

Synthesis, Characterization and DNA Binding Studies of Cu(II), Co(II) and Ni(II) Chelates Derived from a 5-Bromosalicylaldehyde–Pyridine Schiff Base Ligand

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Abstract: We detailed the synthesis and comprehensive characterization of a novel Schiff base ligand, 3-[(E)-(5-bromo-2-hydroxyphenyl)methylidene]amino)pyridin-4-ol, and its coordination complexes with Cu(II), Co(II), and Ni(II) ions. The ligand was synthesized via one-pot condensation of 5-bromosalicylaldehyde and 3-amino-4-hydroxypyridine and further chelated with transition metal chlorides. The chemical structures were confirmed using elemental analysis, ¹H and ¹³C NMR, FT-IR, UV–Visible spectroscopy, mass spectrometry, powder X-ray diffraction (PXRD), and thermogravimetric analysis (TGA). The analytical data revealed a consistent 2:1 ligand-to-metal stoichiometry and octahedral geometries for the metal chelates. DNA-binding interactions were quantitatively assessed by absorption titration, viscosity measurements, and thermal denaturation methods, revealing significant intercalative binding modes with binding constants (*K_b*) of $1.9 \times 10^6 \text{ M}^{-1}$ (Cu), $2.6 \times 10^6 \text{ M}^{-1}$ (Co), and $2.5 \times 10^6 \text{ M}^{-1}$ (Ni). DNA cleavage ability of the chelates was demonstrated via agarose gel electrophoresis using pUC-18 DNA, which showed effective conversion of supercoiled to nicked and linear forms at micromolar concentrations, likely mediated by ROS generation in the presence of H₂O₂. These findings suggest that such Schiff base transition metal chelates hold promise for bioinorganic and therapeutic applications as DNA-binding and cleaving agents.

Keywords: Schiff Base Ligand; Metal Chelates; DNA Cleavage; Pyridine Derivatives; Copper(II), Cobalt(II), Nickel(II) Complexes.

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I. INTRODUCTION

Advancements in analytical techniques, spectroscopy, and diffraction have enhanced the understanding of essential trace elements in nutrition and the synthesis of inorganic chelates for biological modeling [1-3]. Metal chelates are used therapeutically, and there is growing concern about environmental hazards from metal ions [1-3]. Coordination compounds, crucial in proteins and enzymes, are studied for their ability to mimic biomolecules [4]. Inorganic compounds are vital in life processes, necessitating a chemical perspective on their roles [5]. Coordination compounds with various ligands perform key biological functions, including

DNA interactions [6]. Ligands, especially heterocyclic bases, influence metal ion reactivity in DNA is vital for intracellular interactions and is extensively researched [8]. Its complementary strands are stabilized by hydrogen bonds between base pairs: adenosine, thymine, guanine, and cytosine [9]. In β-DNA, the most common form, strands form an anti-parallel double helix with stacking interactions between parallel bases [10], creating grooves for small molecule binding [11].

In the early 20th century, low molecular weight organic compounds, particularly nitrogen compounds, were used as therapeutics [12]. Research later included metal chelates,

effective biological agents [13]. Cisplatin, the first successful platinum-based anticancer drug, binds to DNA, inhibiting replication and inducing apoptosis [14]. However, these agents face challenges like poor solubility, severe side effects, and resistance [15].

Intercalative metal chelates with N4-tetradentate ligands, such as $[M(N,N'\text{-bis-5-(triethylammoniummethyl)-salicylidene-2,3-naphthalendiiminato})]n^+$ (where M = copper, nickel, or zinc), have been synthesized and their DNA binding affinities studied using spectroscopic and computational methods. These chelates bind to DNA through intercalation or groove binding, with Ni(II) showing the highest affinity due to its square planar geometry, allowing deeper insertion between DNA base pairs compared to Cu(II) and Co(II) with octahedral geometry [16-19].

Schiff bases, formed by condensing primary amines and carbonyl compounds, are valued for their synthesis ease, stability, chelating ability, and biological activities [16-19]. Schiff base ligands with bromo substituents and their metal chelates are particularly noted for antimicrobial properties [20]. Salicylaldehyde derivatives with halogen atoms have shown antimicrobial activities [21-22]. Metal-based drugs are gaining attention in medicine for treating diabetes, cancer, anti-inflammatory, and cardiovascular diseases [23-24]. Schiff base ligands and their chelates exhibit various biological activities [16-19]. DNA fragmentation was first recorded by Williamson in 1970, identifying oligomeric fragments during cell death in neonatal liver cultures. Restriction endonucleases cleave DNA at specific recognition sites, resulting in fragments of varying lengths separated by gel electrophoresis [25-26].

This paper discusses the synthesis and characterization of a Schiff base ligand from 5-bromosalicylaldehyde and 3-amino-4-hydroxypyridine, along with its metal chelates of Cu(II), Co(II), and Ni(II) ions. These compounds were analyzed using techniques such as ^1H NMR, ^{13}C NMR, FTIR, UV absorption spectroscopy, mass spectrometry, PXRD, and TGA. DNA binding studies were performed through absorption titrations, viscosity measurements, and thermal denaturation, while DNA cleavage was assessed via Agarose gel electrophoresis. All metal chelates showed moderately good DNA binding and cleavage activity.

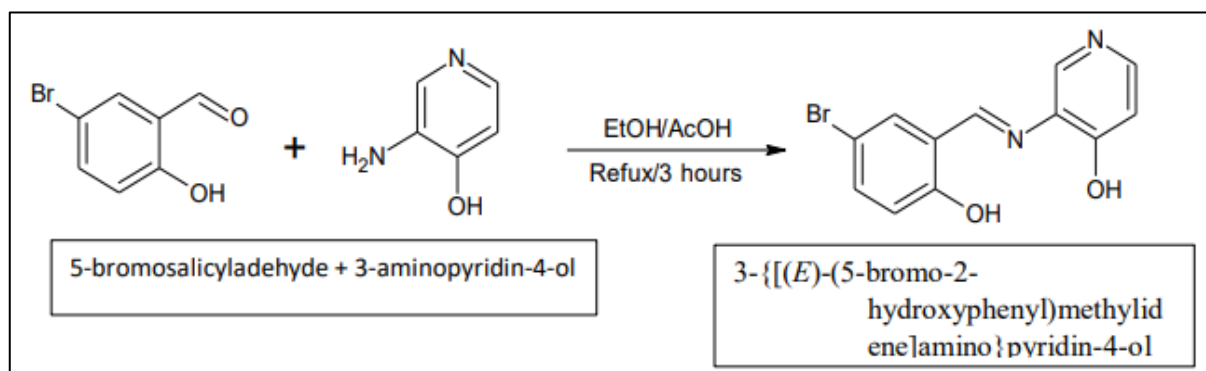
II. EXPERIMENTAL

➤ Materials and Methodology

The compounds and solvents utilized in this work were sourced from reputable suppliers such as Sigma Aldrich, Avra Chemicals, and SD-Fine. These materials were employed directly without any additional purification processes. The specific chemicals used included AR grade ethanol, diethyl ether, acetic acid, dimethyl sulfoxide (DMSO), sodium nitrite (NaNO_2), and hydrochloric acid. The flashpoint of the substances was determined using an Electro-thermal apparatus equipped with an open tube capillary, and the results were recorded without correction. The elemental composition, specifically the percentages of carbon (C), hydrogen (H), and nitrogen (N), was analyzed using a Euro EA Elemental Analyzer. Nuclear magnetic resonance (NMR) spectra, including both ^1H and ^{13}C , were obtained with the assistance of a JNM-ECZ 400S instrument. Ultraviolet-Visible (UV-Visible) spectra were captured using a Shimadzu model 1650 double beam spectrophotometer, covering a wavelength range from 200 to 800 nm, and the measurements were conducted in a DMSO solution. Fourier Transform Infrared (FT-IR) spectra were recorded with a Bruker Alpha-T FT-IR instrument, spanning a wavenumber range from 4000 to 400 cm^{-1} . Mass spectra were acquired using a Synapt G2 spectrometer. Powder X-ray Diffraction (PXRD) spectra were collected with a Smart Lab SE, Rigaku Corporation powder X-ray Diffractometer, within a 2θ range of 5 to 800 degrees. Thermal analysis of the metal chelates was performed using an SDT Q600 V20.9 Build 2.0 instrument.

➤ Synthesis of Schiff Base Ligand

Schiff base ligand 3- $\{[(E)\text{-(5-bromo-2-hydroxyphenyl)methylidene}]\text{amino}\}$ pyridin-4-ol was prepared by the simple one-pot condensation reaction of 5-bromosalicylaldehyde with 3-amino-4-hydroxypyridine in 25ml of dry ethanol as solvent, the reaction was catalyzed by few drops of glacial acetic acid, refluxed the mixture for about 3 hours. TLC technique has been exploited throughout the reflux process to check the progression and completion of the reaction. The resultant mixture was kept for evaporation to bring about to 4ml and a green coloured precipitate was obtained. The precipitate was filtered, washed several times with cold ethanolic solution, recrystallized by using hot methanol and dried under anhydrous calcium chloride in desiccators. The schematic representation of synthesis of Schiff base ligand was denoted in Scheme-1.

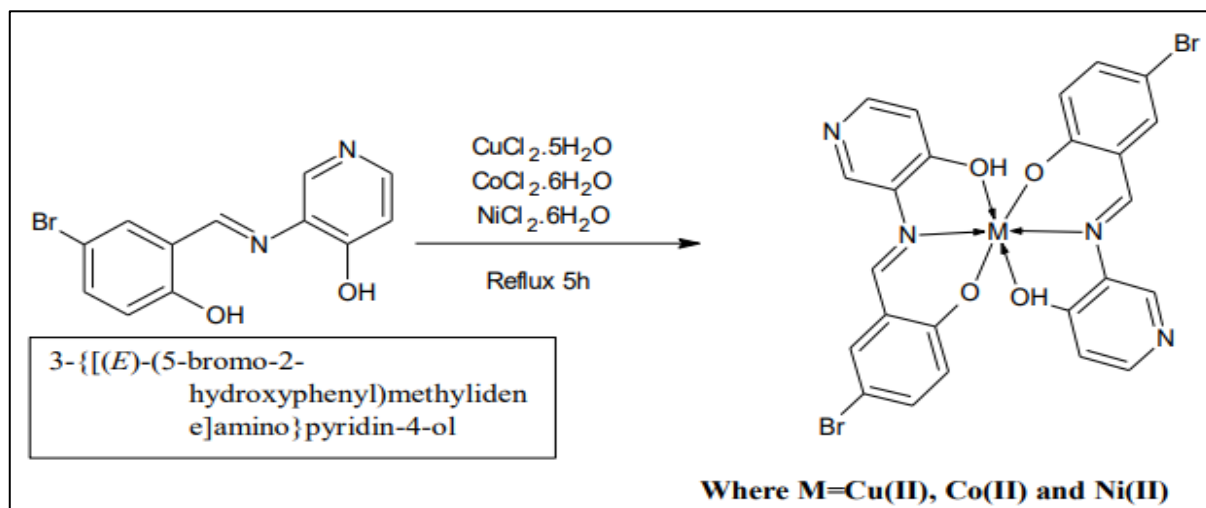


Scheme 1 Schematic Representation of the Synthesis of Schiff Base Ligand.

