated with the complexes [23].

The bands observed in the region 3095 3049 cm^{-1} and the region 2961– 2915cm^{-1} can be assigned to the ・υ(C - H) aromatic and ・(C - H) aliphatic stretching vibrations, respectively[5, 7, 13, 24]. The ligands exhibit the characteristic υ(C=N) band in the 1651 – 1628 cm^{-1}

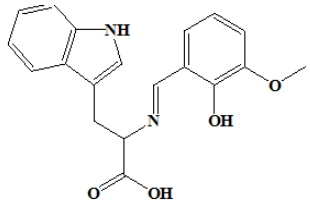
region, while the complexes the (C=N) were observed in the 1626 - 1613 cm^{-1} region The (C=N) stretching frequency is generally shifted to a lower frequency, indicating decrease in the (C=N) bond order due to the coordinate bond of the metal with the imine nitrogen lone pair [5, 7, 13, 25]. The ・υ(C - O) . (phenolic) vibration of ligands are observed around 1293-1244 cm^{-1}, which gets shifted to a lower frequency region in the complexes indicating coordination of phenolic oxygen [26]. The ligands exhibit other two intense bands at (1410 - 1345), (1599 - 1588) cm^{-1} corresponding to symmetric stretching and asymmetric stretching frequencies of (COOH) group, respectively of the organic ligand. Symmetric and asymmetric bands were shifted to a lower frequency upon complex formation [27]. The difference between symmetry and asymmetry stretching vibration of COO^- group showed that amino acid Schiff bases coordinated through COO^- group [28]. Two new bands appeared at 737 - 679 cm^{-1} and 559 - 551 cm^{-1} and it could be attributed to ・υ(M-N) and ・υ(M-O) stretching, respectively [5, 7, 13, 29, 30].

Table 5: IR spectral data of the investigated Schiff base mino acids ligands and their complexes.

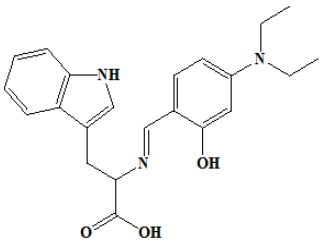
Comp.	υ(OH)/ H_2O	υ(-C=N)	υ_S(COO)	υ_{As}(COO)	υ_{Ar}(C-H)	υ_A(C-H)	υ_{ph}(C-O)	υ (M-N)	υ (M-O)
MSP	3438(s)	1634(s)	1410(m)	1589(m)	3088(m)	2932(m)	1259(m)	-	-
MSPCu	3443(s)	1626(s)	1389(m)	1563(m)	3051(w)	2930(w)	1235(m)	698(w)	559(w)
MST	3389(s)	1651(s)	1362(m)	1588(w)	3090(w)	2917(w)	1293(m)	-	-
MSTFe	3399(s)	1613(s)	1343(w)	1549(w)	3049(w)	2929(w)	1246(m)	737(s)	551(m)
DST	3414(s)	1628(s)	1345(m)	1599(m)	3095(w)	2961(w)	1244(m)	-	-
DSTCu	3416(s)	1620(s)	1391(w)	1551(s)	3053(w)	2915(w)	1240(w)	679(s)	555(s)

S = strong, m = medium, w = weak, Ar = aromatic, A = aliphatic, ph = phenolic

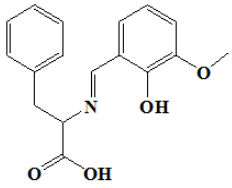
3.3 The proposal structures of ligands



(R, E)-2-(2-hydroxy-3-methoxybenzylideneamino)-3-(1H-indol-3-yl)propanoic acid



(E)-2-(4-(diethylamino)-2-hydroxybenzylideneamino)-3-(1H-indol-3-yl) propanoic acid



(R, E)-2-(2-hydroxy-3-methoxybenzylideneamino)-3-phenylpropanoic acid

Scheme 2

3.4 UV-Visible studies

The wavelengths at maximum absorption band (λ_{max}) and the molar absorptivity (ε) of the different bands in the recorded spectra of the complexes (Fig 1) are given in Table 6. The electronic absorption spectra of the Schiff bases recorded in DMF is composed of three bands in the 200 - 800 nm region. The first band appeard below 300 nm region can be assigned to the π-π* transition of the aromatic rings. The second band observed within the wavelength range 315- 431 nm is due to transition between the π-orbital localized on the central azomethine (-CH = N-) bond [31] while the third band The charge transfer bands being more sensitive to solvent changes than bands resulting from local transitions. A broad band from 498- 621 nm, which indicating that band could be mainly attributed to d → d transition in an octahedral structure of the prepared complexes [5, 7, 13, 33, 34].

