

Synthesis, Characterization, Study of antimicrobial activity and Evaluation of stability constants of Cu(II) complexes with ligands 2-(4,5dihydro-1,5-diphynyl-1H-pyrazole-3-yl)phenol derivatives.

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Abstract- The synthesis and characterization of an unique tetradentate Schiff bases were presented in this report namely: 2-(4,5dihydro-1,5-diphynyl-1H-pyrazole-3-yl)phenol (HL¹) and 2-(4,5dihydro-5-(4-methoxyphenyl)-1-phynyl-1H-pyrazole-3-yl)phenol (HL²) and their copper complexes. On the basis of elemental analysis, magnetic susceptibility, conductivity, electronic, IR, ESR spectral data and powder X-ray powder diffraction observations indicated that for investigated complexes have been proposed octahedral geometry. The stability constants were determined using the p^H metric technique. At various temperatures, a series of titrations using standard NaOH solution were performed. a temperature of 25°C and 30 °C. On the basis of experimental results and analysis it is observed that the values of proton-ligand stability constants and metal-ligand stability constants were found to decrease with increase in temperature. The antimicrobial and antifungal activities of the studied ligands as well as their copper complexes were evaluated using the cup plate technique at various doses in DMSO solvent using the minimum inhibitory concentration (MIC) method. The research indicates that Cu(II) complexes have high microbial activity, whereas ligands (HL¹) and (HL²) have moderate activity.

Key words: Complexes, ligands, conductivity, spectral, stability

I. INTRODUCTION

The solution study of metal complexes has played a crucial role in the medicinal, analytical, environmental and biological sciences [1]. Metal complexes containing physiologically active ligands are a topic of intense fundamental and technical interest. [2]. The specificity of metal ligands and the strength of metal ligand bonds are determined by stability constants. [3]. For the determination of the stability constant, PH-metry has been used effectively. The research of J. Bjerrum and Ido-Laden advanced the understanding of metal-chelates and ionic complexes in solution equilibrium. Schwarzenbach and Ackermann [4] establish that stability of the chelates decreases as the increase of size ring. As the Coordination chemistry of metal-complex plays a crucial role in biological system of organism[5] hence the development, stability and reactivity of these complexes has been a dynamic field of research [6]. The stability constant gives the formation of metal-ligand. Metal ions plays essential role in the formation of stable complexes is of attention to the analytical chemists and bio-inorganic researchers [7]. Metal complex stability constants are widely utilised in a variety of disciplines, including biological processes, medicines, analytical procedures, separation methods, and so on. Metal complexes serve an important function in nature, and they've been widely employed as enzyme inhibitors in therapeutic trials. Stability constants determine the metal-ligand selectivity and potency of metalligand bonds. Complexes are formed by the majority of the d-block and f-block components. There are diverse kinds of ligands used for complexation[8]. In the present work we prepare complexes of copper (II) with 2-(4,5dihydro-1,5-diphynyl-1Hpyrazole-3-yl)phenol (HL¹) and 2-(4,5dihydro-5-(4-methoxyphenyl)-1-phynyl-1Hpyrazole-3-yl)phenol (HL²) have been prepared, characterization and evaluation of stability constant of Cu(II) metal complexes with ligands 2-(4,5dihydro-1,5-diphynyl1H-pyrazole-3-yl)phenol derivatives.

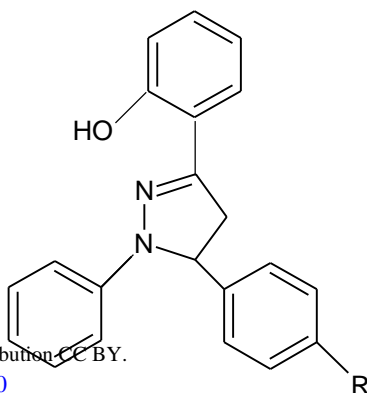


Fig.1: HL¹: R=H, 2-(4,5-dihydro-1,5-diphenyl-1H-pyrazole-3-yl)phenol,
HL²: R = OCH₃, 2-(4,5-dihydro-5-(4-methoxyphenyl)-1-phenyl-1H-pyrazole-3-yl)phenol