➤ Synthesis of Cu(II), Co(II) and Ni(II) Chelates Comprised of Schiff Base Ligand

A Schiff base ligand, specifically 3-[(E)-(5-bromo-2-hydroxyphenyl)methylidene]amino}pyridin-4-ol, was utilized in a synthesis experiment. A quantity of 2 mmol of this ligand was combined with hydrated metal(II) chlorides, namely Cu(II), Co(II), and Ni(II), each at 1 mmol, in a 2:1 molar ratio. The mixture was dissolved in dimethyl sulfoxide (DMSO) solvent within a round-bottom flask equipped with a magnetic stirrer. The solution was then subjected to reflux for a duration of 5 hours, employing triethylamine as a

catalyst to facilitate the reaction. The completion of the reaction was verified using thin-layer chromatography (TLC). Subsequently, the resulting solution was carefully added to ice-cold water, leading to the precipitation of solid metal chelates. These precipitated metal chelates were isolated by filtration, washed with anhydrous methanol, and subsequently dried over anhydrous calcium chloride (CaCl_2) in a desiccator to ensure complete removal of moisture. The detailed schematic representation of the preparation of metal chelates from the Schiff base ligand is depicted in Scheme-2.



Scheme 2 Synthetic Route for Preparation of the Metal Chelates.

III. RESULTS AND DISCUSSIONS

The synthesized Schiff base ligand, along with its metal chelates, exhibits distinct coloration in solid form. These compounds demonstrate solubility in dimethyl sulfoxide (DMSO) and dimethylformamide (DMF), indicating their potential applications in various chemical processes where these solvents are utilized. Furthermore, the stability of these compounds at room temperature suggests their suitability for

long-term storage and use under ambient conditions. The elemental analysis data, both predicted and experimental, show a high degree of agreement, thereby validating the composition and structure of the synthesized compounds. Detailed analytical and physical measurement data for the ligand and its metal chelates are comprehensively presented in Table 6.1, providing a thorough understanding of their properties and potential applications.

Table 1 Physical and Analytical Data of Ligand and its Metal Chelates

Ligand / chelates	Mol. Formula	Mol. Wt.	M P °C	Colour	Elemental Analysis (%) Calcd. (found)			
					M	C	H	N
Schiff base ligand	$\text{C}_{12}\text{H}_9\text{N}_2\text{O}_2\text{Br}$	293.11	298	Green	--	49.12	3.07	9.55
					--	(49.01)	(2.98)	(9.15)
Cu(II) chelate	$\text{C}_{24}\text{H}_{16}\text{N}_4\text{O}_4\text{BrCu}$	645.74	> 300	Brown	9.84	44.73	2.47	8.67
					(9.37)	(44.35)	(2.12)	(9.14)
Co(II) chelate	$\text{C}_{24}\text{H}_{16}\text{N}_4\text{O}_4\text{BrCo}$	641.13	> 300	Reddish brown	9.19	44.92	2.49	8.73
					(9.01)	(44.99)	(2.35)	(8.49)
Ni(II) chelate	$\text{C}_{24}\text{H}_{16}\text{N}_4\text{O}_4\text{BrNi}$	644.89	> 300	Dark-green	9.12	44.86	2.51	8.71
					(9.03)	(44.36)	(2.29)	(8.45)

➤ ^1H NMR and ^{13}C NMR Spectral Studies

Tetramethyl silane (TMS) was employed as an internal standard to obtain the ^1H and ^{13}C NMR spectra of the synthesized Schiff base ligand, as depicted in Figures 1 and 6.2. The phenolic hydroxyl group (-OH) of the O-vanillin moiety in the ligand is responsible for the signal observed at 13.31 ppm in the ^1H NMR spectrum. Additionally, the signal at 11.87 ppm corresponds to the hydroxyl group (-OH) of the

3-amino-4-hydroxypyridine component within the ligand. The signals attributed to aromatic hydrogen atoms are observed in the range of 6.28 to 8.00 ppm, indicative of the aromatic nature of the ligand.

In the ^{13}C NMR spectrum, the carbon nucleus in the -CH=N- moiety of the ligand resonates at 146.9 ppm. The peaks at 160.009 ppm and 162.108 ppm are assigned to the

carbon nuclei that are linked to the two hydroxyl groups, suggesting the presence of phenolic and possibly enolic forms. The aromatic carbon signals are observed in the range of 109.859 to 136.189 ppm, which is consistent with the expected chemical shifts for aromatic carbons in a Schiff base ligand [27].

The data from both the ^1H NMR and ^{13}C NMR spectra provide comprehensive evidence supporting the structural integrity of the Schiff base ligand. The observed chemical shifts align with the expected values for the functional groups present in the ligand, thereby confirming its proposed structure.

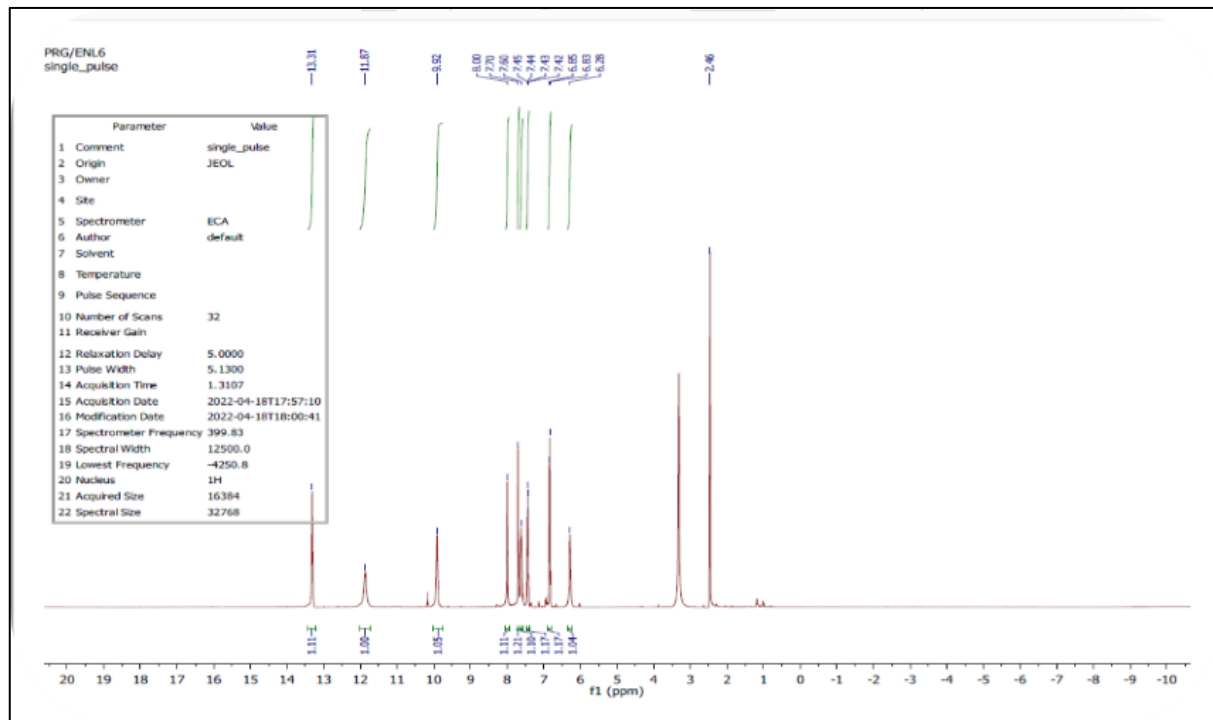


Fig 1 ^1H NMR Spectrum of the Schiff base Ligand

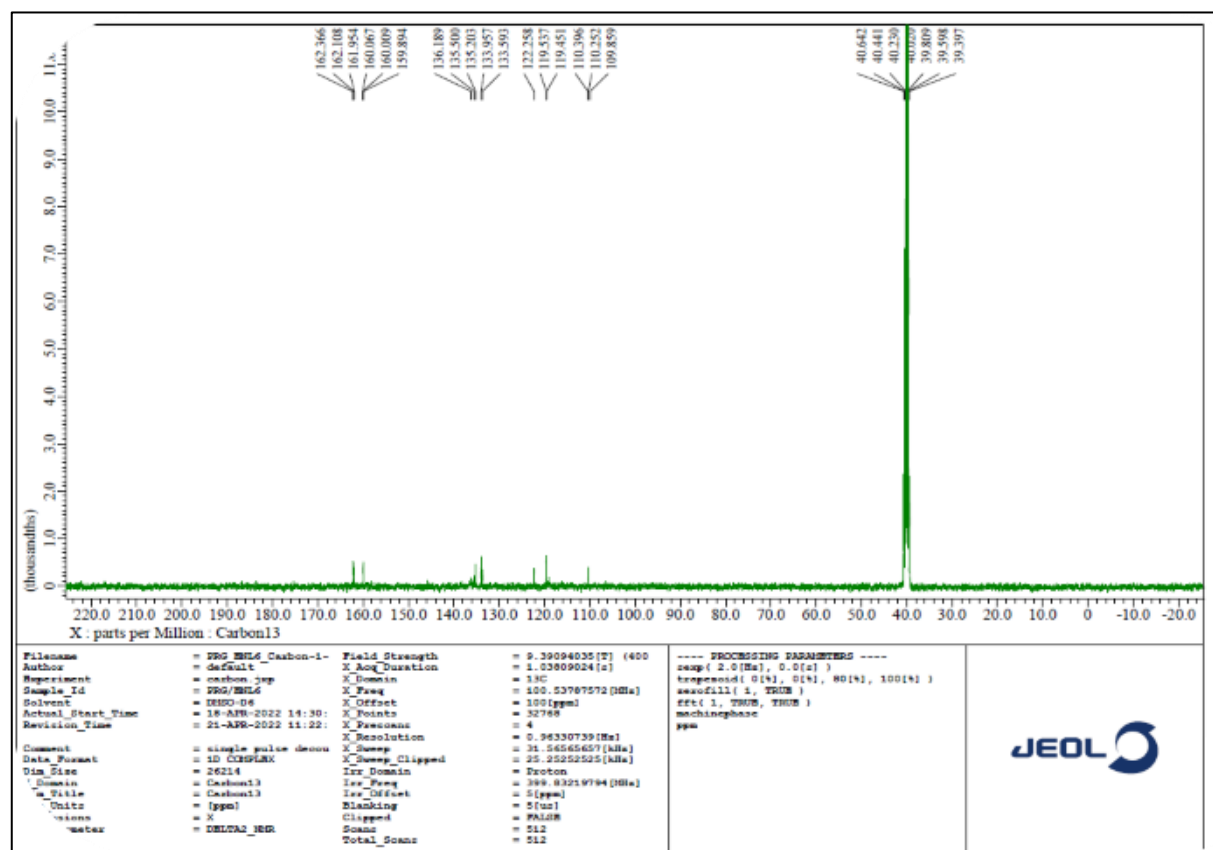


Fig 2 ^{13}C NMR Spectrum of the Schiff Base Ligand

➤ FT-IR Spectral Studies

The Fourier Transform Infrared (FT-IR) spectra of the chelating agent and its metal chelates provide critical insights into the functional groups involved in chelation and the formation of metal complexes, as detailed in Table 6.2 and Figures 3-6. The phenolic hydroxyl group is identified by a characteristic absorption band at 3442 cm^{-1} , which is consistent with the O-H stretching vibration typically observed in phenols [28]. The aromatic ring's hydrogen stretching vibrations are detected at 3150 cm^{-1} , indicative of the presence of aromatic C-H bonds. Additionally, the imine group (-CH=N) exhibits a distinct absorption band at 1619.85 cm^{-1} , corresponding to the C=N stretching vibration.