3.5 Thermogravimetric Analysis

The Copper (II) and iron (II) complexes are subjected to thermogravimetric analysis in dynamic air in 40–750 °C temperature range, at a heating rate of 10 °C/min. The experimental results are given in Table 7. The MSPCu, DSTCu and MSTFe complexes have weight losses of 5.5%,

Table 6: Molecular electronic spectra, λ_{max} (nm) and ε_{max} ($dm^3mol^{-1}cm^{-1}$) of the prepared Schiff base amino acids ligands and their complexes in DMF at 298 K.

Compound	λ_{max}(nm)	ε_{max} ($dm^3mol^{-1}cm^{-1}$)	Assignment
MSP	249	116	π→π*
	419	136	n→π*
MSPCu	620	80	d-d band
	375	712	LMCT band
	242	1236	Intraligand band
MST	431	100	n→π*
	315	31	n→π*
	247	37	π→π*

MSTFe	498	6550	d-d band
	254	11900	Intraligand band
DST	397	93	n→π*
	247	111	π→π*
DSTCu	621	31	d-d band
	385	171	LMCT band
	239	233	Intraligand band

Table 7: Thermal analysis data of the prepared complexes

Compound	Temperature °C	Fragment loss %		Weight loss %	
		Molecular formula	Molecular weight	Found	Calc
MSPCu	29.5-105.5	$2H_2O$	36.0	4.5	5.2
	106.7-217.3	C_7H_7	91.0	12.6	13.1
	218.9-253.9	C_7H_7	91.0	13.4	13.1
	255.2-323.9	$C_8H_8O_2$	136.0	19.2	19.6
	325.2-404.9	$C_8H_8O_2$	136.0	19.7	19.6
	406.7-508.8	$C_4H_2N_2O_4$	142.0	20.4	20.4
Residu	>750.0	Cu	63.5	9.1	9.1
DSTCu	24.9-220.4	$2.5H_2O$	45.0	5.1	5.2
	221.6-287.4	$C_{12}H_{10}N_2O_2$	214.0	24.8	24.0
	289.1-401.7	$C_{21}H_{29}N_2O_2$	341.0	39.4	39.4
	402.9-521.5	$C_{11}H_9N_2O_2$	201.0	23.2	23.3
Residu	>750.0	Cu	63.5	7.2	7.4
MSTFe	28.3-101.4	$2H_2O$	36.0	4.6	4.7
	103.4-269.5	C_9H_8N	130.0	16.9	16.9
	270.3-442.4	$C_{11}H_9N_2O_2$	201.0	26.2	26.2
	443.6-613.3	$C_{10}H_9NO_4$	207.0	26.9	27.0
	614.5-750.5	$C_8H_8O_2$	120.0	15.6	15.7
Residu	>750.0	Fe	56.0	7.3	7.3

5.1% and 4.6 % which are due to the existence of 2, 2.5 and 2 water molecules of hydration at temperature range of 9.5-105.5 °C, 24.9-220.4 °C and 28.3-101.4 °C respectively. The weight losses of 12.9 %, 13.4 %, 19.2 %, 19.7 and 20.4 % is attributed to the gradual decomposition of the ligand moiety around the metal ion at temperature range 106.7 - 508.5 °C for MSPCu complex, The weight loss of 24.8 %, 39.4 % and 23.2 % is attributed to the gradual decomposition of the ligand moiety around the metal ion at temperature range 221.6 - 521.5 °C for DSTCu complex and the weight losses of 16.9, 26.2, 26.9 and 15.6 % are attributed to the gradual decomposition of ligand moiety around the metal ion at temperature 103.4 °C - 613.3 °C for MSTFe complex. The final product can be observed as a metal residue at temperature up to 750 °C [35].

