

Synthesis, Characterization and Antimicrobial Properties of Benzimidazole Derivatives and Their Metal Complexes.

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Abstract: Four benzimidazole derivatives namely bis(2-benzimidazolyl-methyl)amine (L1), bis(2-benzimidazolyl-phenyl)amine (L2), bis(2-benzimidazolyl-methyl-6-sulfonate)amine (L3) and bis(2-benzimidazolyl-phenyl-6-sulfonate)amine (L4) were synthesized through condensation reaction of 1, 2-diaminocompounds and dicarboxylic acids. The benzimidazole derivatives and their corresponding oxovanadium (IV), Copper (II) and Zinc (II) complexes were characterized using ¹H and ¹³C NMR, UV-Visible and Infra-red spectroscopies, metal analysis, conductivity and magnetic susceptibility measurements. Spectra analyses of the ligands and metal complexes showed the coordination of the ligands to the metal ions via the nitrogen and oxygen atoms. The in-vitro antibacterial and antifungal activities of the benzimidazole derivatives and their metal (II) complexes was assayed against six bacterial isolates namely, *Citrobacterfreundii*, *Salmonella enterica*, *Pseudomonas aerugmosa* and three strains of *Escherichia coli* and four fungal isolates namely *Candida albicans*, *Alternaria spp.*, *Aspergillusflavus* and *Tricophytatonsurans* using agar well diffusion method. Most of the test isolates were sensitive to the ligands and their respective metal chelates at varied concentrations.

Keywords: Anti-microbial agents, Benzimidazole, Metal complexes, Syntheses,

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I. Introduction

Benzimidazoles are heterocyclic compounds formed from benzene and imidazole rings containing nitrogen, oxygen, sulphur and its derivatives are of wide interest because of their diverse biological activities and clinical applications,[1-3]. Benzimidazoles are regarded as a promising class of bioactive heterocyclic compounds that exhibit a range of biological activities like anti-bacterial, anti-fungal, anti-viral, anti-diabetic, anti-cancer, numerous anti-oxidant, anti-HIV, anti-convulsant, anti-inflammatory, anti-proliferative, and analgesic properties.[4]

In addition to their biological importance, benzimidazoles form stable complexes with various transition metals.[5]. The coordination chemistry of benzimidazole and its derivatives continues to receive considerable attention because of their biological significance and interesting spectral, magnetic and structural properties.[2][6][7] Due to the increasing incidence of fungal and bacterial infections across the globe as well as resistance and side effects of some of the existing drugs, there is the need for further research for more potential compounds possessing antimicrobial activity.[8][9][10].

Thus, the necessity of this study for the syntheses of more derivatives of benzimidazole and their metal complexes as possible antimicrobial agents. The synthesis of benzimidazoles from 1, 2-diamino compounds and dicarboxylic acids is very important for the synthesis of various useful compounds, [11]. A total of four benzimidazole derivatives and twelve metal(II) complexes were prepared in this study and characterized by their physical, spectral and analytical data. The synthesized compounds were further evaluated for their antimicrobial properties against various pathogens using agar well diffusion method.

II. Experimental

2.1. Reagents and Instrumentation

All the reagents and solvents were purchased from Sigma-Aldrich and they were used without further purification. The synthetic reactions were monitored by thin layer chromatography (plates coated with 0.2 mm Merck 60 F254 silica gel) and were visualized by UV irradiation (254 nm). Elemental analysis was carried out by standard methods. ¹H and ¹³C-NMR spectra of the compounds were recorded with Agilent-VNMRS-400 using TMS as internal standard and DMSO as solvent. Infrared spectra (KBr pellets) of the compounds were recorded on a Shimadzu FT-IR 8000 Spectrophotometer. The melting points of compounds were determined with a Gallenkamp melting point apparatus. UV/Visible absorption spectra were recorded using a Shimadzu

UV-1700 spectrophotometer at room temperature. Conductance measurements were recorded using HANNA TDS conductivity meter. The magnetic susceptibility measurements of the metal complexes were made at room temperature using MSB-MK1 Sherwood Susceptibility Balance.

2.2. Synthesis of Bis(2-benzimidazolyl-methyl)amine (L_1) (Scheme 1)

The methods reported in the Literature, [12-14] were adapted and used as appropriate. *o*-phenylenediamine (0.046 mol) and iminodiacetic acid (0.023 mol) in 5ml ethylene glycol was stirred for 4 hours at 180 °C. The reaction mixture was cooled and the product was triturated with water, filtered and recrystallized out of methanol-water mixture and finally dried, yield 60%, m.p. 258-260°C

2.3. Synthesis of Bis(2-benzimidazolyl- phenyl)amine (L_2) (Scheme 2)

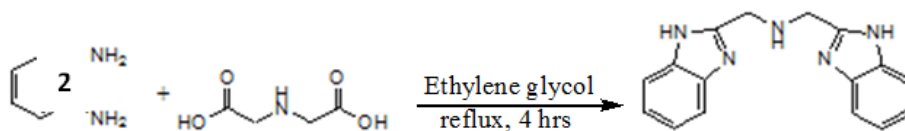
o-phenylenediamine (0.004 mol) and 2'2'-iminodibenzoic acid (0.002 mol) were stirred in ethanol in the presence of ammonium chloride at 80-90 °C for about 4 hours. The reaction mixture was then allowed to cool and poured on ice cold water. The product obtained was filtered and dried in the oven, yield 57%, m.p. 308-310°C.

2.4. Synthesis of Bis(2-benzimidazolyl-methyl-6-sulfonate)amine (L_3) (Scheme 3)

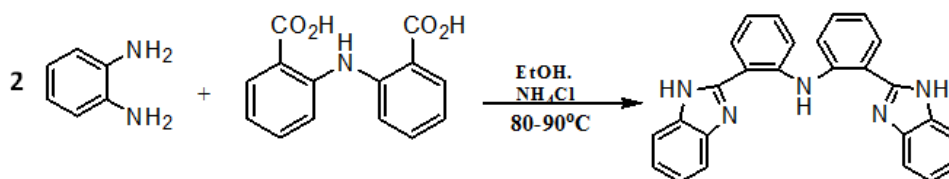
4-sulfo-*o*-phenylenediamine (0.004 mol) and iminodiacetic acid (0.002 mol) were mixed thoroughly with silica in a mortar. The resulting mixture was then irradiated using domestic microwave at 160-560 W for about 15-20mins. The product was then cooled to room temperature, yield 70%, m.p. 210-212°C.

2.5. Synthesis of Bis(2-benzimidazolyl-phenyl-6-sulfonate)amine (L_4) (Scheme 4)

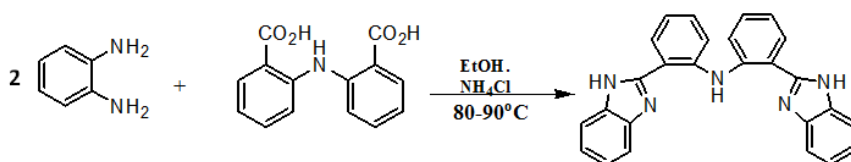
4-sulfo-*o*-phenylenediamine (0.004 mol) and 2'2'-iminodibenzoic acid (0.002 mol) were stirred in ethanol in the presence of ammonium chloride at 80-90 for about 4 hours. The reaction mixture was then allowed to cool and poured on ice cold water. The product obtained was filtered and dried, yield 51%, m.p. 260-263°C.