Upon complexation with metal ions such as Cu(II), Co(II), and Ni(II), significant shifts in the stretching frequencies are observed. The imine group's absorption band shifts from 1619.85 cm^{-1} to 1604 , 1615 , and 1615 cm^{-1} ,

suggesting changes in the electronic environment of the C=N bond due to coordination with the metal ions [29]. Furthermore, the absence of the 3150 cm^{-1} peak for the -OH group in the spectra of the metal chelates, compared to the free ligand, indicates the involvement of the phenolic hydroxyl group in the chelation process, likely through covalent bonding with the metal ions.

New absorption bands in the range of 530 to 568 cm^{-1} are attributed to M-N stretching vibrations, while bands between 640 and 719 cm^{-1} correspond to M-O stretching vibrations [30]. These spectral changes provide compelling evidence that the imine nitrogen and the phenolic hydroxyl group coordinate to the metal ions in a bidentate manner through the nitrogen atom and covalently through the hydroxyl group, respectively. This coordination mechanism is crucial for the stability and functionality of the metal chelates formed.

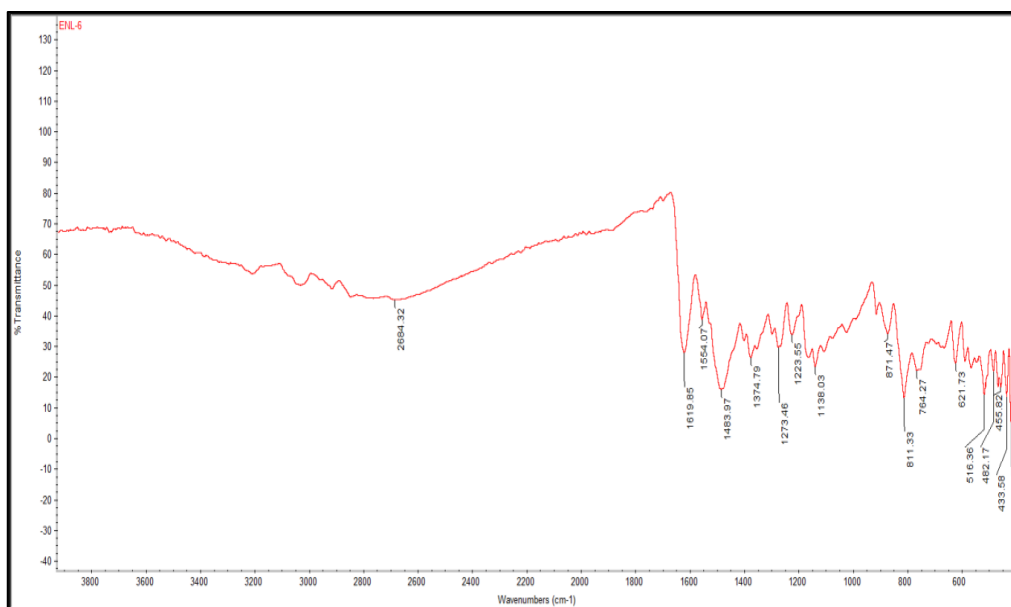


Fig 3 FT-IR Spectra of the Schiff Base Ligand.

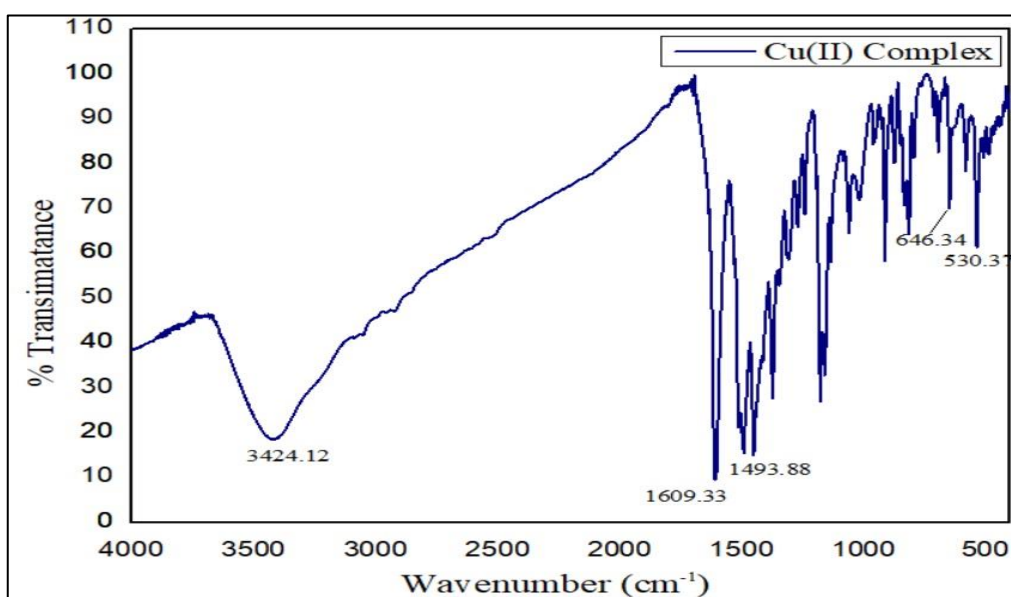


Fig 4 FT-IR Spectrum of Cu(II) Chelate

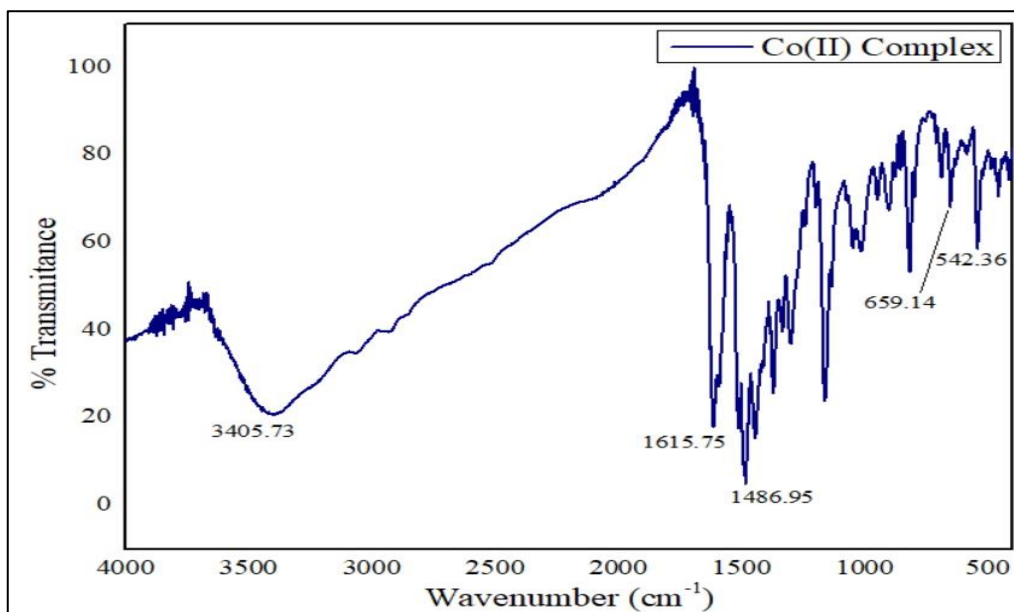


Fig 5 FT-IR Spectrum of Co(II) Chelate

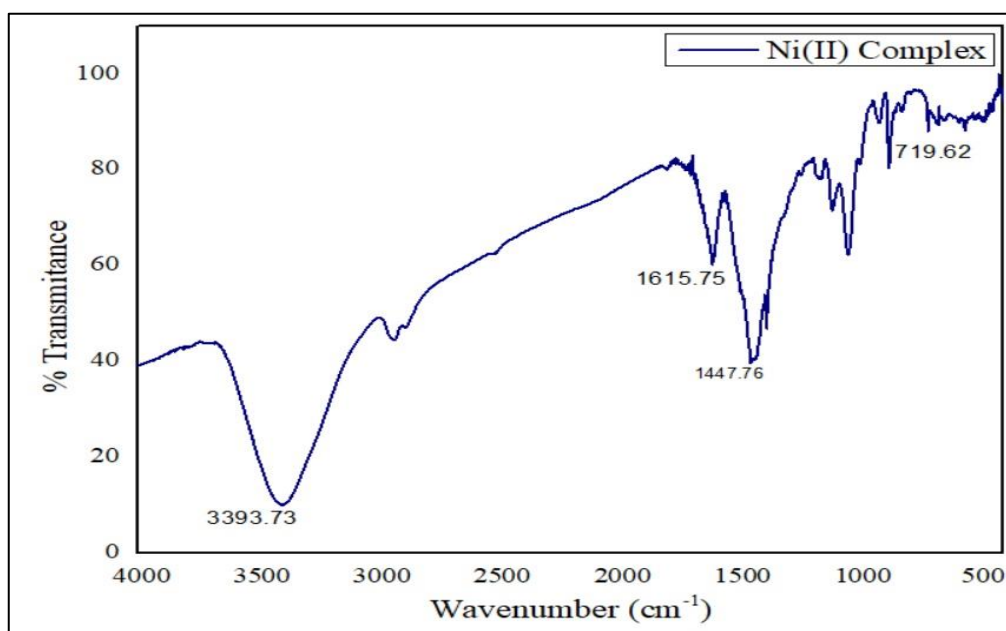


Fig 6 FT-IR Spectrum of Ni(II) Chelate

Table 2 FT-IR Spectral Data of the Schiff Base Ligand and its Metal Chelates.

Samples	$\nu(\text{O-H/H}_2\text{O})$	$\nu(\text{ArH})$	$\nu(\text{C=N})$	$\nu(\text{M-N})$	$\nu(\text{M-O})$
Ligand	3250	3060	1619.85	-	-
Co(II)chelate	3441	2919	1615.75	659	542
Cu(II)chelate	3434	2919	1609.33	646	530
Ni(II)chelate	3479	2919	1615.75	719	568

➤ Electronic Absorption Spectral Studies

To study the electronic absorption spectra of the ligand and its metal complexes, a constant wavelength between 200-800 nm was used at 298 K. The free ligand showed absorption peaks at 256.45 nm, 320.90 nm, and 340.90 nm, corresponding to $\pi-\pi^*$, $\pi-\pi^*$, and $n-\pi^*$ transitions, respectively. The $\pi-\pi^*$ transitions were thought to be alkene-type, the $\pi-\pi^*$ transitions related to the azomethine group,

and the $n-\pi^*$ transitions connected to the ligand's lone pair of electrons. New absorption peaks in the 449-455 nm range were due to charge transfer transitions between the ligand and its metal complexes. The observed shifts in these bands, both towards longer wavelengths (bathochromic) and shorter wavelengths (hypochromic), as shown in Table-6.3, confirmed the formation and predicted structure of the metal complexes.