3.6 Kinetic Data for TGA

The data are summarized in Table 8. The activation energies of decomposition were in the range 12.06 - 165.82

Table 8: The kinetic and thermodynamic data for the decomposition of complexes

Complex	T (^{o}C)	E* J/mol	A S^{-1}	Thermodynamic Parameters (J/mol)		
				S*	H*	G*
MSPCu	56.1	0.6	0.7	-234.9	-465.7	12713.9
	206.3			-245.7	-1713.7	48977.6
	242.6			-247.1	-2015.2	57909.3
	288.9			-248.5	-2400.8	69417.4
	378.4			-250.8	-3143.9	91751.9
	442.9			-252.1	-3680.3	107980.4
DSTCu	185.9	0.5	0.6	-246.0	-1545.1	44299.0
	264.6			-249.4	-2198.4	63792.8
	455.8			-251.7	-2873.2	84150.9
	345.8			-253.9	-3787.1	111958.2
MSTFe	53.1	0.4	0.5	-235.9	-440.7	12085.7
	195.2			-246.7	-1621.9	46543.8
	344.7			-251.5	-2863.9	83825.6
	473.5			-254.1	-3934.1	116374.6
	667.0			-256.9	-5542.7	165819.9

Table 9: The formation constant (K_f), stability constant (pK) and Gibbs free energy (ΔG^*) values of the prepared complexes in aqueous - ethanol at 298K.

Complex	Type of complex	K_f	pK	ΔG^* kJ mol^{-1}
MSPCu	1:2	7.60×10^9	22.8	-56.3
MSTFe	1:2	6.88×10^8	20.4	-50.4
DSTCu	1:2	7.16×10^9	22.7	-56.2

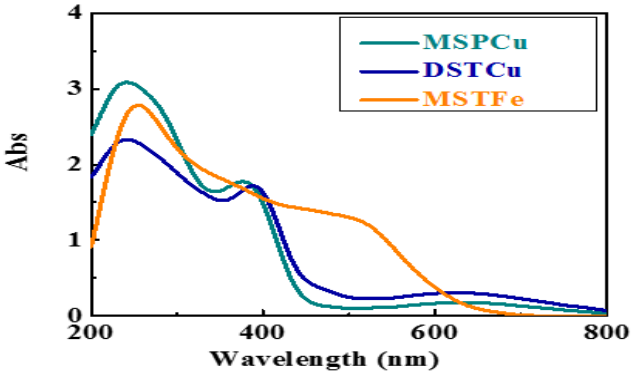


Figure 1: Molecular electronic spectra of [DSTCu] = 10^{-2} mol dm^{-3}, [MSPCu] = 2.5×10^{-3} mol $dm^{-3}$and [MSTFe] = 2×10^{-4} mol dm^{-3}.

KJ /mol. The high values of the activation energies reflect the thermal stability of the complexes. The entropy of activation had negative values in all complexes, which indicates that the decomposition reactions proceed with a lower rate than the normal one [14].

3.7 Determination of the stoichiometry and formation constant of the prepared complexes

The stoichiometry of the various complexes formed in solutions via the reaction of M(II) with the studied ligands was determined by applying the spectrophotomertic molar ratio and continuous variation methods as shown in Figs 2, 3. Complexation of ligands with metal ions were studied in solution using ethanol as a solvent, in order to determine [M:L] mole ratio in the complex follow molar ratio method [5, 7, 13, 36]. A series of solutions were prepared having a constant concentration of the metal ion and the [M: L] mole ratio was determined from the relationship between the absorption of the absorbed light and the mole ratio of [M: L]. Continuous variations method [5, 7, 13, 37, 38], the total number of moles of reactants is kept constant for a series of measurements. Each measurement is made with a different mole ratio or mole fraction of reactants. The maximum change will occur when the mole ratio of the reactants is close to the optimum ratio which is the stoichiometric ratio in the chemical equation. Maximumes of Gibbs free energy mean that the reaction is spon in the curve at X_{ligand} = 0.65 - 0.70 implicates a 1:2 (metal ion to ligand) molecular association.