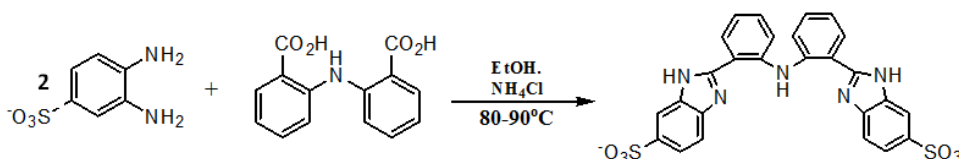
Scheme 1. Synthesis of L_1



Scheme 2. Synthesis of L_2



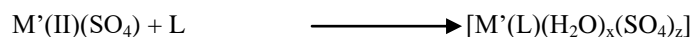
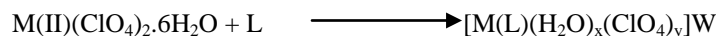
Scheme 3. Synthesis of L_3



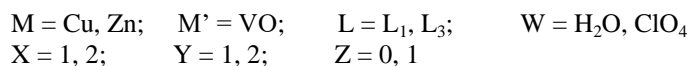
Scheme 4. Synthesis of L_4

2.6. Synthesis of the Metal Complexes (Schemes 5 and 6)

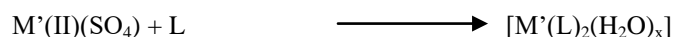
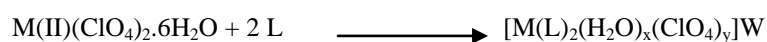
Aqueous solution of metal(II) salts (0.18 mole) was added drop wise to a hot magnetically stirred methanolic solution of Ligands (L1 & L3) (0.0018 mol) and NaOH (0.36 mmol), The resulting solution was stirred for 4 hours and then evaporated to dryness. This was repeated for L2 and L4 in ratio 1:2 of the Ligand to metal.



Where;



Scheme 5. Synthesis of metal complexes of L1 and L3



Where;



Scheme 6. Synthesis of metal complexes of L2 and L4.

2.7. Antimicrobial Study

The antimicrobial sensitivity testing of the ligands (L₁-L₄) and their metal complexes against six strains of bacteria and four fungi were carried out. The susceptibility of bacteria to the compounds was determined using agar well diffusion method. The test organisms used include bacteria namely *Citrobacterfreundii*, *Salmonella enterica*, *Pseudomonas aeruginosa* and three strains of *Escherichia coli* and fungi namely *Candida albicans*, *Aspergillus flavus*, *Tricophyta tonsurans* and *Alternaria spp*.

The bacterial isolates were first enriched in nutrient broth for 24 hours while the fungi were grown on potato dextrose agar before use. Using sterile swab sticks, plates of Mueller Hinton agar and potato dextrose agar were seeded with standardized bacterial inocula (10⁶ CFU/ml) and fungi spores, respectively. Seeded plates were allowed to stand for a while at room temperature before wells were bored on them using cork borer (6mm). Each of the bored well was filled with 5 µl of each compound, reference antibiotic (streptomycin) and antifungal drug (fluconazole).

The plates were allowed to stand on the laboratory bench for 1 hour to allow proper diffusion of the compounds into the media and incubated at 37 °C for 24 hours and 25 °C for seven days for bacteria and fungi, respectively. The diameters of the zones of inhibition were measured using a transparent calibrated ruler to the nearest millimetre (mm).

III. Results And Discussion

3.1. IR Spectra

The Infrared spectra of the complexes were compared with those of the free ligands in order to determine the coordination sites. Thus, characteristic peaks in the spectra of the ligands and complexes were considered and compared. The important IR spectral bands of the ligands and their metal complexes along with their tentative assignments are given in Table 1.

The IR spectra of Ligands 1 and 3, (Figs 7 and 9), exhibit bands at 3419-3452 cm⁻¹ and 1581-1587 attributed to N-H stretching and bending of the benzimidazole ring, respectively, [15] The spectra also exhibit bands at 1435-1462 cm⁻¹ and 1531-1541 cm⁻¹ which are characteristic of =C-H and C=N vibrational frequencies, respectively. On coordination, there was a shift in the stretching frequencies of N-H and C=N. There was a downward shift in the frequency of the N-H stretching vibrations in the complexes than in the free ligands, (Figs. 11- 13). In analogous ammine complexes as reported by Ibrahim *et al*, [11], the N-H stretching frequency becomes lower as coordination to the metal ion occurs and the stability of the complex increases, indicating that the N-H bond is weaker in the complexes containing stronger M-N bonds. Increased strength of the metal-ligand bond means lower electron density on the nitrogen of N-H group; this results in a decrease in the N-H bond strength.

Similarly, as a result of coordination, there was a considerable shift in the stretching frequencies of the C=N bonds in the imidazole units of the benzimidazole rings in the IR spectra of the complexes as compared to their values for the uncoordinated ligands, suggesting coordination through the pyridine nitrogens of the benzimidazole rings,[16]. Further evidence of coordination of the ligands with the metal ions was shown by the appearance of new bands at 466-547 and 626-653 cm^{-1} assigned to metal-nitrogen (M-N)[17] and metal-oxygen (M-O)[11] vibrations respectively. These bands were absent in the spectra of the ligands, thus confirming participation of the O and N atoms in the coordination.

For the metal ion complexes of L2, L3 and L4,(Figs.14-22), there was disappearance of spectral bands for C=N stretching frequencies in all the complexes upon coordination with the appearance of new bands between 464-520 cm^{-1} which is characteristic of M-N stretching frequencies. These suggest evidence of coordination through the pyridine nitrogen of the benzimidazole rings, [16]. The stretching frequencies of the second N-H sandwiching the two phenyl rings in the ligands remained constant all through indicating that there was no coordination through this site, hence coordination took place only on the pyridine nitrogen of the benzimidazole units.

Furthermore, for the metal (II) perchlorate complexes, there were appearance of new bands with a split pattern at 1120-1180 cm^{-1} assigned to coordinated ClO_4^- and the corresponding M- OClO_3 is observed at around 620 cm^{-1} . This suggests participation of the perchlorate ions in the coordination in these complexes, [18]. The spectra of the VO(II) complexes, (Figs.11,14,17,20), also exhibit a strong band in the region 877-974 cm^{-1} , which has been assigned to V=O stretching vibration with a monomeric square pyramidal coordination geometry.[19]

The presence of coordinated water molecule in the complex is indicated by the appearance of a broad band at 3421-3510 cm^{-1} assigned to the O-H stretching vibrations of the coordinated water molecules. The corresponding in-plane bending mode is probably overlapped to the very strong band at around 1620 cm^{-1} . [4] The strong bands observed for VO(II) complex at 1116 and 1051 cm^{-1} can be attributed to asymmetric and symmetric stretching vibration of the coordinated sulfate ion,[11].

3.2. $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$

The $^1\text{H-NMR}$ spectrum of the free ligand L1, (Fig. 5) showed peaks for the benzimidazole protons between 6.77-7.13 ppm. The signal at 3.63 ppm corresponds to the methylene proton. The signal at 3.3 ppm is the solvent peak. The $^{13}\text{C-NMR}$ spectrum, (Fig. 6), showed signals at 122.26 and 139.79 ppm for two methine carbons on the phenyl part of the ring while signals for two quaternary carbons appeared at 115.46 and 154.60 ppm. The signal for the methylene carbon appeared upfield with a chemical shift of 47.05 ppm with that of solvent at 39.15-40.61 ppm.

3.3. Electronic Spectra and Magnetic Moments

The electronic absorption spectra of the ligands and complexes were recorded in methanol and aqueous solution. The electronic spectra of the ligands in UV region showed absorption bands 242, 274 and 281 nm for L1; 218, 298, 332 nm for L2, 230 nm, 283 nm for L3 and 294 nm, 362 nm for L4, (Table 2). The bands correspond to $n \rightarrow \sigma^*$, $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions of the major chromophores, -N-H, -C=N and C=C present in the ligand molecules. However, on coordination, shifts in the bands were observed.