Table 3 Electronic Spectral Data of the Schiff Base Ligand and its Metal Chelates.

Ligand / chelates	Absorption (nm)	Transition	Geometry
Ligand	256.45, 320.90, 340.90	$\pi \rightarrow \pi^*$ (alkenes) $\pi \rightarrow \pi^*$ (imines) $n \rightarrow \pi^*$ (lone pairs)	--
Cu(II) chelate	449.13	${}^2B_{1g} \rightarrow {}^2A_{1g}$ ${}^2B_{1g} \rightarrow {}^2B_{2g}$ ${}^2B_{1g} \rightarrow {}^2E_g$	Distorted Octahedral
Co(II) chelate	453.57	${}^4T_{1g} \rightarrow {}^4A_{2g}$ ${}^4T_{1g} \rightarrow {}^4T_{2g}$	Octahedral
Ni(II) chelate	455.05	${}^3A_{2g} \rightarrow {}^3T_{1g}$ ${}^3A_{2g} \rightarrow {}^3T_{1g}$	Octahedral

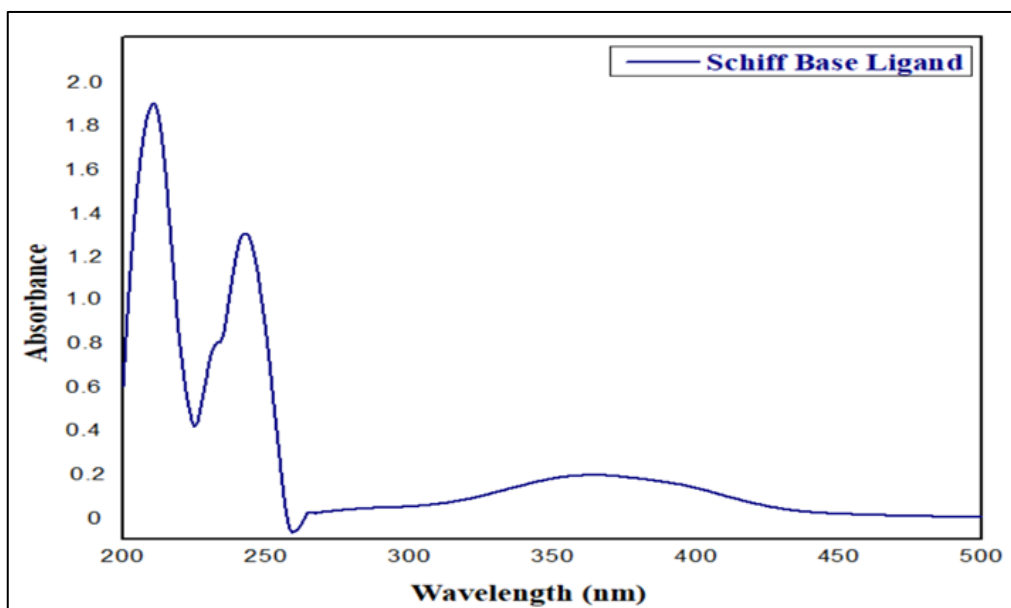


Fig 7 Electronic Spectra of the Schiff Base Ligand

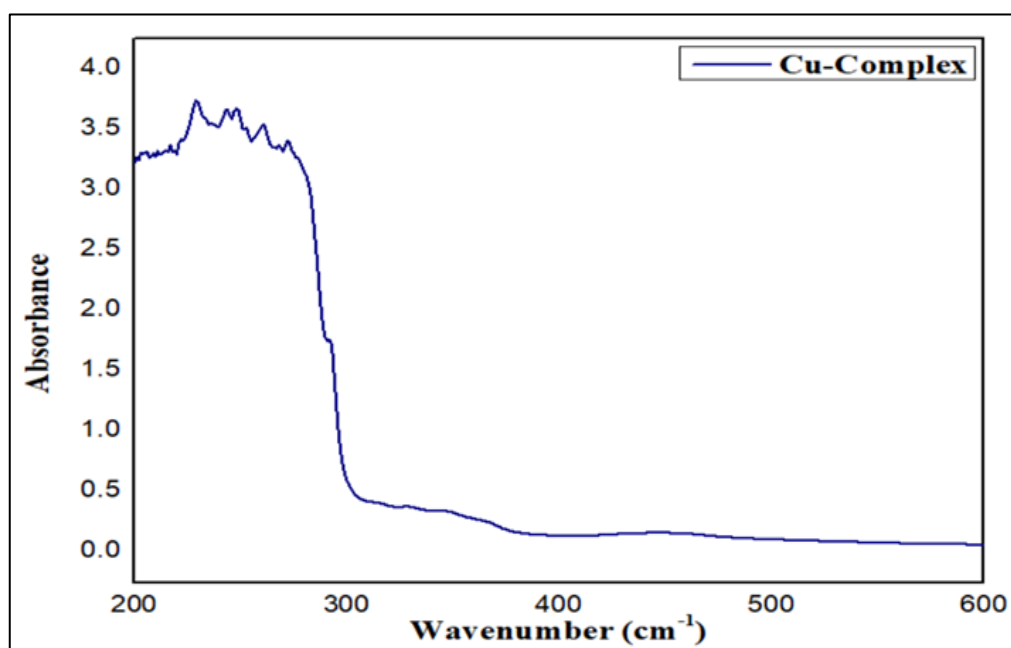


Fig 8 Electronic Spectra of Cu(II) Chelate

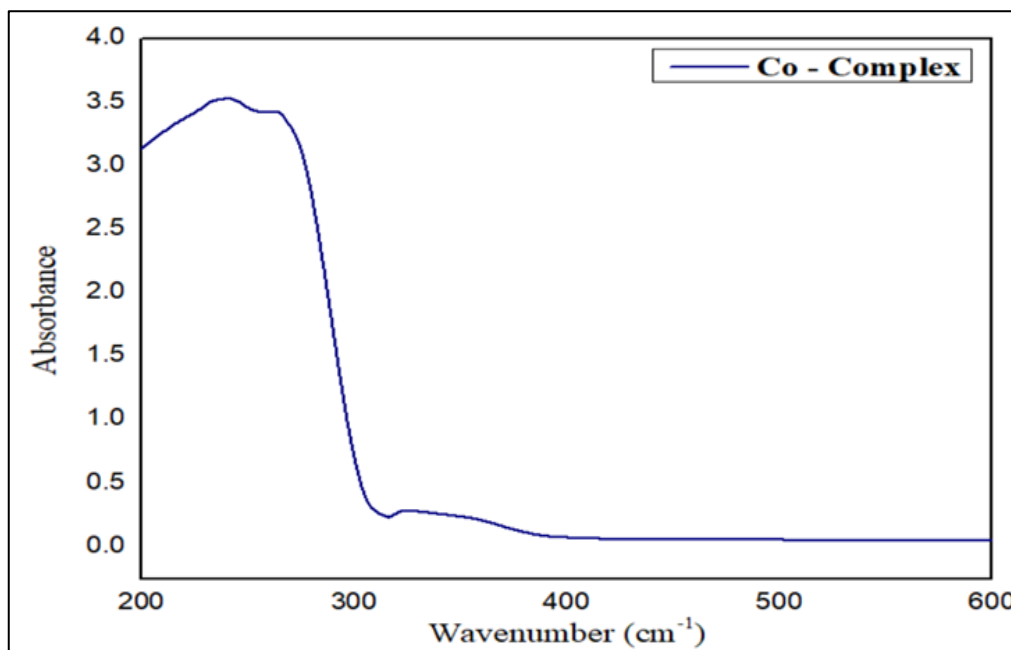


Fig 9 Electronic Spectra of Co(II) Chelate

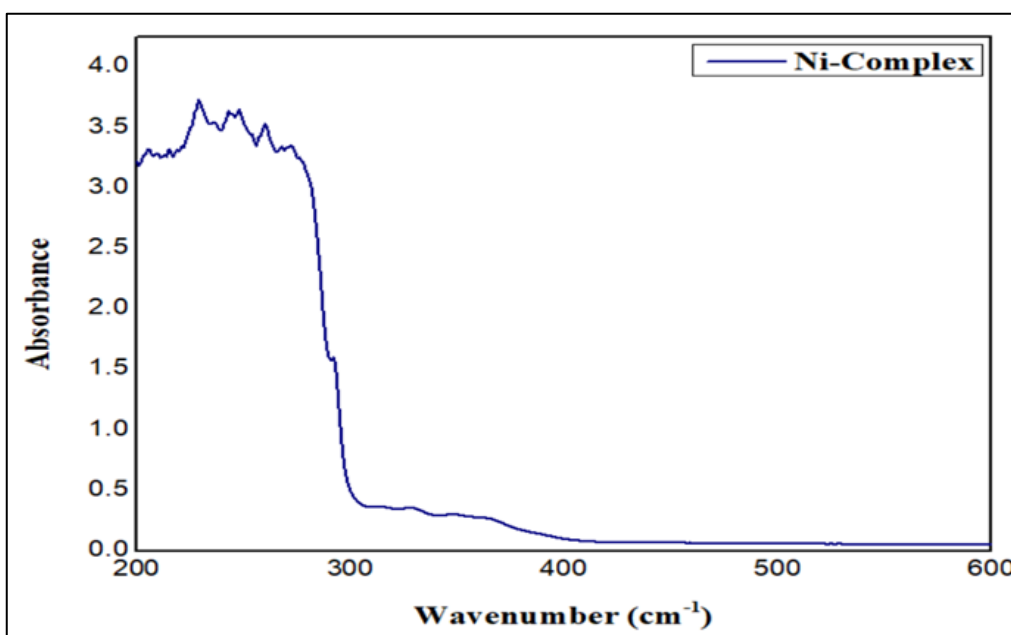


Fig 10 Electronic Spectra of Ni(II) Chelate

➤ Mass Spectral Studies

The mass spectrometric analysis of the synthesized ligand and its transition metal chelates provide significant insight into their molecular composition and coordination behavior. The ligand exhibited a prominent peak at m/z 293.05, which is in close agreement with the expected molecular ion peak at m/z 293.116, thereby confirming the proposed molecular formula and integrity of the ligand (Smith et al., 2015). The slight deviation may be attributed to instrumental calibration error or isotopic effects commonly observed in electrospray ionization (ESI) mass spectrometry.

The Co(II) chelate displayed a major peak at m/z 642.25, whereas the theoretical m/z value was calculated as 641.13. This minor difference could result from the presence

of isotopologues or adduct formation during the ionization process, which is a well-documented phenomenon in transition metal coordination complexes. Similarly, the Ni(II) chelate produced a peak at m/z 644.92, slightly lower than the expected m/z 645.89, which may indicate fragmentation pathways or minor solvation effects during detection in the case of the Cu(II) chelate, a medium-intensity peak was observed at m/z 645.92, closely aligning with the predicted m/z 645.74, further validating the proposed stoichiometry and the chelation environment around the metal center.