The apparent formation constants (K_f) of the studied complexes formed in solution were determined from the spectrophotometeric measurements using the continuous variation method according to the following relation:

$$K_f = \frac{(A/A_m)}{4C^2 (1-A/A_m)^3} \qquad \text{(M: L) (1:2)}$$

Where A_m is the absorbance of the maximum formation of the complex, A is the arbitrary chosen absorbance values on either sides of the absorbance pass and C is the initial concentration of the metal. The obtained K_f values indicate the high stability of the prepared complexes. The values of K_f for the studied complexes increase in the following order: MSPCu > DSTCu > MSTFe. Moreover, the values of the stability constant (pK) and Gibbs free energy (ΔG^*) of the investigated complexes are cited in Table 9. The negative valutaneous and favorable [5, 7, 13].

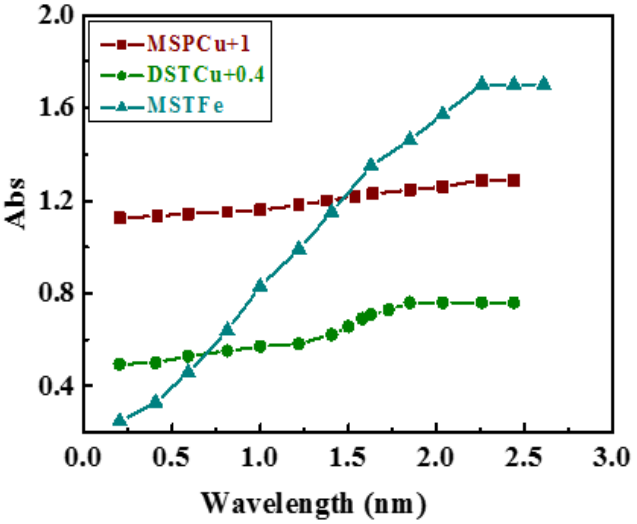


Figure 2: Molar ratio plots for the studied complexes in aqueous-alcoholic mixtures at 298K.

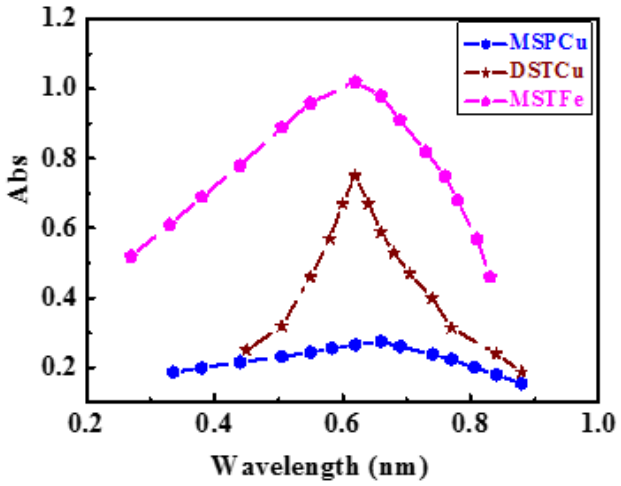


Figure 3: Continuous variation plots for the studied complexes in aqueous-alcoholic mixtures at 298K.

3.8 Particle Size measurements

Fig. (4 (a-d)) shows TEM images of MSPCu and MSTFe complexes. The images of MSPCu complex show that this complex has a wire shape. Moreover, the calculated histogram for the particle size shows that the prepared complexes have 75 and 26 nm for MSPCu and MSTFe complexes respectively. This concludes that the prepared complexes have high surface area and could be possessed catalytic activity [39].

3.9 Stability range of the investigated complexes

The stability range of the studied complexes was found to be in the range pH = 3 – 11 for MSPCu, pH = 4 - 12 for DSTCu and pH = 4 - 9 for MSTFe, according to the obtained pH - absorbance curves (Fig. 5). This means that M(II) ion greatly stabilizes the tested Schiff base amino acids in this range. Accordingly, these ligands can be used as masking reagents of M(II) ions in that range of pH [5, 7 , 13].