II. MATERIALS AND METHODS

Sisco-Chem Industries provided all of the chemicals and solvents, which were AR grade. Commercial sources have been used to get the metal salts. Standard techniques were used to estimate the metal content [8]. In a DMF solution, The electromagnetic spectra were acquired in the 200-1000nm region using an Elico SL 159 spectrophotometer (10-3). The HERAEUS C, H, N-O fast analyzer was used to acquire elemental analyses. On a VARAN E-109 GHz, ESR measurements were taken. The experiment has been carried out with DPPH as a reference and a 3200 Gauss field. A model 300 Lewis Coil Force Magnetometer with a one Tesla field strength was used to test magnetic susceptibilities at room temperature using the Faraday approach. With Hg [Co (SCN)₄][9], the instrument was calibrated. Using an Electro-thermal IA 9100 equipment, all melting points were measured without correction (Shimadzu, Japan). A Perkin-Elmer 1650 spectrophotometer was used to record IR spectra as potassium bromide pellets (Shimadzu). pH measurements were taken using a Horiba F.8 pH metre that had been calibrated with standardized buffer solutions. Merck, in Germany, supplied the copper(II) sulphate pentahydrate. Most of the other compounds were acquired from Merck in Germany and were analytical grade. All throughout research, double-distilled water was utilised.[10].

Preparation of Ligands:

2-(4,5dihydro-1,5-diphenyl-1H-pyrazole-3yl)phenol (HL¹) and 2-(4,5dihydro-5-(4-methoxyphenyl)-1-phenyl-1H-pyrazole-3yl)phenol(HL²):

Phenyl hydrazine (0.021mol) was treated to a solution of 2'-hydroxy chalcones (0.02mol) in 30 ml of methanol and 6-9 hours refluxed. The product was precipitated and filtered after the reaction mixture had completely cooled. From methanol 2-(4,5dihydro-1,5-diphenyl-1H-Pyrazole-3-yl)Phenol(HL¹) and 2-(4,5dihydro-5-(4methoxyphenyl)-1-phenyl-1H-Pyrazole-3-yl)Phenol(HL²) the crude product was crystallized [11].

Preparation of copper complexes with (HL¹) and (HL²):

Copper chloride (CuCl₂.2H₂O) in (0.01 moles) was refluxed with an ethanolic ligand solution (0.02 moles) in an ethanolic medium for around 5-6 hours, and the mixture of reactions was introduced along with 1 g of Sodium Acetate. The reaction mixture was added to distilled water (100ml) with a constant stirring. Separate metal (II) complexes were filtered and cleaned over fused calcium chloride with distilled water containing little quantity of ethyl alcohol, and vacuum-dried. Table-1 illustrates the physical features of ligands and also the metal complexes.

pH metric titrations:

The readings were taken with a digital pH metre version L-120 with combined glass electrode (reading precision 0.001 pH units). Standard buffer (BDH) solutions were used to standardise the instrument at various pH levels. Throughout the titration, the experimental solution's temperature was maintained at a constant temperature. The temperature was maintained with a Hakkin-1101 thermostat. For temperature regulation, the reaction mixture (50ml) was put in a cell with an external thermostatic jacket. The activated combination glass electrode was dipped into the reaction mixture and pre-purified nitrogen gas was pushed across it throughout the pH titration[13]. To keep the reaction mixture homogeneous, a magnetic stirrer (REMI 1 MLH) was used to agitate it constantly. Individually, A standard carbonate-free NaOH solution was used to titrate the three solutions.

1. Titration of free acid (A): HCl (1x10⁻³ M), 2. Titration of free acid + ligand (A + L): HCl (1x10⁻³ M) + L (1x10⁻³ M) and 3. Titration of free acid + ligand + metal (A +L+M): A (1x10⁻³ M)+L (2x10⁻³ M) + M (1x10⁻³ M). At 0.1 M ionic strength of NaClO₄, these titrations were performed at varied temperatures of 25 ±1 °C and 30 ±1 °C.

Determination of Stability Constants: Titration values for Cu²⁺ metal ions were obtained at different temperatures using NaClO₄ with a 0.1 M ionic strength. The stability constants of generated compounds were investigated in the pH range of 3-13. Using Irving and Rossotti's[14] equation as a reference, The standard curve of solutions 1 and 2 was used to calculate the mean values of A (the average number of protons associated with the ligand) for the examined ligands (HL¹ and HL²) at various pH levels. On plotting log $\bar{n}_A/(1 - \bar{n}_A)$ vs. pH, a straight lines having an intercept equal to P^{Ka} on the pH axis are obtained. From the titration curves of solutions 1-3, the metal + ligand formation number values \bar{n} (the average number of ligand molecules coordinated to the metal ion) of the metal complexes were obtained at various pH values. The \bar{n} values were found to be around or equal to 2, indicating the formation 1:2 complexes. The \bar{n} values were calculated using the Irving and Rossotti formulation[14]. The free ligand exponent P_L was calculated from the obtained values of \bar{n} .