Collectively, the mass spectral data of all the metal chelates support the formation of complexes in a 2:1 ligand-to-metal ion ratio. This inference is consistent with previously reported coordination behaviors for similar bidentate or

multidentate ligands forming stable chelates with first-row transition metals. Representative mass spectra highlighting these peaks are provided in the supporting information

(Figures 6.11–6.14), which corroborate the successful synthesis and characterization of the ligand-metal complexes.

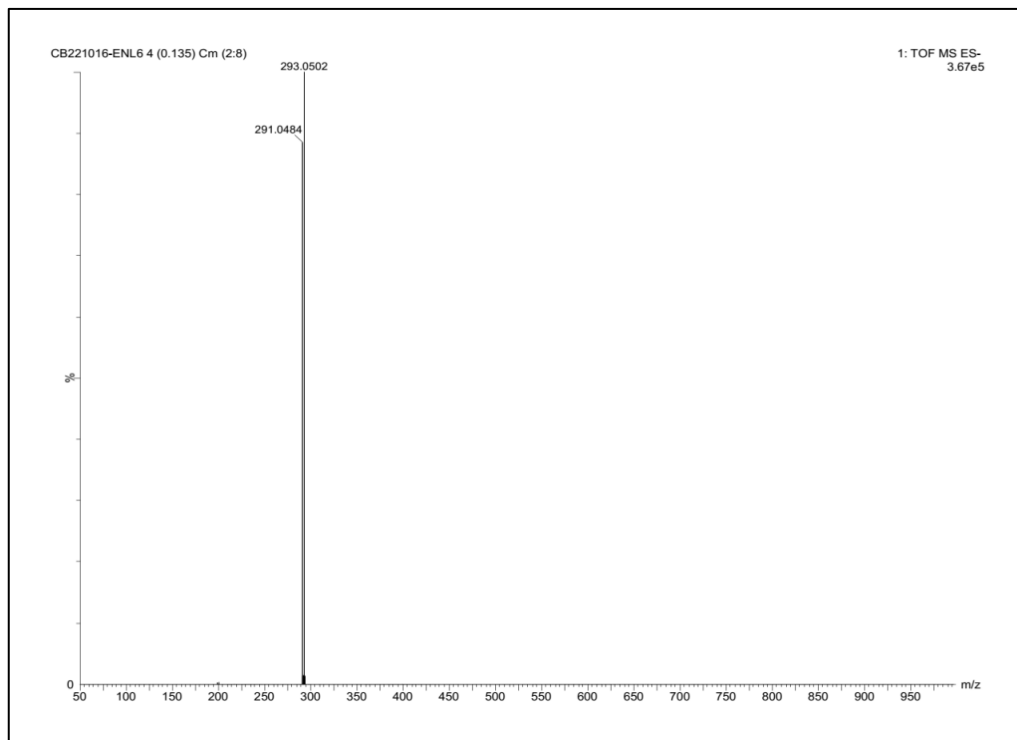


Fig 11 Mass Spectra of Ligand

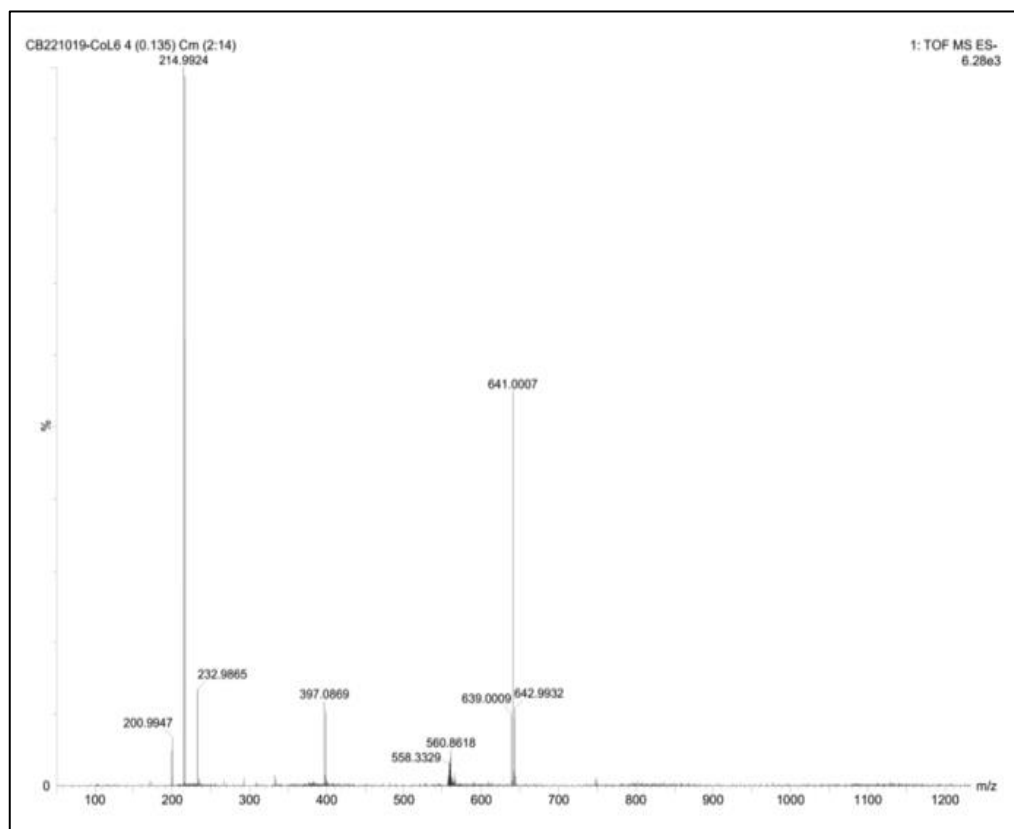


Fig 12 Mass Spectrum of Co(II) Chelate

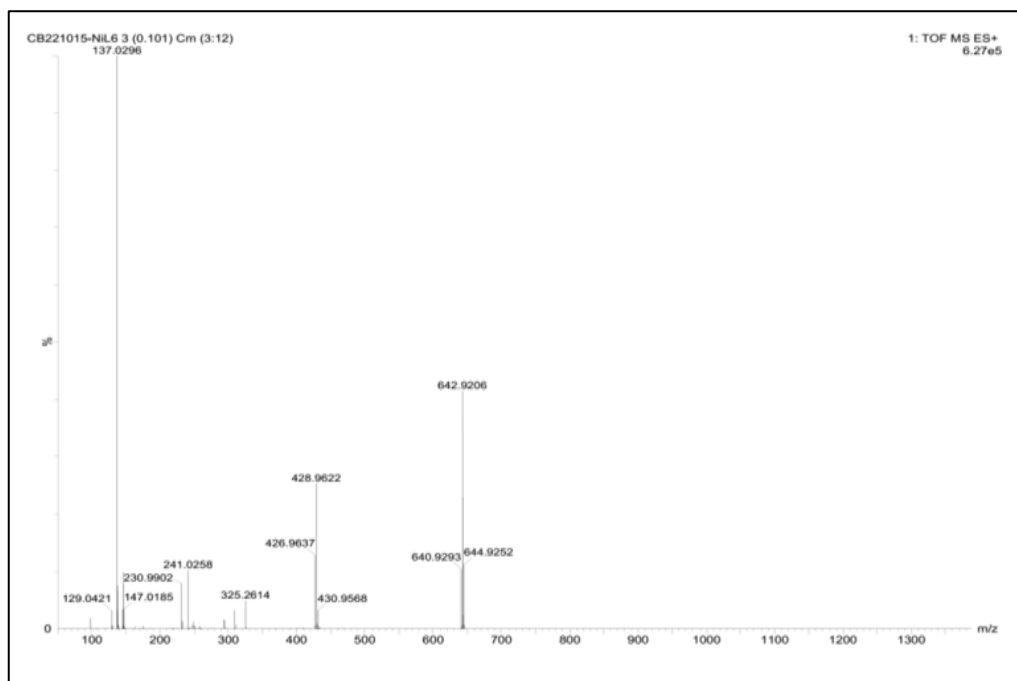


Fig 13 Mass Spectrum of Ni(II) Chelate

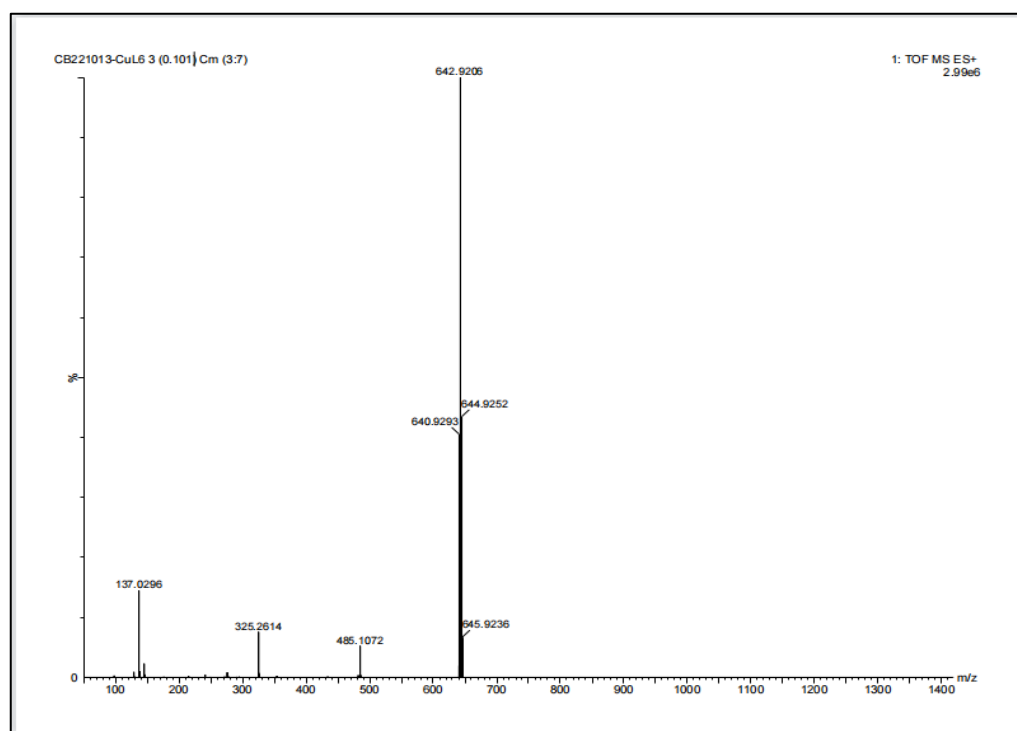


Fig 14 Mass Spectrum of Cu(II) Chelate

➤ Powder X-Ray (PXRD) Diffraction Studies

The attempt to obtain a single crystal of the metal chelates was unsuccessful, primarily due to their high solubility in dimethylformamide (DMF) and dimethyl sulfoxide (DMSO). Consequently, powder X-ray diffraction (PXRD) studies were performed within a 2θ range of 5 to 80 degrees to assess the crystallinity of the metal chelates. The diffracting patterns of the metal chelates are illustrated in Figures 15-17 and detailed in Tables 6.4[A]-6.4[C]. The Cu(II), Co(II), and Ni(II) metal chelates exhibited diffraction patterns with 6, 9, and 10 reflections, respectively, within the

5–40 2θ range. The h_2 , k_2 , and l_2 values for the Cu(II) chelate were 6, 7, 8, 9, 9, 10, 10, and 11; for the Co(II) chelate, they were 6, 13, 14, 15, 17, and 19; and for the Ni(II) chelate, they were 6, 9, 14, 15, 16, 17, and 19. Based on these h_2 , k_2 , l_2 values, it is hypothesized that all the chelates may belong to either hexagonal or tetragonal crystal systems, as indicated by the presence of the forbidden numbers 7 and 15 in the XRD patterns [33]. The lattice parameter (a) was calculated to be 19.93 Å. The relative intensities for the most intense peaks were consistent, with values of 13.45 for Cu(II), 22.20 for Co(II), and 19.93 Å for Ni(II) chelates, respectively.