3.10 Antimicrobial evaluation

The inhibition zones based upon zones size around discs were measured. The antimicrobial screening results exhibited marked enhancement in activity on coordination with the M(II) ion against more testing bacterial and fungal strains Figs. 6, 7.

Mode of action of antimicrobials may involve various targets in microorganisms. (i) Interference with the cell wall synthesis, damage as a result of which cell permeability may be altered (or) they may disorganize the lipoprotein leading to the cell death. (ii) Deactivate various cellular enzymes, which play a vital role in different metabolic pathways of these microorganisms. (iii) Formation of a hydrogen bond through the azomethine group with the active center of cell constituents, resulting in interference with the normal process. Effect of azomethine (C=N) group. The mode of action of the compounds may involve formation of a hydrogen bond through azomethine group (C=N) with the active centers of cell constituents [40] resulting in interferences with the normal process.

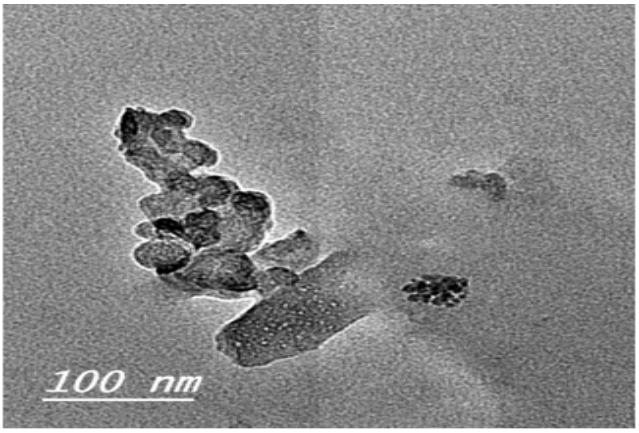


Figure. 4 (a): TEM image of MSTFe complex.

The antimicrobial activity of the metal complexes generally depends on the following factors: the chelation ability of the ligand, the nature of nitrogen donor ligands, the total charge of the complex, the existence and the nature of the metal ion neutralizing the ionic complex and the nuclearity of the metal center in the complex. The susceptibilities of certain strains of bacteria to Schiff base amino acids ligands and their complexes were evaluated by measuring the size of the bacteriostatic diameter. The antimicrobial screening results showed marked enhancement in activity on coordination with the complexes.

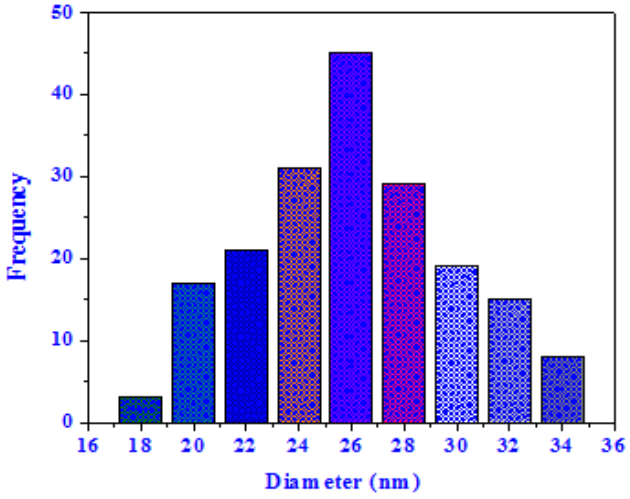


Figure. 4 (b): Calculated Histogram for the particles size of MSTFe complex

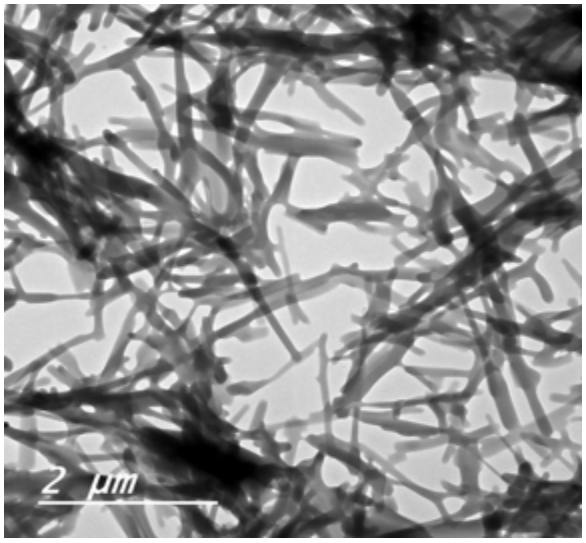


Figure. 4 (c): TEM image of MSPCu complex .