The electronic spectra of oxovanadium (IV) complexes,(Figs. 25, 26), displayed three bands for the complexes of L1, L2, L3 and L4. The electronic spectra exhibit three bands between 792 nm and 565 nm (Table 2). These bands can be assigned to $^2\text{B}_2 \rightarrow ^2\text{E}$, $^2\text{B}_2 \rightarrow ^2\text{B}_1$ and $^2\text{B}_2 \rightarrow ^2\text{A}_1$ transitions respectively, while the two d-d- transition bands observed for that of L1 complex can be attributed to $^2\text{B}_2 \rightarrow ^2\text{E}$ and $^2\text{B}_2 \rightarrow ^2\text{B}_1$ transitions, the band at 448 nm is due to charge transfer. These spectral characteristics suggest that the synthesized oxovanadium (IV) complexes are five coordinate, square pyramidal structures, [20] Also the magnetic moments for VO(IV) complexes (1.71-2.01) are within the range of a square pyramidal geometry, [21].

The electronic absorption spectra of Cu(II) complexes of L1 and L4 displayed two broad bands at 816 nm and 808 nm, and 889 nm and 914 nm respectively, with a slight shoulder. This is consistent for Cu(II) complex in an octahedral environment with $^2\text{E}_g \rightarrow ^2\text{T}_{2g}$ transition, [18]. This broadening may be due to Jahn-Teller effect arising from unequal occupation of the e_g pair orbital. Accordingly, the electronic spectrum of the five-coordinated copper(II) complex in the square pyramidal geometry with three d-d bands, [23]. These bands have been assigned to the transitions $dz^2 \rightarrow dx^2-y^2$, $dxy \rightarrow dx^2-y^2$, and $dxz, dyz \rightarrow dx^2-y^2$. The energy level sequence will then depend on the amount of distortion due to ligand field and Jahn-Teller effect,[23].

The electronic spectra of the Cu(II) complexes of L2 and L3, (Figs. 23, 24), showed two characteristic bands at 610 - 633 nm and 673 - 687 nm. These may be assigned to $dxz, dyz \rightarrow dx^2-y^2$ and $dxy \rightarrow dx^2-y^2$ transitions respectively. Because of the low intensity of $dz^2 \rightarrow dx^2-y^2$ transition, this band is usually not observed as a separate band in the tetragonally distorted complexes. However, the band displayed by L3 at 452 nm is due to charge transfer.[11] For copper (II) complexes, the magnetic moment values are usually not used for the prediction of the geometry but could give information on the number of metal centers involved in the

complex. A moment of 1.7-2.1 BM is usually observed for mononuclear copper(II) complexes regardless of stereochemistry. Higher values can be obtained due to orbital contribution and spin-orbit coupling, [24]. The magnetic moments values of the copper(II) complexes ranges from 1.72-1.80 indicating their mononuclear nature with one unpaired electron.

The electronic absorption spectra of Zn(II) complexes of L1, L2, L3 and L4 displayed a band at 427-454 nm due to charge transfer, (Fig. 27). However the Zn(II) complexes are diamagnetic as expected for d^{10} system, (Table 2)

3.5. Conductivity Measurements

The molar conductivities for the complexes were measured in distilled water and are shown in Table 3. Some are in the range ($116-139 \Omega^{-1} \text{cm}^2 \text{mol}^{-1}$) indicating their electrolytic/ionic nature while others are in the range ($20-41 \Omega^{-1} \text{cm}^2 \text{mol}^{-1}$) indicating their non-electrolytic/non-ionic nature.[26] For those in the range of $116-139 \Omega^{-1} \text{cm}^2 \text{mol}^{-1}$, these values showed that they are 1:1 electrolytes.

The results obtained were consistent and in agreement with the most commonly observed geometries (square pyramidal and octahedral) in metal complexes.

Table 1. The Important Infrared Frequencies (cm-1) of the Ligands and their VO(IV), Cu(II) and Zn(II) Complexes.

Assignments	ν -O-H (H ₂ O)	ν -N-H	ν -C=C (Ar)	ν -N-H (bend)	ν C=N	ν -CH ₂	ν -S=O	M-N	M-O	M=O (M=V)
L1	-	3419	1622	1587	1531	1435	-	-	-	-
[VO(L1)(H ₂ O)(SO ₄)]	3421	3201	1626	1529	1529	1475	1116,1051	547	653	974
[Cu(L1)(ClO ₄) ₂ H ₂ O]H ₂ O	3450	3261	1610	1500	1545	1473	-	495	626	-
[Zn(L1)ClO ₄ (H ₂ O) ₂]ClO ₄	3450	3261	1624	1498	1545	1456	-	488	626	-
L2	-	3392	1600	1523	1643	-	-	-	-	-
[VO(L2) ₂](SO ₄)	-	3462	1608	1550	-	-	-	520	-	877
[Cu(L2) ₂ ClO ₄]ClO ₄	-	3462	1608	1548	-	-	-	520	696	-
[Zn(L2) ₂ ClO ₄]ClO ₄	-	3468	1608	1548	-	-	-	518	696	-
L3	-	3452	1629	1581	1541	1462	1151, 1064	-	-	-
[VO(L3)(H ₂ O) ₂]	3356	3192	1626	1529	-	1479	1149, 1111	470	605	966
[Cu(L3)(H ₂ O) ₂]H ₂ O	3439	-	1620	1498	-	1460	1143, 1087	466	600	-
[Zn(L3)(H ₂ O) ₂]H ₂ O	3510	3399	1662	1581	-	1460	1130, 1099	476	569	-
L4	-	3342	1604	1579	1668	-	1165, 1084	-	-	-
[VO(L4) ₂](H ₂ O)]	3464	3350	1608	1575	-	-	1145, 1112	520	646	949
[Cu(L4) ₂](H ₂ O) ₂]	4339	3282	1604	1575	-	-	1145, 1122	464	636	-
[Zn(L4) ₂](H ₂ O)]	3441	3385	1608	1577	-	-	1145, 1120	466	626	-

Table 2. Electronic Spectra and Magnetic Moments of the Ligands and their VO(IV), Cu(II) and Zn(II) Complexes

Compounds	Intra-Ligand Transitions (nm)	Ligand Field Transition (nm)	Magnetic Moments (μ_{eff} in BM)
L1	242, 274, 281	-	-
[VO(L1)(H ₂ O)(SO ₄)]	237, 271, 278	573, 624	1.80
[Cu(L1)(ClO ₄) ₂ H ₂ O]H ₂ O	239, 271, 278	816, 889	1.78
[Zn(L1)ClO ₄ (H ₂ O) ₂]ClO ₄	240, 272, 278	-	Diamagnetic
L2	218, 298, 332	-	-
[VO(L2) ₂](SO ₄)	218, 298, 336	695, 708, 745	1.90
[Cu(L2) ₂ ClO ₄]ClO ₄	212, 297, 335	633, 687	1.80
[Zn(L2) ₂ ClO ₄]ClO ₄	220, 298, 329	449	Diamagnetic
L3	230, 283	-	-
[VO(L3)(H ₂ O) ₂]	260, 280	565, 625, 696	2.01
[Cu(L3)(H ₂ O) ₂]H ₂ O	260	610, 673	1.75
[Zn(L3)(H ₂ O) ₂]H ₂ O	241, 278	-	Diamagnetic
L4	294, 362	-	-
[VO(L4) ₂](H ₂ O)]	288, 352	711, 752, 792	1.71
[Cu(L4) ₂](H ₂ O) ₂]	247, 272, 280, 294, 372	808, 914	1.72
[Zn(L4) ₂](H ₂ O)]	294, 356	-	Diamagnetic

Table 3. Physical and analytical Data of Ligands and their VO(IV), Cu(II) and Zn(II) Complexes.