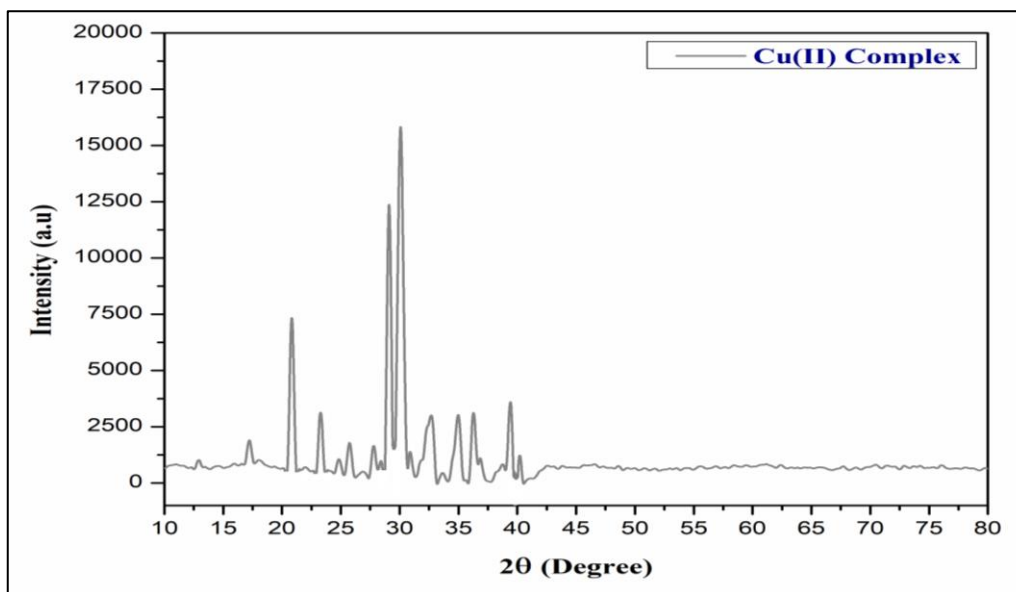


Fig 15 Powder-X-Ray Diffraction Pattern of the Cu(II) Metal Chelate.

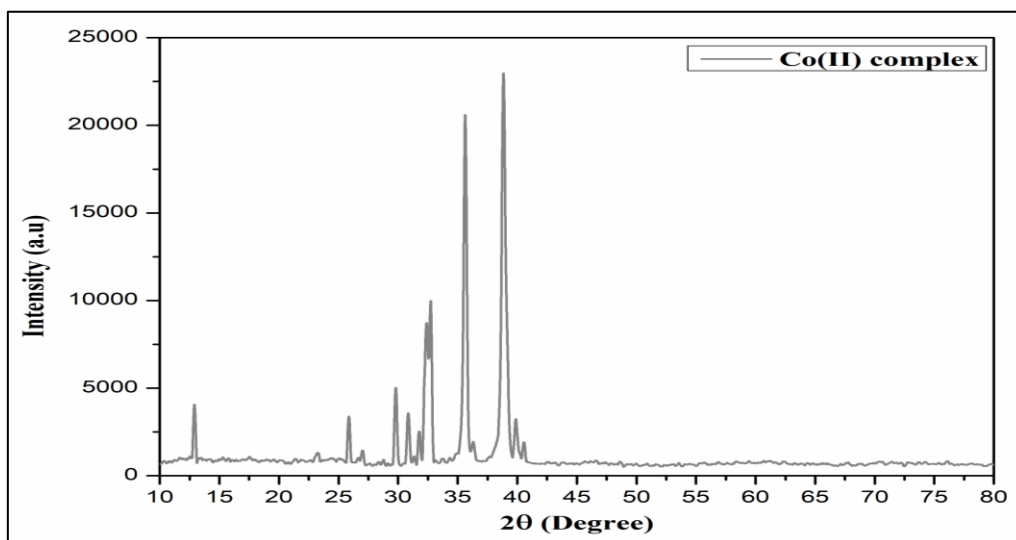


Fig 16 Powder-X-Ray Diffraction Pattern of the Co(II) Metal Chelate.

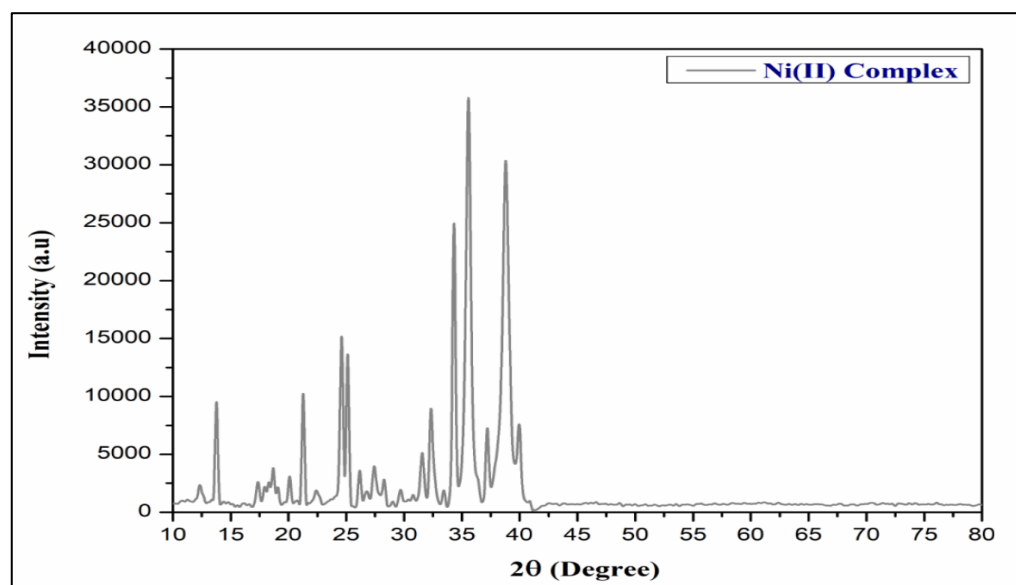


Fig 17 Powder-X-Ray Diffraction Pattern of the Ni(II) Metal Chelate.

Table 4 [A] Powder XRD Data of Cu(II) Chelate.

PEAK	2 θ	θ	$\sin\theta$	$\sin 2\theta$	$1000*\sin 2\theta$	$h^2+k^2+l^2$	m	h k l	d in A*	a in A*
1	21.04	10.52	0.183	0.3672	367.2173	5.9991	6	211	4.219	12.65
2	23.12	11.56	0.201	0.4035	403.5201	6.5922	7	-	3.843	13.45
3	29.39	14.69	0.256	0.5129	512.9523	8.3799	8	220	3.036	12.14
4	31.08	15.54	0.271	0.5424	542.4483	8.861	9	300	2.875	12.93
5	32.07	16.03	0.279	0.5597	559.7271	9.1441	9	300	2.788	12.54
6	34.89	17.44	0.304	0.6089	608.9454	9.9481	10	122	2.569	12.84
7	36.45	18.22	0.318	0.6361	636.1725	10.393	10	122	2.462	12.65
8	39.14	19.57	0.341	0.6831	683.1219	11.16	11	113	2.299	13.45

Table 4 [B] Powder XRD Data of Co(II) Chelate.

PEAK	2 θ	θ	$\sin\theta$	$\sin 2\theta$	$1000*\sin 2\theta$	$h^2+k^2+l^2$	m	h k l	d in A*	a in A*
1	12.26	6.13	0.1067	0.2134	213.4	6.000	6	112	7.213	21.6
2	26.81	13.40	0.2317	0.4634	463.4	13.029	13	230	3.323	21.6
3	29.89	14.94	0.2578	0.5156	515.6	14.496	14	123	2.987	20.9
4	32.02	16.01	0.2758	0.5516	551.6	15.508	15	-	2.792	22.3
5	35.79	17.98	0.3086	0.6172	617.2	17.353	17	223	2.495	21.2
6	38.44	19.22	0.3291	0.6582	658.2	18.506	19	240	2.339	22.2

Table 4 [C] Powder XRD Data of Ni(II) Chelate.

PEAK	2 θ	θ	$\sin\theta$	$\sin 2\theta$	$1000*\sin 2\theta$	$h^2+k^2+l^2$	m	h k l	d in A*	a in A*
1	13.78	6.89	0.1202	0.2405	240.5064	6.00015	6	112	6.421116	19.26335
2	21.56	10.78	0.1881	0.3762	376.293	9.387767	9	300	4.118405	18.53282
3	32.07	16.035	0.2798	0.5597	559.7271	13.96408	14	123	2.788674	19.52072
4	34.57	17.285	0.301	0.6033	603.3603	15.05264	15	-	2.592512	19.44384
5	35.78	17.89	0.3122	0.6244	624.4788	15.57952	16	400	2.507564	20.06051
6	38.34	19.17	0.334	0.6691	669.1592	16.6942	17	223	2.345814	19.93942

➤ Thermogravimetric Studies

Thermogravimetric analysis (TGA) was employed to investigate the physico-chemical properties of metal chelates, specifically focusing on the presence of water molecules and the thermal stability of these compounds [34]. The analysis was conducted under a nitrogen atmosphere, where a controlled linear heating rate of 10 °C/min was maintained, and weight loss was monitored between 27 and 1000 °C [35]. The breakdown curve of the metal chelates is illustrated in Fig. 18.

The Cu(II) chelate demonstrated remarkable thermal stability, exhibiting no weight loss up to 95 °C. Beyond this temperature, the chelate underwent disintegration in four distinct phases. The initial phase, occurring at 95 °C, involved the dissolution of water molecules either from the coordination sphere or the lattice structure. At 213.63 °C, the degradation of the ligand began. The third phase, spanning from 316.52 to 418.25 °C, saw the decomposition of a portion of the organic moiety. The final phase, at 549.09 °C, resulted in the most significant decomposition of organic molecules, with the residual material transforming into Copper Oxide (CuO).