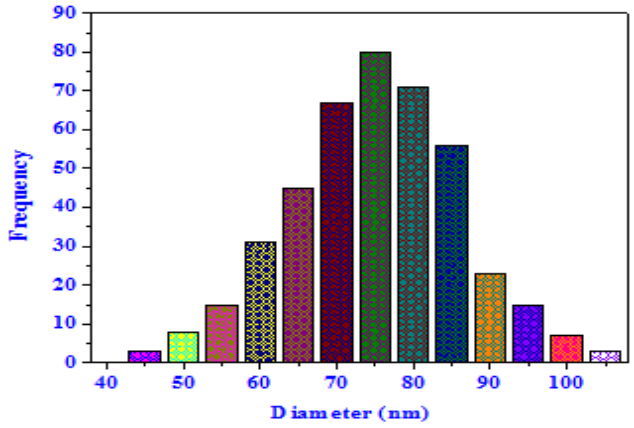


Figure. 4 (d): Calculated Histogram for the particles size of MSPCu complex

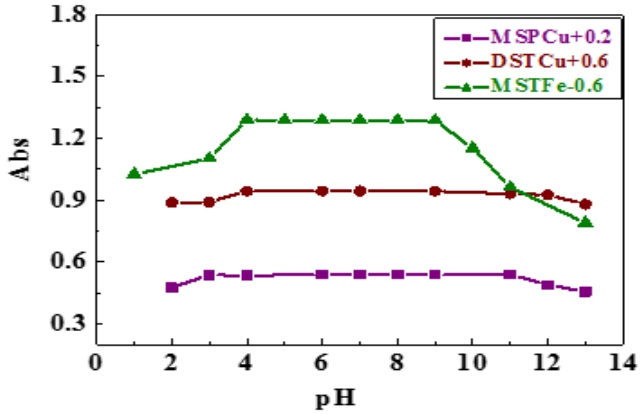


Figure 5: pH profile of the prepared Schiff base amino acid complexes at 298 K.

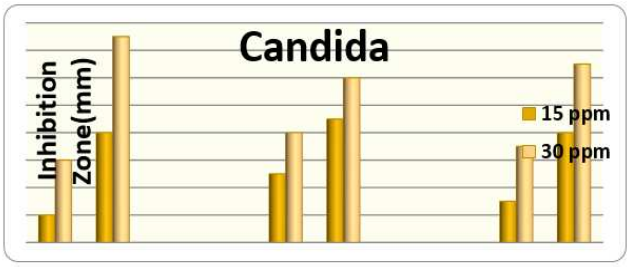


Figure 6: Antifungal evaluation of the investigated Schiff base amino acids ligands and their complexes against *Candida glabrata* fungi.

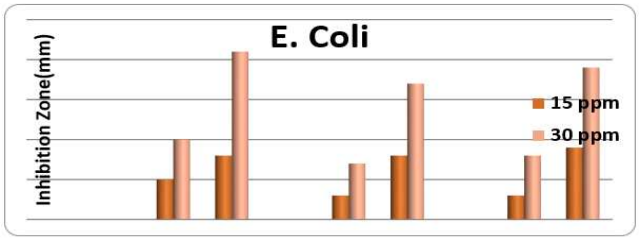


Figure 7: Antibacterial evaluation of the investigated Schiff base amino acids ligands and their complexes against *Escherichia coli* bacteria.