Compounds	Yield (%)	Melting Point (°C)	Molar Conductance ($\Omega^{-1}\text{cm}^2\text{mol}^{-1}$)	% Metal Found (Calculated)
L1	60	258-260	-	-
[VO(L1)(H ₂ O)(SO ₄)]	71	>340	40.36	-
[Cu(L1)(ClO ₄) ₂ H ₂ O]H ₂ O	55	>340	30.24	11.54(11.33)
[Zn(L1)ClO ₄ (H ₂ O) ₂]ClO ₄	39	> 340	116.70	11.21(10.70)
L2	57	308-310	-	-
[VO(L2) ₂](SO ₄)	35	>320	139.96	-
[Cu(L2) ₂ ClO ₄]ClO ₄	38	>320	120.72	6.08(5.94)
[Zn(L2) ₂ ClO ₄]ClO ₄	41	>320	133.10	6.59(6.13)
L3	70	210-212	-	-
[VO(L3)(H ₂ O) ₂]	62	>320	30.22	-
[Cu(L3)(H ₂ O) ₂]H ₂ O	55	>320	20.14	11.32(11.49)
[Zn(L3)(H ₂ O) ₂]H ₂ O	69	>320	35.64	12.05(11.79)
L4	51	260-263	-	-
[VO(L4) ₂ (H ₂ O)]	58	>320	43.25	-
[Cu(L4) ₂ (H ₂ O) ₂]	60	>320	32.90	5.30(5.30)
[Zn(L4) ₂ (H ₂ O)]	43	>320	39.06	5.59(5.44)

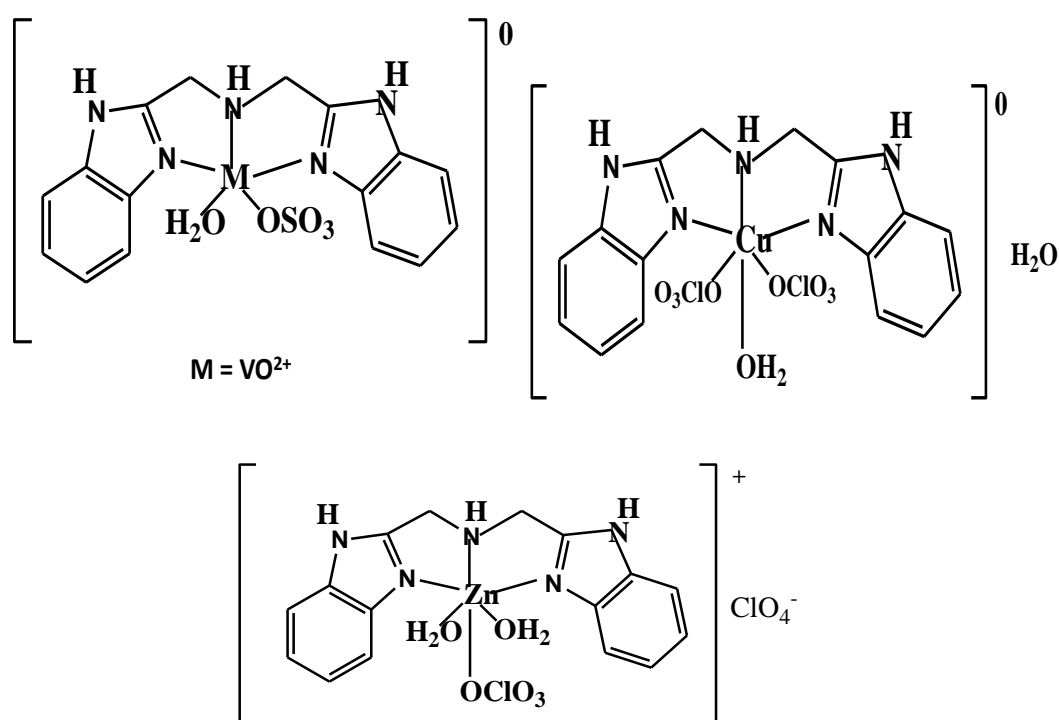
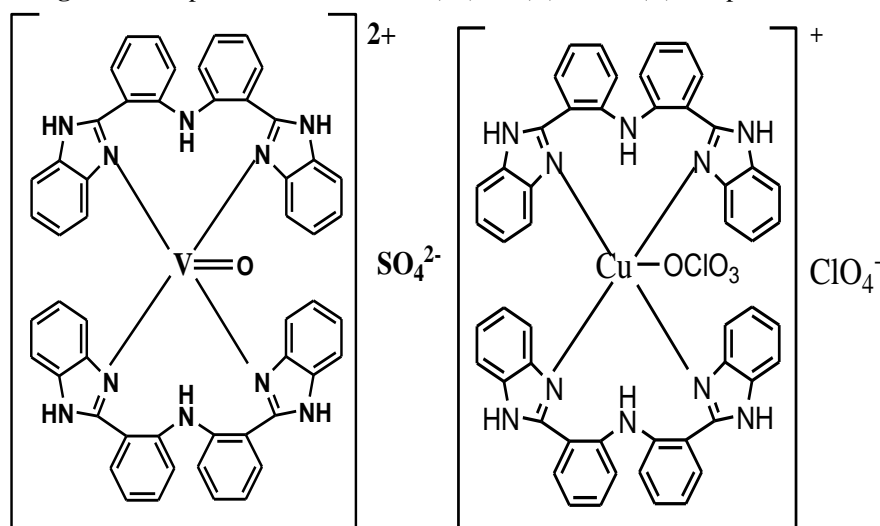