III. RESULTS AND DISCUSSION

Table 1. lists the analytic and physical properties of the complexes and their ligands. The results of the complexes' elemental analysis match stoichiometry for metal:ligand ratios of 1:2 molar ratios. The molar conductance values of these complexes in DMF correspond to non-electrolytes. The magnetic moment of copper(II) complexes is 1.80-1.82 B.M., which is consistent with a spin-only value. The magnetic moment of copper (II) complexes with deformed octahedral geometry and no spin interaction is reported to be in the range of 1.80-1.93 B.M[15-17]. As a result, it's possible to deduce that copper (II) complexes have a lot of spin interaction.

Table 1: Analytical data of ligands and copper complexes

Ligands/ Complexes	Yield (%)	M.p (°C)	Elemental analysis				
			Cu	C	H	N	Cl
HL ¹ C ₂₁ H ₁₆ ON ₂	67	162- 163	-	80.72 (80.75)	8.92 (8.96)	5.21 (5.15)	-
HL ² C ₂₂ H ₁₈ O ₂ N ₂	67	167- 168	-	77.25 (77.18)	9.29 (9.34)	5.35 (5.29)	-
Cu(L ¹) ₂ Cl ₂ Cu(C ₂₁ H ₁₄ ON ₂) ₂ Cl ₂	80	190	8.36 (8.42)	66.79 (66.84)	3.75 (3.71)	7.48 (7.43)	9.45 (9.40)
Cu(L ²) ₂ Cl ₂ Cu(C ₂₂ H ₁₆ O ₂ N ₂) ₂ Cl ₂	82	192	7.75 (7.80)	64.87 (64.82)	3.87 (3.93)	6.95 (6.88)	8.65 (8.71)

The values in the parentheses were calculated one

A broad band detected at 3300 cm⁻¹ in the ligands HL¹ and HL² is attributed to ν_{OH} vibrations, which were found to be absent in all of their corresponding complexes, showing that phenolic oxygen is included in binding with the metal ion through deprotonation. The moderate frequency sharp bands, which occurred at 1617 cm⁻¹ in the case of ν_{C=N} in the case of ligand, has shifted negative 23-21 cm⁻¹ in the case of all complexes and now appears in the range 1594-1596 cm⁻¹. When compared to their ligand, all complexes' bands move to the lower frequency side, indicating that the azomethine function's nitrogen atom is co-ordinated in bonding with the metal atom. Srinivas et al [18], have reported ν_{M-Cl} vibration in the range 320-300cm⁻¹ for chloro bridged Cu(II) complexes. ν_{M-Cl} vibrations are ascribed to the bands seen in the range 315-306 cm⁻¹ in all complexes of the ligands HL¹ and HL². Chohan et al [19], have reported the bands in the region 525-510 cm⁻¹ and 450-445 cm⁻¹ for ν_{M-O} and ν_{M-N} vibrations respectively. Bipin et al [20], have reported the ν_{M-O} vibrations in the region 568-527 cm⁻¹ and ν_{M-N} vibrations around 414 cm⁻¹. Under the present investigation the bands observed in the region 569-536 cm⁻¹ and 437-407 cm⁻¹ in all the complexes of the ligands HL¹ and HL² are assigned ν_{M-O} and ν_{M-N} vibrations.

Cu(II) is a d⁹ ion that illustrates the John Teller effect well. Three bands have been discovered in this study at 14824.35 cm⁻¹, 18135.86 cm⁻¹ and 32467.53 cm⁻¹ for Cu(L¹)₂Cl₂ and 14705.88 cm⁻¹, 18181.81 cm⁻¹ and 32258.06 cm⁻¹ for Cu(L²)₂Cl₂ which may be assigned to the transitions ²B_{1g} → ²A_{1g}, ²B_{1g} → ²B_{2g} and ²B_{1g} → ²E_g respectively. These findings suggest that The ESR spectra of the copper complex [Cu(L¹)₂]Cl₂ as polycrystalline sample have been recorded at room temperature (ESR chart was calibrated with DPPH). The observed g values of the [Cu(L¹)₂]Cl₂ complexes are as follows: g_{||} = 2.47038, g_⊥ = 2.16656, g_{av} = 2.27235 and G = 2.85494. The isotropic g values have been calculated by Kneubuhl's methods and methods reported earlier. The g_{||} values indicate that the complex is covalent in nature g_{||} > g_⊥; it is evident that the unpaired electron lies predominantly in the dx²-y² orbital of Cu(II) ion. The G = (g_{||} - 2) / (g_⊥ - 2) which measures the exchange interaction between copper centers in a polycrystalline solid, has been calculated[21].