The Schiff base amino acids with nitrogen and oxygen donor systems might inhibit enzyme production, since the enzymes which require these groups for their activity appear to be especially more susceptible to deactivation by the metal ions upon chelating. The theory states that the polarity of the metal ion is reduced on complexation due to the partial sharing of its positive charge with donor groups. Consequently, the positive charge is delocalized over the whole ring, which causes the improved lipophilicity of the compound through cell membrane of the pathogen [7, 13, 41].

3.11 DNA binding analysis using absorption spectral studies

The application of electronic absorption spectroscopy in

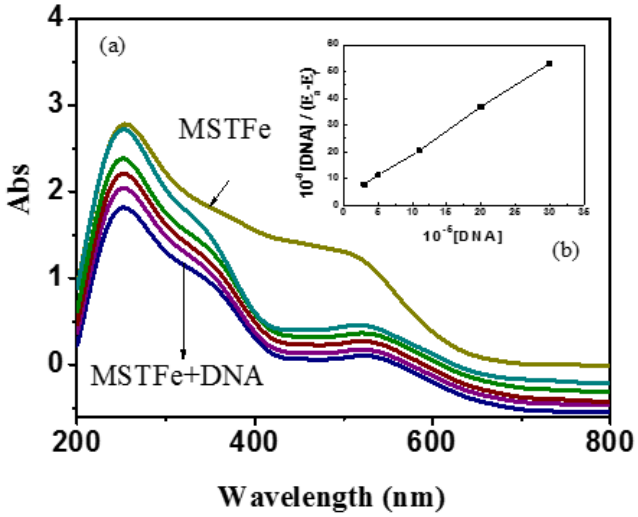


Figure 8: a) Spectral scans of the interaction of MSTFe complex (10^{-2} mol dm^{-3}) in 0.01 mol dm^{-3}Tris buffer (pH 7.2, 298 K) with CT-DNA (3-30 μ M intervals). b) Plot of [DNA] / (ε_a - ε_f) versus [DNA] for the titration of DNA with MSTFe complex.

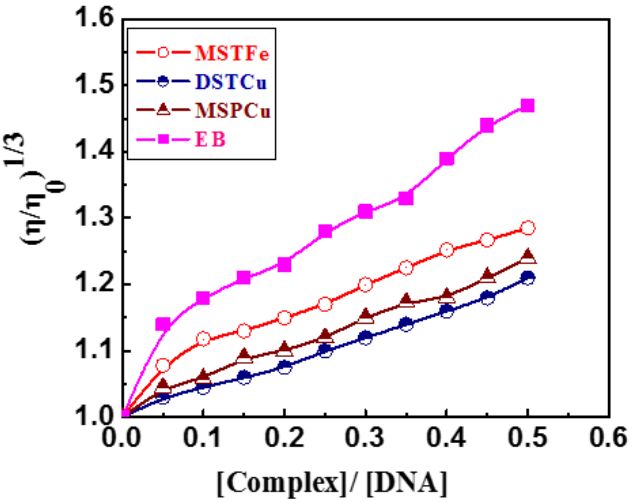


Figure 9: The effect of increasing concentration of the synthesized complexes on the relative viscosities of DNA at [DNA] = 0.5 mM, [complex] and [EB] = 25 -250 μM and 298 K.

CT-DNA binding studies is one of the most important techniques [5, 7, 13, 42].

The DNA binding studies were characterized by absorbance maximum at 378 nm for MSPCu, 389 nm for DSTCu and 518 nm for MSTFe. The addition of increasing higher concentration of DNA led to hypochromic (except DSTCu, the addition of increasing higher concentration of DNA led to hyperchromic) in its visible absorption spectra as a result of the formation of more stable complexes. The interaction of complexes with DNA resulted in the decrease of absorption intensity accompanied by a shift towards lower wavelengths (2 to 34 nm). The percentage of the hypochromism is about 35.0 % to 38.8 % reduction. The spectral changes were used to evaluate the intrinsic binding constant (K_b), it observed $1.7x10^5$, $1.3x10^5$ and $5.4x10^5$ M^{-1} for MSPCu, DSTCu and MSTFe, which are intercalated into DNA base pairs [5,7, 13, 43] .