Figure 1: Proposed structures for VO(IV), Cu(II) and Zn(II) complexes of L₁



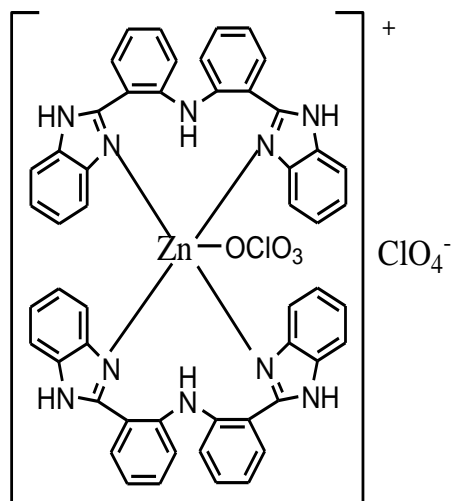


Figure 2. Proposed structure of Vo(IV), Cu(II) and Zn(II) complexes of L₂

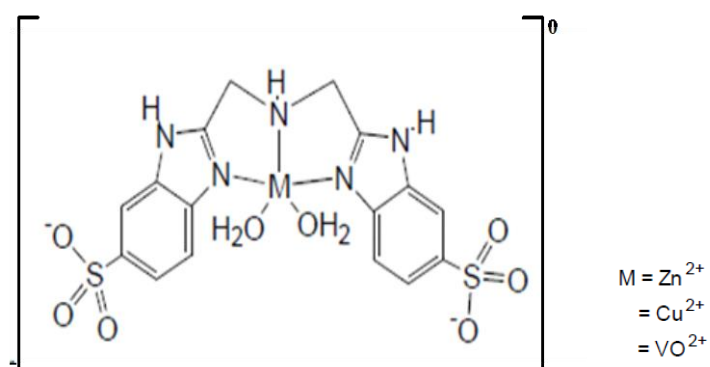
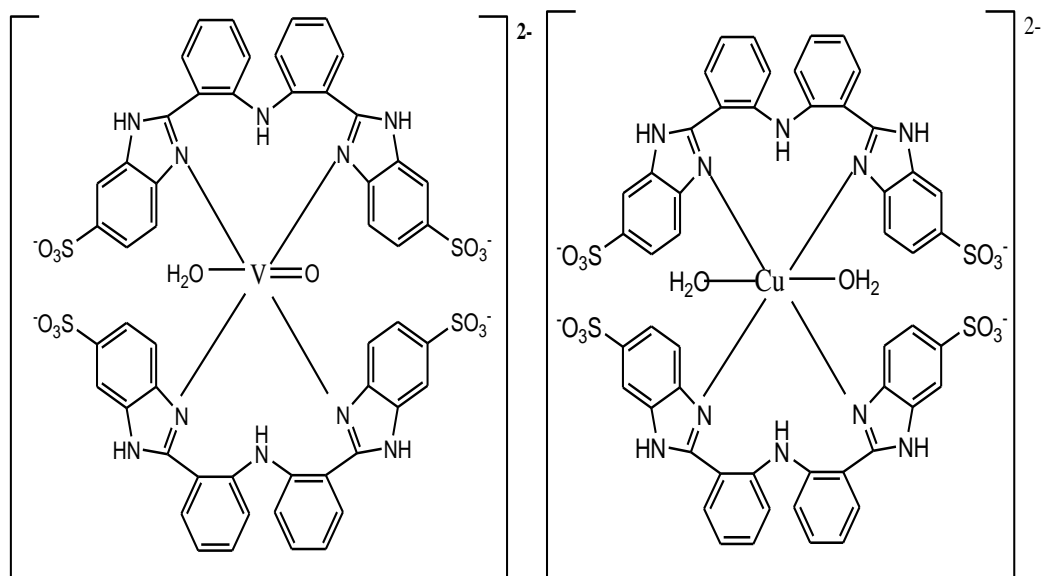


Figure 3: Proposed structure of Vo(IV), Cu(II) and Zn(II) complexes of L₃



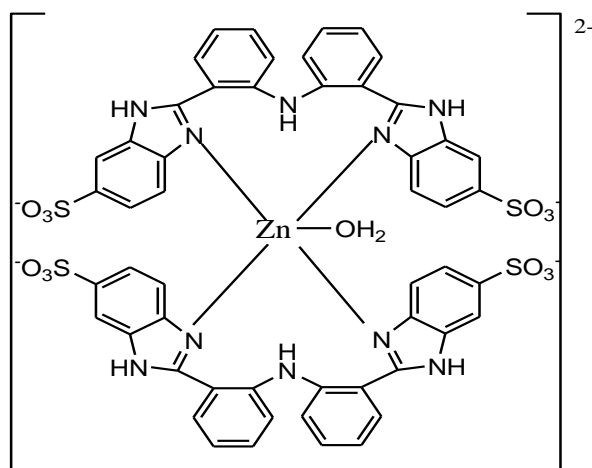


Figure 4: Proposed structure of Vo(IV), Cu(II) and Zn(II) complexes of L₄

3.6. Antimicrobial Activity

The results of the antimicrobial susceptibility of the ligands and their corresponding metal complexes at varied concentrations (20, 10 and 5 mg/mL) against six strains of bacteria and four fungi with reference to Streptomycin and Fluconazole, respectively are shown in Tables 4 -7. Most of the isolates were sensitive to the ligands and their respective metal chelates at varied concentrations. L1 and its metal complexes have a considerable effect on the fungi than the bacteria as evident in Table 4. At low concentration (5 mg/ml), *Pseudomonas aeruginosa* was resistant to L1 and its VOL1 complex. Similarly, *Escherichia coli* (2) was resistant to L1 and its ZnL1 complex at 5 mg/ml. ZnL1 possessed greater inhibitory effect against *Escherichia coli* (3) than the reference streptomycin at high concentration. At all concentrations, *Candida albicans* was appreciably sensitive to L1 and its metal complexes. Most of the bacterial isolates showed significant resistance against L2 and its metal complexes. At low concentration, *Escherichia coli* (2) was resistant to ZnL2. At high concentration, CuL2 showed higher activity against *Alternaria spp.* than the reference fluconazole. L2 and its metal complexes showed appreciable activity against *Candida albicans*. L3 and its metal complexes demonstrated considerable activity against *Tricophytatonsurans* at all concentrations (Table 5). *Aspergillus flavus* was completely resistant to ZnL3 at all concentrations (Table 6). Meanwhile, L4 and its metal complexes have appreciable inhibitory effect against *Candida albicans* at all concentrations. All the ligands and their corresponding metal complexes showed variable antimicrobial activities against the test isolates (Table 7). By careful study of the results obtained, it was observed that the ligands showed lower inhibitory effect against most of the test isolates. The antimicrobial activities of the ligands become more pronounced on coordination with the metal ions under the same experimental conditions. This is consistent with previous works, [27-30] A comparative study of the ligands and their complexes as antifungal and antibacterial agents indicates that the metal complexes are more active than the free ligands. Such increased activity of the metal chelates can be explained by the reduced polarity of the ligand due to overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with electron releasing group. Thus, reducing the total electron density on the free ligand makes diffusion to proceed faster through the bacterial cells, [26]. It is generally observed that metal chelates have higher antimicrobial activity than the free ligand due to increase in cell permeability. The lipid membrane which surrounds the cells favours only the passage of the lipid soluble material and it is known that liposolubility is an important factor in controlling antimicrobial activity, [28-30]. Generally, the antimicrobial activities of all the compounds and complexes compare favourably with those of streptomycin and fluconazole standards.

Table 4. Diameter of Zones of Inhibition of Bis(2-benzimidazolyl-methyl)amine (L1) and Metal Complexes on Selected Bacterial and Fungal Isolates (mm)

Bacteria	L1		VOL1		CuL1		ZnL1		STREP			FLUCO				
	20	10	5	20	10	5	20	10	5	20	10	5	20	10	5	
<i>Citrobacter freundii</i>	26	26	24	19	15	14	26	24	22	26	22	20	30	26	30	NA
<i>Escherichia coli</i> (1)	18	16	10	12	12	16	18	18	16	16	14	12	28	24	26	NA
<i>Salmonella enterica</i>	20	20	18	16	16	14	24	21	18	22	20	18	36	30	28	NA
<i>Escherichia coli</i> (2)	15	15	0	20	16	12	20	20	18	22	18	0	32	28	28	NA
<i>Pseudomonas aeruginosa</i>	15	15	0	12	12	0	24	20	17	22	18	14	28	26	26	NA
<i>Escherichia coli</i> (3)	13	13	12	14	14	12	20	20	15	32	20	20	30	26	24	NA
Fungi																
<i>Candida albicans</i>	30	30	30	30	30	30	28	28	25	32	30	30	NA	35	30	28
<i>Alternaria spp</i>	14	12	18	14	10	20	14	16	20	14	14	12	NA	20	24	24

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<i>Aspergillus flavus</i>	20 14 12	22 16 14	20 12 12	22 22 16	NA	34 34 38
<i>Tricophyta tonsurans</i>	22 22 18	23 22 18	20 20 12	26 26 22	NA	32 30 30
Negative Control	+++	+++	+++	+++	+++	+++

Key: +++: No zone of inhibition produced (there was no growth around well). (STREP): Streptomycin. (FLUCO): Fluconazole.