The powder x-ray diffraction for [Cu(L²)₂]Cl₂ that is Cu(C₂₁H₁₄ON₂)₂Cl₂ have been studied. The complexes are found to consist of some reflections between 8 and 69° (2θ), which result from x-ray diffraction by planes of metal ions. Using Bragg's equation, nλ = 2d Sinθ, where λ is the wavelength of the x-ray utilised (Cu kλ = 1.54056 Å⁰), the inter planar spacing (d) was determined from the sites of intense peak. In the table, the estimated spacing as well as corresponding value with regard to the most intense peaks are noted. All of the major peaks have an index of [22-23], and their Sin2θ values are compared to those calculated. When these numbers are compared, it is clear that the computed and observed values are in good agreement. The amorphous character of the complexes is shown by the broad peak of the complex.

Copper (II) complexes have been given an octahedral geometry based on elemental analysis, magnetic susceptibility, conductivity, electronic, IR, and ESR spectrum data, as well as powder X-ray powder diffraction data.

The average value and straight-line techniques were used to calculate the mean p^{Ka} values from the corresponding various experimental formation curves (shown in figure 2). For the studied ligands [HL¹] and [HL²], the findings obtained for proton-ligand complexes (pKa values) were 7.820 and 8.890 at 25±1°C and 6.467 and 9.298 at 30±1°C, respectively. The formation curves for the complexes were obtained by plotting the relation between average number of ligands attached per metal ion (\bar{n}) and free ligand exponent (p_L), \bar{n} and p_L were calculated as previously mentioned. To compute successive stability constants (logk and logβ), the method of interpolation is used[14] where logk and logβ are equal to the values of (P_L) when (\bar{n}) = 0.5 and 1.5 respectively. logk and logβ for all complexes at different temperatures are given in Table 2.

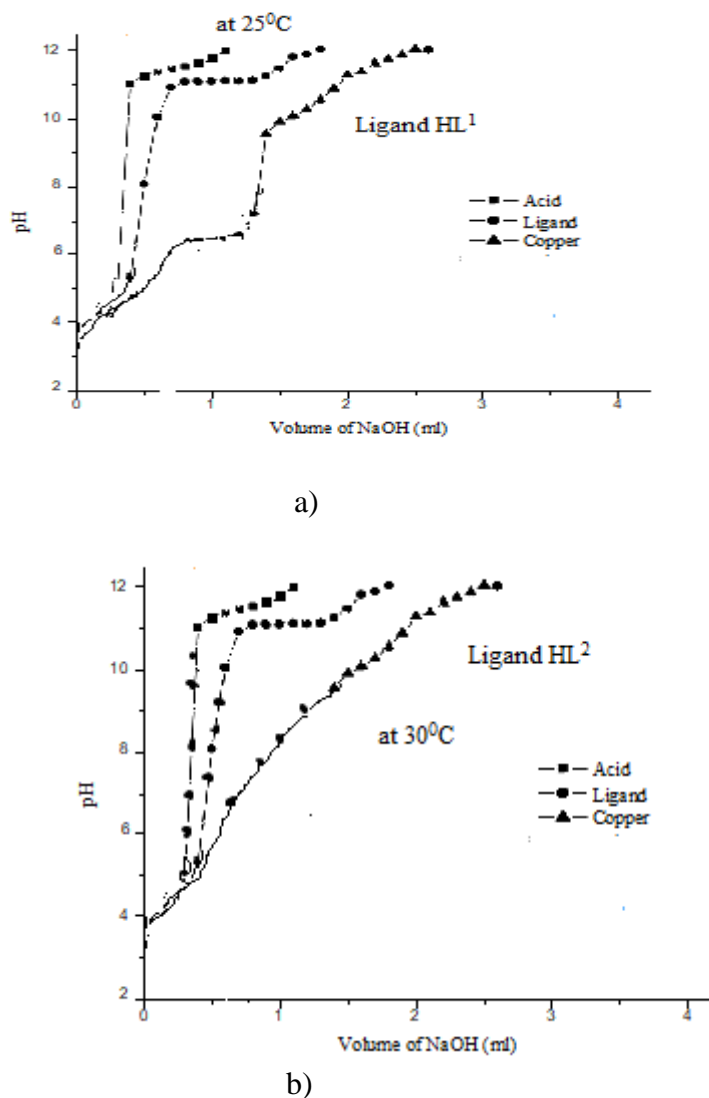


Fig.2. Titration curves p^H vs volume of NaOH

It is possible to investigate the relationships between the ligands' ionisation constants (p^{K_a}) and the stability constants ($\log k$, $\log \beta$) of their complexes with Cu^{2+} ions. The stability constant ($\log k$, $\log \beta$) of the complexes appears to grow as the pK_a of the ligands rises. Table 2 shows that the stability constants of Cu^{2+} ions complexes with the studied ligands (HL^1 and HL^2) decreases with the increasing temperature.