The complexes may bind to DNA through intercalative interaction, which is brought by the π-π interaction between the complex which possesses an aromatic ring moiety in the ligands and the aromatic heterocyclic bases of DNA. The electronic absorption spectra of the prepared complexes in the absence and presence of different concentration of buffered CT-DNA are given in Table 10 and Fig. 8.

3.12 DNA binding analysis using viscosity measurements

Viscosity measurements were carried out to provide information on binding model between complexes and DNA. By increasing concentrations of complexes, the relative specific viscosity of DNA increased, but the increase is less than that observed for the typical intercalator EB, indicating that intercalative as shown in Fig. 9. A classical intercalation model results in lengthening of the DNA helix as base pairs to an increase of DNA viscosity [5, 7, 13, 44].

3.13 Agarose gel electrophoresis of CT-DNA interaction with the investigated complexes

The cleavage efficiency of complexes was compared to that of the control as function of their efficient DNA binding ability Fig. 10.

Table 10: Spectral parameters for DNA interaction with the prepared complexes

Comp.	λ_{max} free (nm)	λ_{max} bound (nm)	Δn (nm)	Chromism (%) [a]	Type of Chromism	$10^5 K_b$ $mol^{-1}dm^3$	ΔG^* kJ mol^{-1}
MSPCu	630	640	10	87.8	Hypo	1.7	-29.9
	378	371	7	38.2	Hypo		
MSTFe	253	251	2	35.0	Hypo	5.4	-32.9
	518	522	4	61.5	Hypo		
DSTCu	623	630	7	38.7	Hypo	1.3	-29.3
	389	355	34	38.2	Hyper		

[a] Chromism (%) = $(A_{free} - A_{bound}) / A_{free}$

Control experiment using DNA alone does not show any significant cleavage of CT-DNA although on longer exposure time. The variation in DNA–cleavage efficiency of ligands / transition metal complexes was due to the difference in their binding ability. It was clearly concluded that when complexes cleaved the DNA, the pathogenic growth was arrested through destruction of the genome of the organism [13].

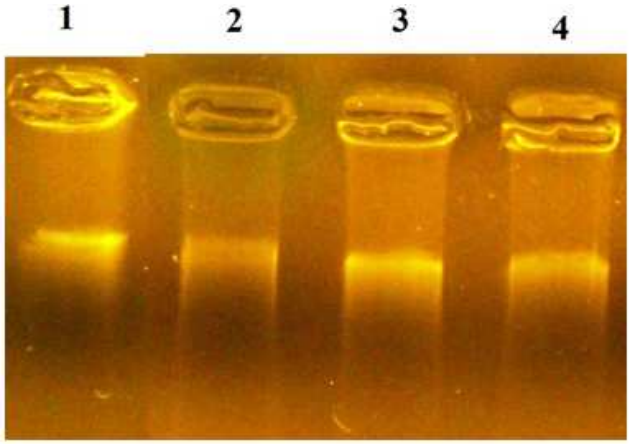


Figure 10: DNA binding results of Schiff base amino acid complexes based on gel electrophoresis. Lane 1: CT - DNA, Lane 2: CT - DNA + MSTFe, Lane 3: CT –DNA+MSPCu, Lane 4: CT – DNA+DSTCu.

4 Conclusion

In this study new dibasic tridentate ONO amino acid Schiff bases ligands and its iron (II) and copper (II) complexes have been synthesized. The structure of these complexes has been confirmed by analytical data, IR, electronic, ^{1}H NMR, magnetic susceptibility, molar conductance and thermal studies. Based on the analytical and spectral studies, octahedral geometry was proposed for the Fe(II) and Cu(II) complexes with general formula $[M(HL)_2].nH_2O$. The prepared complexes have non-electrolytic nature. The obtained K_f values increased in the following order

MSPCu > DSTCu > MSTFe. Thermal study reveals that the complexes are thermally stable. The synthesized Schiff base metal complexes show better antibacterial and antifungal activities than those of the ligands. The DNA binding study takes place via intercalative mode. The information obtained in this study could be helpful in understanding the mechanism of the interactions of metal (II) complexes with nucleic acids and should be useful in the development of potential probes for investigation of the structure and conformation of DNA or new therapeutic agents for some diseases.

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