NA: Not Applicable

Table 5. Diameter of Zones of Inhibition of Bis(2-benzimidazolyl-phenyl)amine (L2) and Metal Complexes on Selected Bacterial and Fungal Isolates (mm)

Bacteria	L2	VOL2	CuL2	ZnL2	STREP	FLUCO
	Concentrations (mg/ml)					
	20 10 5	20 10 5	20 10 5	20 10 5	20 10 5	20 10 5
<i>Citrobacter freundii</i>	14 12 12	18 12 12	18 14 14	18 14 14	32 30 30	NA
<i>Escherichia coli (1)</i>	14 14 12	18 18 14	18 18 14	18 16 16	32 30 28	NA
<i>Salmonella enterica</i>	16 12 12	18 16 16	14 14 12	16 14 14	36 30 28	NA
<i>Escherichia coli (2)</i>	14 14 12	14 12 12	20 20 18	22 18 0	32 28 28	NA
<i>Pseudomonas aeruginosa</i>	16 11 11	12 12 12	15 12 12	14 12 12	30 32 32	NA
<i>Escherichia coli (3)</i>	14 12 20	25 20 20	12 11 20	12 14 22	36 32 32	NA
Fungi						
<i>Candida albicans</i>	24 22 22	26 24 24	26 22 22	30 28 28	NA	34 30 35
<i>Alternaria spp</i>	14 14 14	18 18 15	25 20 19	16 14 12	NA	22 22 22
<i>Aspergillus flavus</i>	14 12 20	16 14 22	20 12 22	16 22 22	NA	38 34 34
<i>Tricophyta tonsurans</i>	15 12 12	17 14 14	15 16 13	15 12 12	NA	32 34 34
Negative Control	+++	+++	+++	+++	+++	+++

Key: +++: No zone of inhibition produced (there was no growth around well). (STREP): Streptomycin. (FLUCO): Fluconazole.

NA: Not Applicable

Table 6. Diameter of Zones of Inhibition of Bis(2-benzimidazolyl-methyl-6-sulfonate) amine (L3) and Metal Complexes on Selected Bacterial and Fungal Isolates (mm)

Bacteria	L3	VOL3	CuL3	ZnL3	STREP	FLUCO
	Concentrations (mg/ml)					
	20 10 5	20 10 5	20 10 5	20 10 5	20 10 5	20 10 5
<i>Citrobacter freundii</i>	18 14 14	17 12 12	20 18 14	16 15 16	34 32 32	NA
<i>Escherichia coli (1)</i>	18 16 10	20 14 14	18 18 16	16 14 12	30 28 28	NA
<i>Salmonella enterica</i>	16 14 12	20 20 16	24 21 18	22 20 18	36 30 28	NA
<i>Escherichia coli (2)</i>	12 12 12	16 14 13	15 12 12	16 11 12	32 28 28	NA
<i>Pseudomonas aeruginosa</i>	20 16 15	16 12 12	20 16 12	20 15 16	34 32 30	NA
<i>Escherichia coli (3)</i>	16 14 12	14 12 12	18 14 14	15 16 14	34 32 32	NA
Fungi						
<i>Candida albicans</i>	26 24 22	28 24 24	30 28 38	30 26 26	NA	36 30 35
<i>Alternaria spp</i>	16 12 12	20 18 12	20 16 16	14 14 12	NA	24 24 24
<i>Aspergillus flavus</i>	16 16 12	30 30 30	25 25 25	0 0 0	NA	38 38 38
<i>Tricophyta tonsurans</i>	26 22 20	38 38 36	26 29 24	32 30 20	NA	32 34 30
Negative Control	+++	+++	+++	+++	+++	+++

Key: +++: No zone of inhibition produced (there was no growth around well). (STREP): Streptomycin. (FLUCO):

Fluconazole. NA: Not Applicable

Table 7. Diameter of Zones of Inhibition of Bis(2-benzimidazolyl-phenyl-6- sulfonate) amine (L4) and Metal Complexes on Selected Bacterial and Fungal Isolates (mm)

Bacteria	L4			VOL4			CuL4			ZnL4			STREP			FLUCO		
	20	10	5	20	10	5	20	10	5	20	10	5	20	10	5	20	10	5
<i>Citrobacter freundii</i>	15	12	12	18	14	12	15	12	12	20	14	13	35	35	32	NA		
<i>Escherichia coli (1)</i>	18	16	10	17	15	12	18	18	15	16	12	13	34	32	32	NA		
<i>Salmonella enterica</i>	16	15	15	16	15	16	15	15	16	18	16	16	34	32	32	NA		
<i>Escherichia coli (2)</i>	20	12	12	14	14	12	20	20	18	18	16	16	32	30	30	NA		
<i>Pseudomonas aeruginosa</i>	14	12	12	16	14	14	15	12	12	12	12	14	35	34	34	NA		
<i>Escherichia coli (3)</i>	12	12	14	25	20	20	12	14	20	18	16	16	36	32	32	NA		
Fungi																		
<i>Candida albicans</i>	28	28	25	24	24	22	26	24	24	32	30	28	NA			36	35	32
<i>Alternaria spp</i>	16	14	12	18	15	14	26	22	19	13	14	12	NA			24	24	24
<i>Aspergillus flavus</i>	14	12	20	16	14	22	20	12	22	16	22	22	NA			38	38	34
<i>Tricophyta tonsurans</i>	25	12	12	17	17	14	15	13	14	16	14	15	NA			34	34	32
Negative Control	+++			+++			+++			+++			+++			+++		

Key: +++: No zone of inhibition produced (there was no growth around well). (STREP): Streptomycin. (FLUCO): Fluconazole. NA: Not Applicable