Table 2: Stability constants of copper complexes of ligands HL^1 and HL^2 at $25 \pm 1^\circ C$ and $30 \pm 1^\circ C$ and at 0.1 M ionic strengths in aqueous media.

Copper complexes with	Stability constants			
	$25 \pm 1^\circ C$		$30 \pm 1^\circ C$	
	$\log k$	$\log \beta$	$\log k$	$\log \beta$
HL^1	7.010	5.680	5.765	4.935
HL^2	6.630	5.443	6.254	5.142

Antimicrobial activities:

Both the investigated ligands, 2-(4,5dihydro-1,5-diphenyl-1H-pyrazole-3-yl)phenol (HL^1), 2-(4,5dihydro-5-(4-methoxyphenyl)-1-phenyl-1H-pyrazole-3-yl)phenol (HL^2) and The cup plate technique was used to test their copper complexes for antibacterial and antifungal activities, at different concentrations in DMSO solvent by minimum inhibitory concentration (MIC) method.

Antibacterial and antifungal activity against *Aspergillus niger* and *Cladosporium* were evaluated, as well as antibacterial and antifungal activity against *Escherichia coli* and *Staphylococcus aureus*. Gentamycine and fluconazole common antibiotics were also evaluated for antimicrobial and antifungal activity at the same proportion as the test compound under the same conditions. The results (Table 3) demonstrate that the Cu(II) complexes have high bacterial and fungal activity, which is attributed to the Cu(II) complexes' quicker diffusion [24-25], while the ligands (HL¹) and (HL²) have moderate activity.

Table 3: The antimicrobial activity of ligands and its copper (II) complexes evaluated by (mm).

Ligands / Complexes	Zone of inhibition in mm			
	Antibacterial activity		Antifungal activity	
	<i>Escherichia Coli</i>	<i>Staphylococcus Aureus</i>	<i>Aspergillus Niger</i>	<i>Cladosporium</i>
HL ¹	13	12	16	13
Cu(II) with HL ¹	22	23	26	21
HL ²	15	14	13	14
Cu(II) with HL ²	24	25	24	22
Gentamycine	27	29	-	-
Fluconazole	-	-	30	25

IV. CONCLUSION

In the present work reported that the preparation and characterization of a new tetradentate Schiff bases namely: 2-(4,5dihydro-1,5-diphynyl-1H-pyrazole-3-yl)phenol (HL¹) and 2-(4,5dihydro-5-(4-methoxyphenyl)-1-phynyl-1H-pyrazole-3-yl)phenol (HL²) and their complexes. All the complexes are colored, stable in air and insoluble in water and common solvents but soluble in polar solvent like DMF and DMSO. The analytical data obtained suggested 1:2 (M:L) stoichiometry for all complexes. The prepared complexes have electrolytic nature. On the basis of elemental analysis, magnetic susceptibility, conductivity, electronic, IR, ESR spectral data and powder X-ray powder diffraction observations data suggested octahedral geometry has been assigned to copper (II) complexes.

The classical Calvin-Bjerrum technique as modified by Irving and Rossotti was used for determination of the stability constants. The technique involved a series of titrations of standard alkali with (i) hydrochloric acid (ii) hydrochloric acid + ligands HL¹ / HL² (iii) hydrochloric acid + ligands HL¹ / HL² + copper ion in presence of an inert electrolyte such as NaClO₄. Series of titrations were carried out with standard NaOH solution at different temperatures 25°C and 30 °C. The proton-ligand stability constants and metal ligand stability constants were determined by using half integral and graphical methods. The substituents in the ligand aromatic ring alter the electron density at various sites, affecting the proton-ligand and metal-ligand stability constants. The values of proton-ligand stability constants and metal-ligand stability constants were found to decrease with increase in temperature. Both the investigated ligands and their copper complexes were screened for their antibacterial and antifungal activity by cup plate method, at different concentrations in DMSO solvent by minimum inhibitory concentration (MIC) method. The results suggest that the Cu(II) complexes have exhibited good bacterial and fungal activities, whereas, ligands (HL¹) and (HL²) have shown moderate activity.

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