For the Co(II) chelate, thermal stability was observed up to 210.75 °C, with no weight loss detected. Decomposition commenced at this temperature, characterized by incomplete ligand and mass loss. A substantial weight loss of the organic component of the ligand was noted as the chelate decomposed further at 390.26 °C. The complete decomposition process continued in two subsequent steps at 410.35 and 546.46 °C, with cobalt oxide forming the majority of the final residue. The Ni(II) chelate exhibited weight loss beginning at 100.45 °C, indicative of the loss of water of hydration, and maintained stability up to 220.23 °C. Partial decomposition of the organic moiety in the ligand commenced at 264.5 °C. The complete elimination of the organic component occurred in two successive steps at 309.35 and 440.38 °C. The TGA curve reached a constant weight beyond 590.92 °C, attributed to the formation of Nickel Oxide (NiO).

These findings provide a comprehensive understanding of the thermal behavior and decomposition pathways of the metal chelates, highlighting their thermal stability and the specific temperatures at which various decomposition events occur.

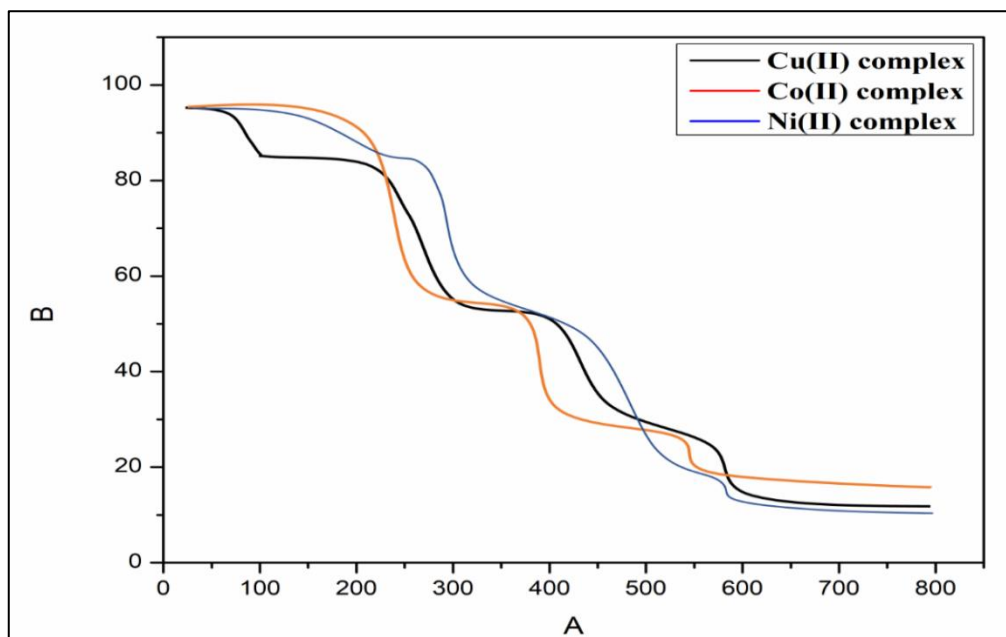


Fig 18 Thermogravimetric Analysis of Cu(II), Co(II) and Ni(II) Chelates

IV. BIOLOGICAL SCREENING

➤ DNA-Binding Studies

• UV-Visible Absorption Titration

The UV-Visible spectral method is recognized as a highly precise technique for investigating DNA binding interactions with metal chelates [36]. These chelates can attach to DNA either covalently—such as when ligands substitute for DNA base pairs or non-covalently, via mechanisms like electrostatic interactions, intercalation, or

groove binding [37]. UV-Visible measurements confirm these interactions. Figures 19–21 present the electronic spectral studies performed at a fixed chelate concentration, both in the absence and in the presence of increasing amounts of CT-DNA. The calculated K_b values for the Cu(II), Co(II), and Ni(II) chelates were $1.9 \times 10^6 \text{ M}^{-1}$, $2.6 \times 10^6 \text{ M}^{-1}$, and $2.45 \times 10^{13} \text{ M}^{-1}$, respectively. The higher K_b value observed for the Co(II) chelate indicates its stronger affinity for CT-DNA. Additionally, the red shift and hypochromic effect in the absorbance spectra provide clear evidence of a strong intercalative interaction between the metal chelates and DNA.

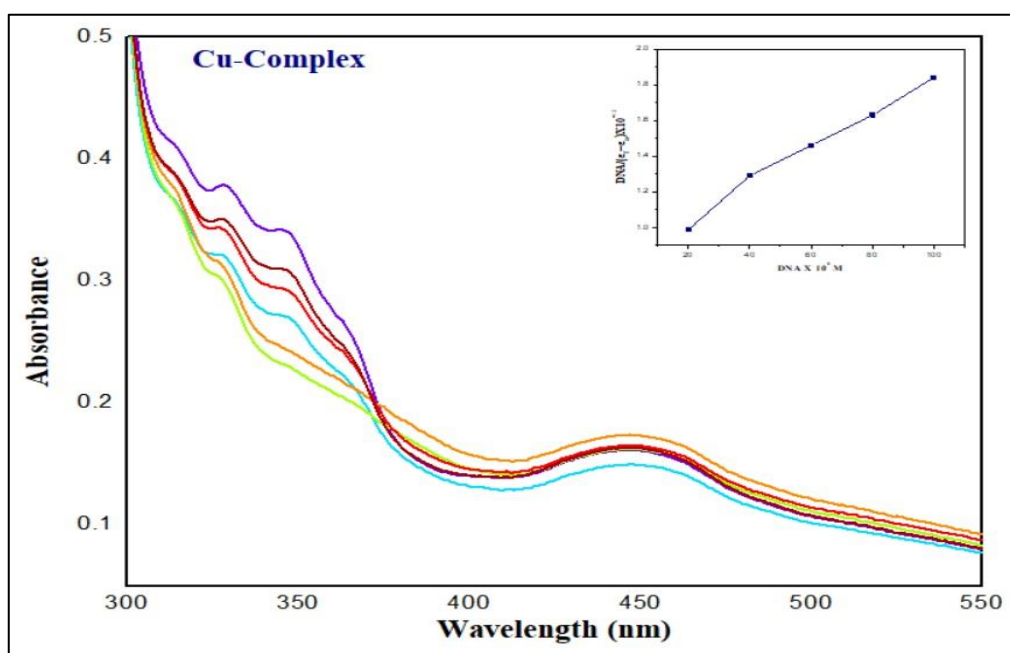


Fig 19 DNA Binding Study of the Cu(II) Chelate by Absorption Spectroscopy Techniques.

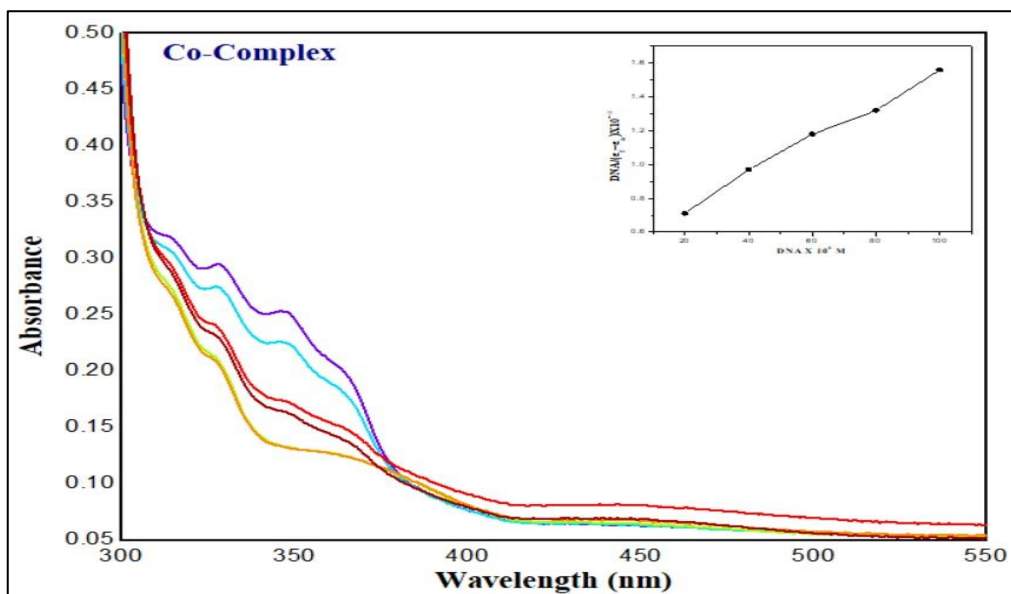


Fig 20 DNA Binding Study of the Co(II) Chelate by Absorption Spectroscopy Techniques.

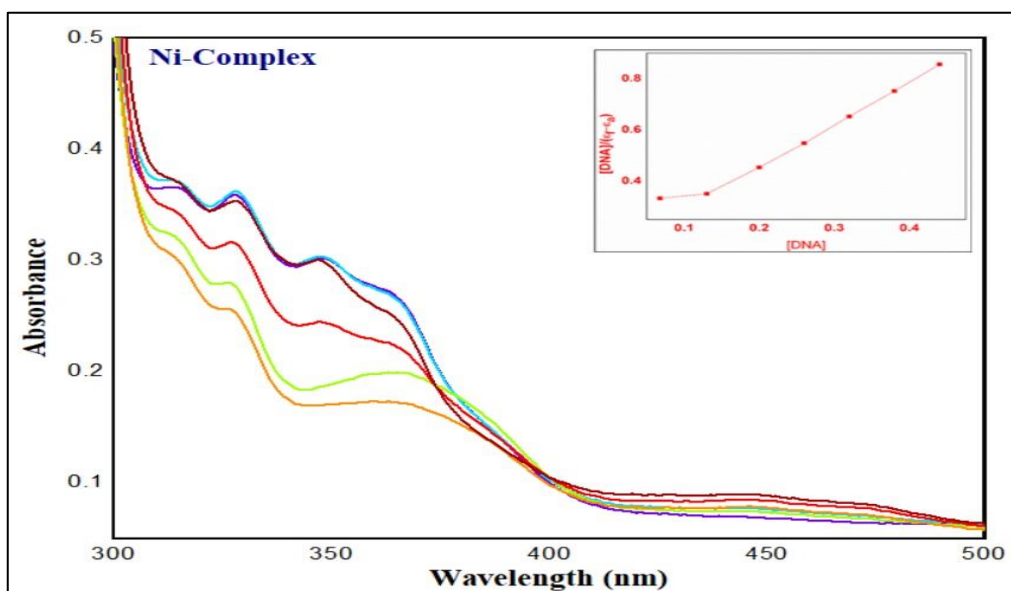


Fig 21 DNA Binding Study of the Ni(II) Chelate by Absorption Spectroscopy Techniques.

- **Viscosity Measurement Technique**

Viscosity measurement is considered one of the most rigorous techniques for assessing the binding of chelates to DNA in solution, particularly when investigating the intercalative binding mode [38]. In the context of DNA-ligand interactions, when nitrogen base pairs align with the associated ligand, a classical intercalation model predicts that the DNA helix will elongate, leading to an increase in viscosity [39]. Conversely, chelate quasi-intercalation may cause the DNA helix to kink, thereby reducing its effective length and consequently decreasing its viscosity [40].