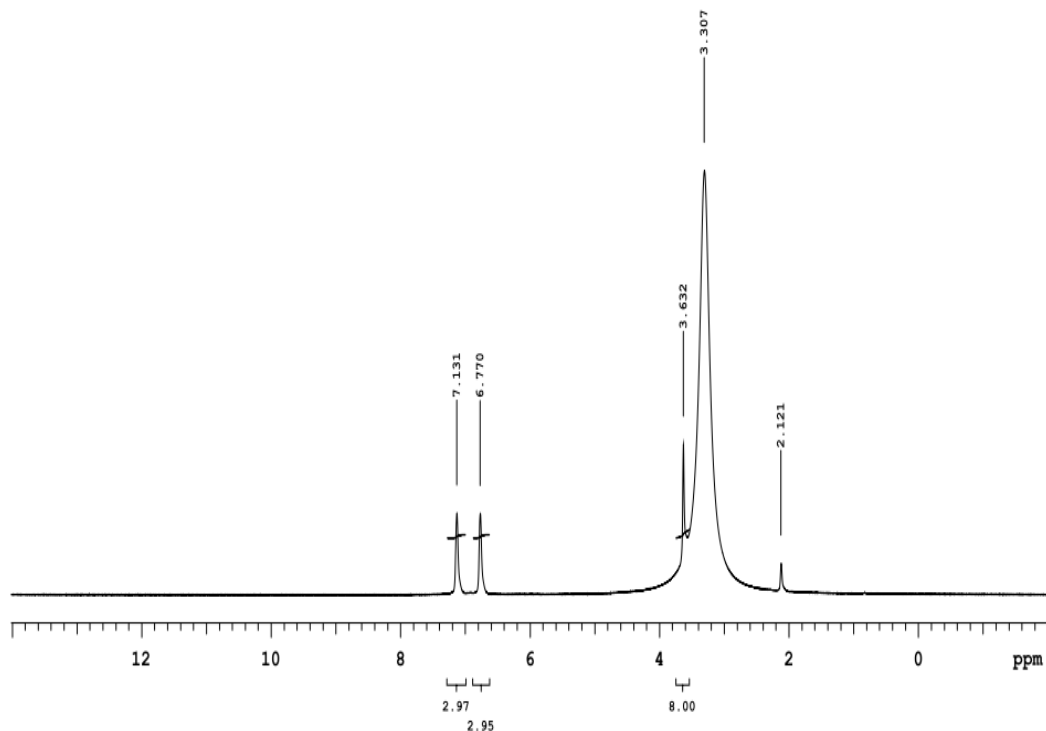


Figure 5 ¹H NMR spectrum of L₁

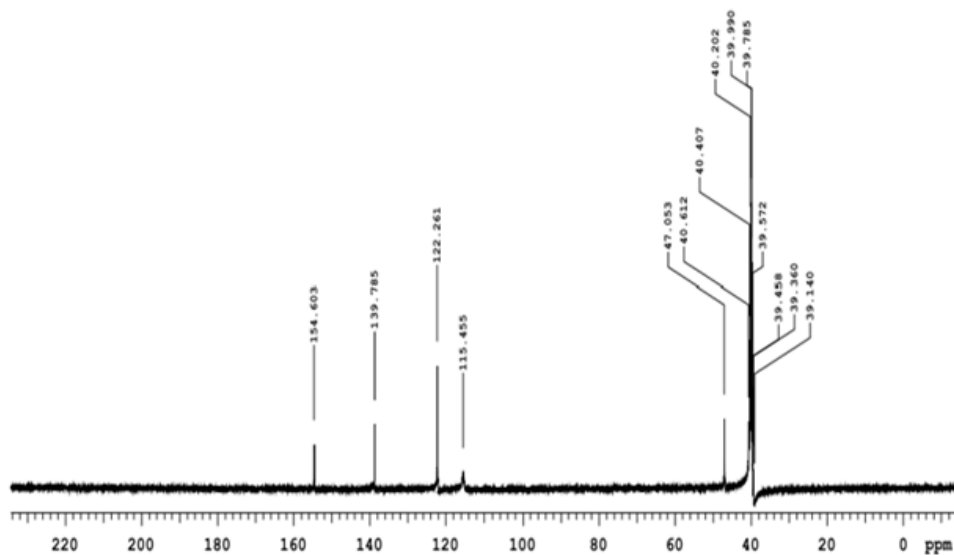


Figure 6: ^{13}C NMR spectrum of L_1

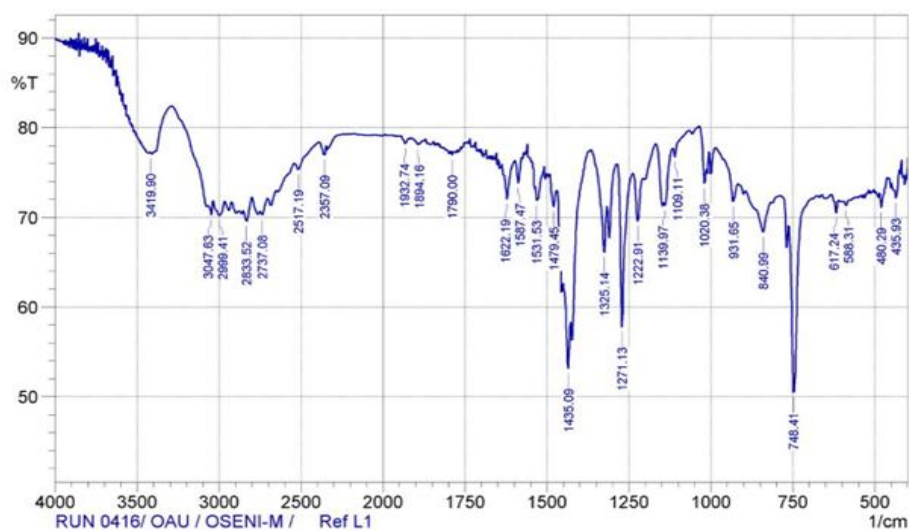


Figure 7: IR spectrum of L_1

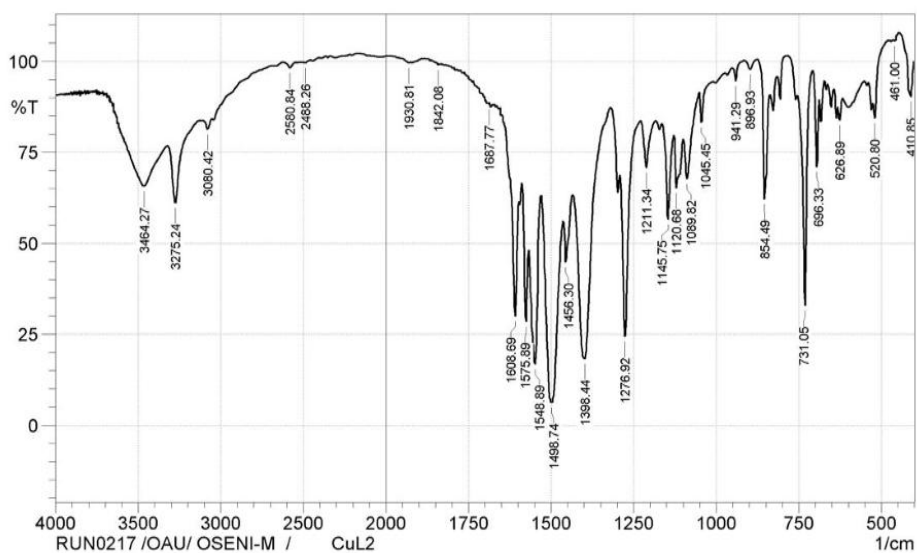


Figure 8: IR spectrum of L_2