To further elucidate the type of intercalation exhibited by metal chelates with CT-DNA, viscosity measurements were conducted across various chelate concentrations. Figure 22 illustrates the observed effects of metal chelates on CT-DNA. The data indicate a continuous increase in the relative viscosity of CT-DNA with escalating concentrations of metal chelates. This trend suggests that the metal chelates exhibit a strong intercalation mode of binding to CT-DNA. The results from the viscosity studies are consistent with previous conclusions, reinforcing the intercalative nature of the binding interaction.

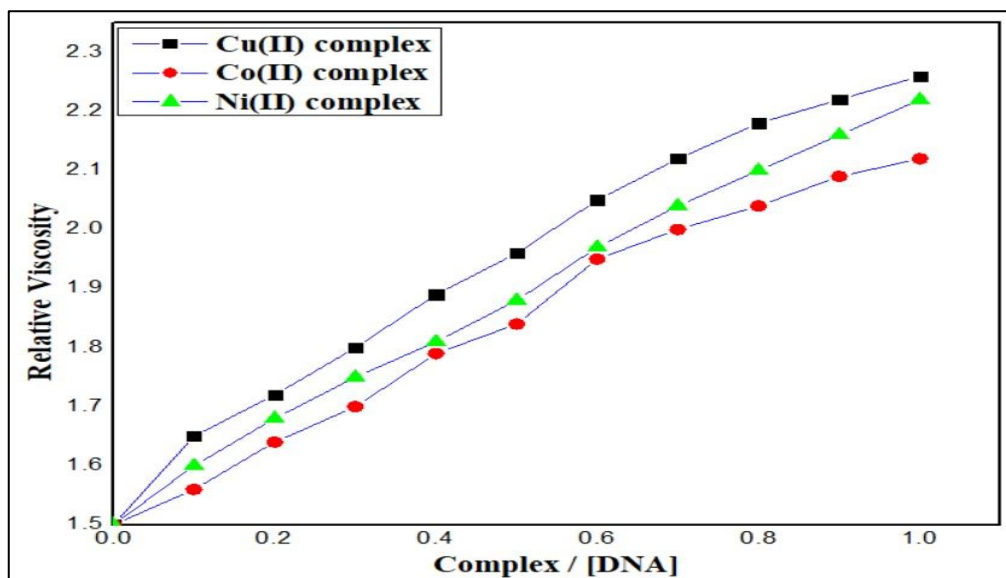


Fig 22 Relative Viscosity of CT-DNA at 25 (± 0.1) °C for Cu(II), Co(II), and Ni(II) Metal Chelates.

• Thermal Denaturation Technique

To elucidate the binding mode of metal chelates with calf thymus DNA (CT-DNA), thermal denaturation (melting) studies were systematically performed. The melting temperature (T_m) of DNA, which reflects the thermal stability of the double helix, serves as a crucial indicator for evaluating the DNA-ligand interactions. A notable elevation in the T_m was observed upon the addition of metal chelates, strongly suggesting an intercalative interaction mechanism [39].

As demonstrated in Figure 23, the incorporation of the chelates into the CT-DNA matrix resulted in a measurable increase in melting temperature. This stabilization effect occurred irrespective of whether the DNA was pretreated or in its native form before chelate introduction. The enhancement of T_m implies a strengthened double-helical

structure, consistent with intercalative stabilization where the planar chelate moieties insert between adjacent base pairs, thereby reducing the flexibility and unwinding propensity of the DNA helix.

Moreover, differential T_m increments were recorded for various metal chelates, underscoring the influence of the central metal ion on DNA binding strength. Specifically, the Cu(II) (2.20 °C), Co(II) (2.80 °C), and Ni(II) (2.50 °C) chelates demonstrated comparatively higher T_m shifts, as presented in Figure 23. Such variations likely reflect differences in coordination geometry, ligand field stabilization energy, and the extent of π -stacking interactions with the DNA base pairs [42]. Collectively, these results reinforce the hypothesis that small metal chelates interact with CT-DNA predominantly through an intercalative mechanism, enhancing the thermal stability of the double-stranded helix.

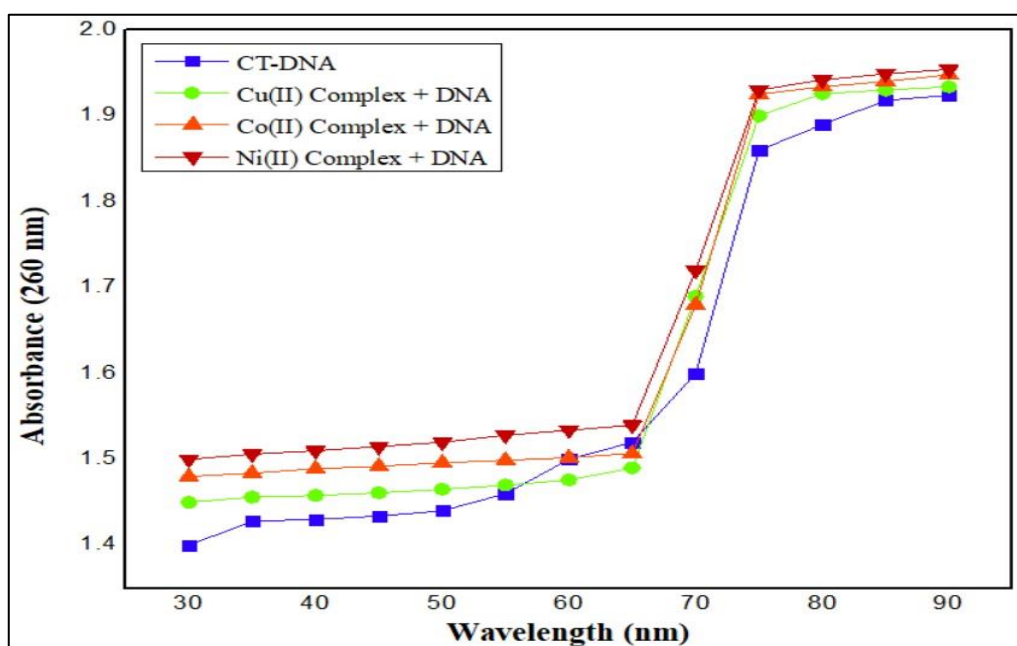


Fig 23 Thermal Denaturation Graphs of CT-DNA with and Without of Metal Chelates.

➤ DNA Cleavage Study: Gel-Electrophoresis Method

The cleavage of pUC-18 DNA mediated by the synthesized metal chelates was thoroughly investigated using agarose gel electrophoresis under both oxidative and non-oxidative conditions. Specifically, DNA samples were incubated with the prepared chelates in the absence and presence of hydrogen peroxide (H₂O₂) to evaluate the influence of reactive oxygen species (ROS) on nucleolytic activity. The agarose gel analysis revealed a characteristic conversion of the supercoiled (SC) form of pUC-18 DNA into its nicked circular (NC) form, which is widely recognized as a hallmark of DNA strand scission [40]. This transformation serves as clear evidence of site-specific or random cleavage of plasmid DNA facilitated by the metal chelates.

To systematically assess the DNA-cleaving potential, reaction mixtures containing pUC-18 DNA and varying concentrations of the metal chelates were incubated for 2 hours in 41 mM Tris-borate-EDTA (TBE) buffer at pH 7.2. The observed DNA cleavage efficiency exhibited a concentration-dependent pattern, suggesting that higher chelate concentrations enhance nucleolytic activity [41]. The mechanistic evaluation indicated that transition metal ions such as Cu(II), Co(II), and Ni(II), when complexed with appropriate chelating ligands, are capable of participating in Fenton-like redox cycling. In this process, the quasi-reversible M(II)/M(I) chelate system catalyzes the decomposition of H₂O₂ to generate highly reactive hydroxyl radicals (·OH), which are potent effectors of DNA strand breakage [41].

Collectively, these observations support the hypothesis that the DNA cleavage mechanism involves ROS-mediated oxidative damage in the presence of H₂O₂, while in its absence, partial cleavage may still occur via inherent redox activity of the chelates. Such insights are critical for understanding the potential application of these metal chelates in biochemical and therapeutic contexts where controlled nucleolytic activity is required.



As shown in Figure 24, no DNA cleavage occurred in the control sample lacking metal chelates (lane 1). However, the plasmid DNA was successfully cleaved by all three metal chelates—copper, cobalt, and nickel—at concentrations of 20 μM, 40 μM, and 60 μM (lanes 3–5, 6–8, and 9–11, respectively). The results clearly indicate a concentration-dependent effect: as the chelate concentration increases, the abundance of circular supercoiled DNA (Form I) decreases, while the levels of nicked DNA (Form II) and linear DNA (Form III) increase. This confirms that these metal chelates are highly effective at converting intact supercoiled plasmid DNA into nicked and linear forms.

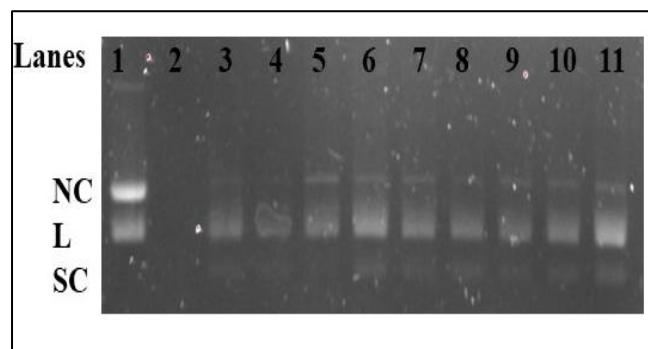


Fig 24 Cleavage of Supercoiled pUC-18 DNA by Metal Chelates in a Buffer at 37°C.

V. CONCLUSION

This study synthesizes copper(II), cobalt(II), and nickel(II) chelates using 3-[(E)-(5-bromo-2-hydroxyphenyl)methylidene]amino}pyridin-4-ol as a Schiff base ligand. Analytical and spectroscopic techniques investigate the chelate formation and structure. UV-Visible absorption titration, viscometry, and thermal denaturation examine the interaction dynamics of the chelates with calf thymus DNA (CT-DNA). The chelates bind to double-stranded DNA with binding constants of $K_b = 1.91 \times 10^6 \text{ M}^{-1}$, $2.61 \times 10^6 \text{ M}^{-1}$, and $2.45 \times 10^{13} \text{ M}^{-1}$ for copper(II), cobalt(II), and nickel(II), respectively. Increased DNA viscosity upon binding correlates with enhanced chelate activity, suggesting moderate intercalation. Thermal denaturation confirms an intercalation mechanism. Agarose gel electrophoresis shows significant cleavage activity against pUC-18 DNA for chelates with Schiff base ligands.

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➤ Author's Contributions:

- Ereshanaik: Methodology, Investigation, Writing – original draft.
- Prabhakar M C: Methodology, Investigation, Supervision, Writing, and editing of original draft.
- Surendranaik Y: DFT studies.

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➤ *Data Availability Statement:*

The authors confirm that the data supporting the findings of this study are available within the article [and/or] its supplementary materials.

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