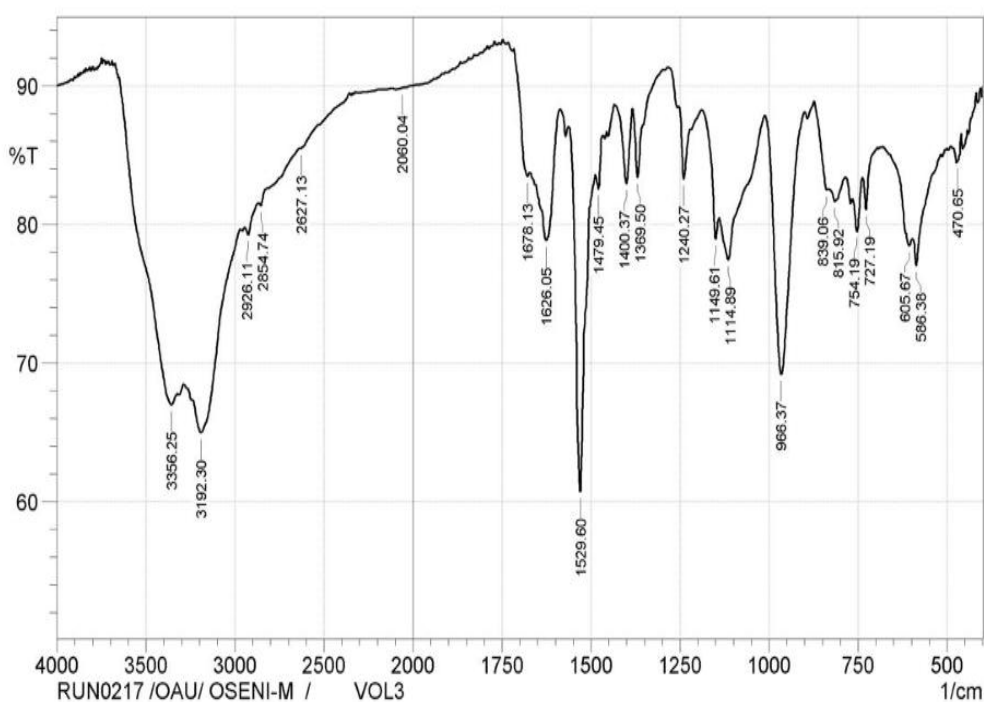


Figure 9: IR spectrum of L₃

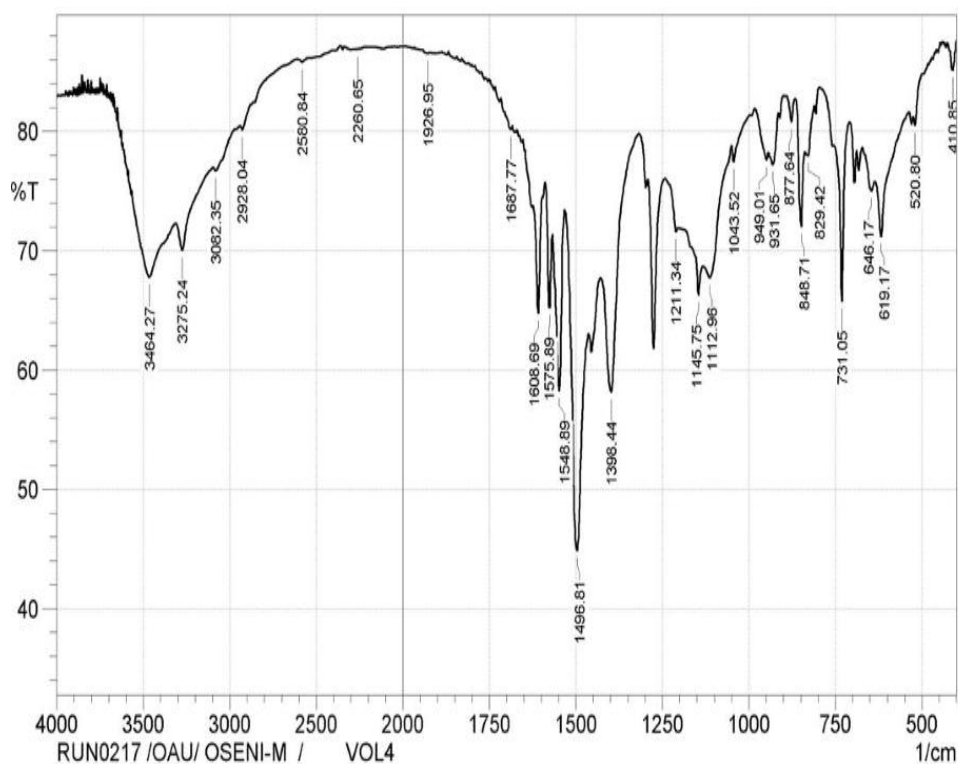


Figure 10: IR spectrum of L₄

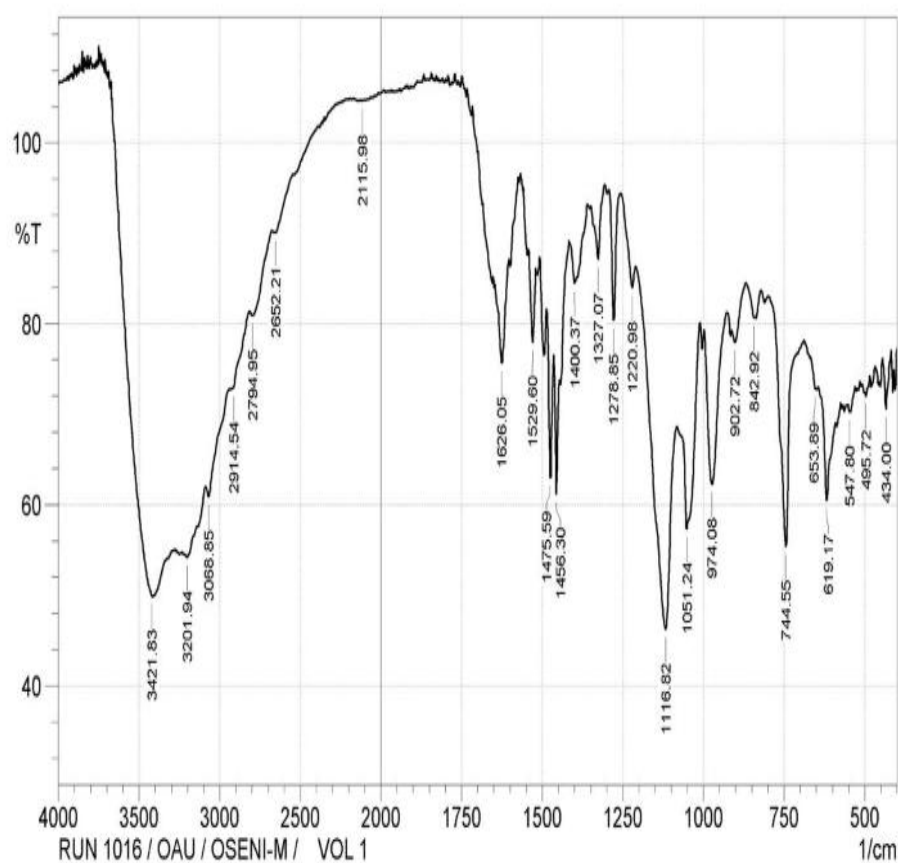


Figure 11: Infrared Spectrum of [VO(L1)(H₂O)(SO₄)]

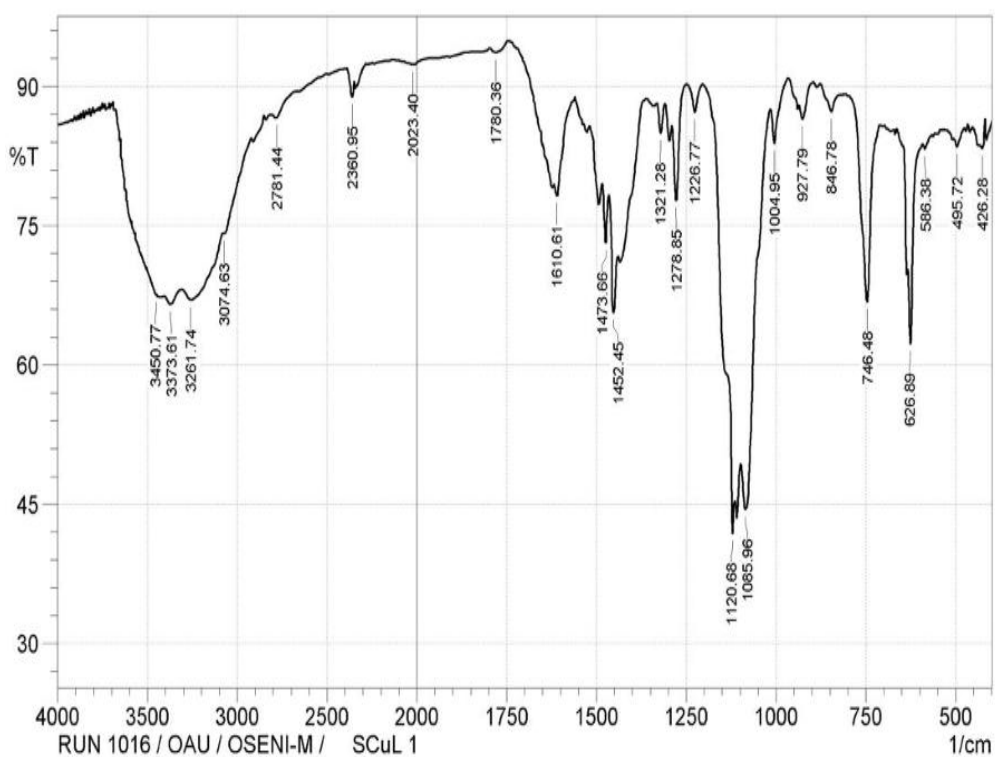


Figure 12: Infrared Spectrum of [Cu(L1)(ClO₄)₂H₂O]H₂O

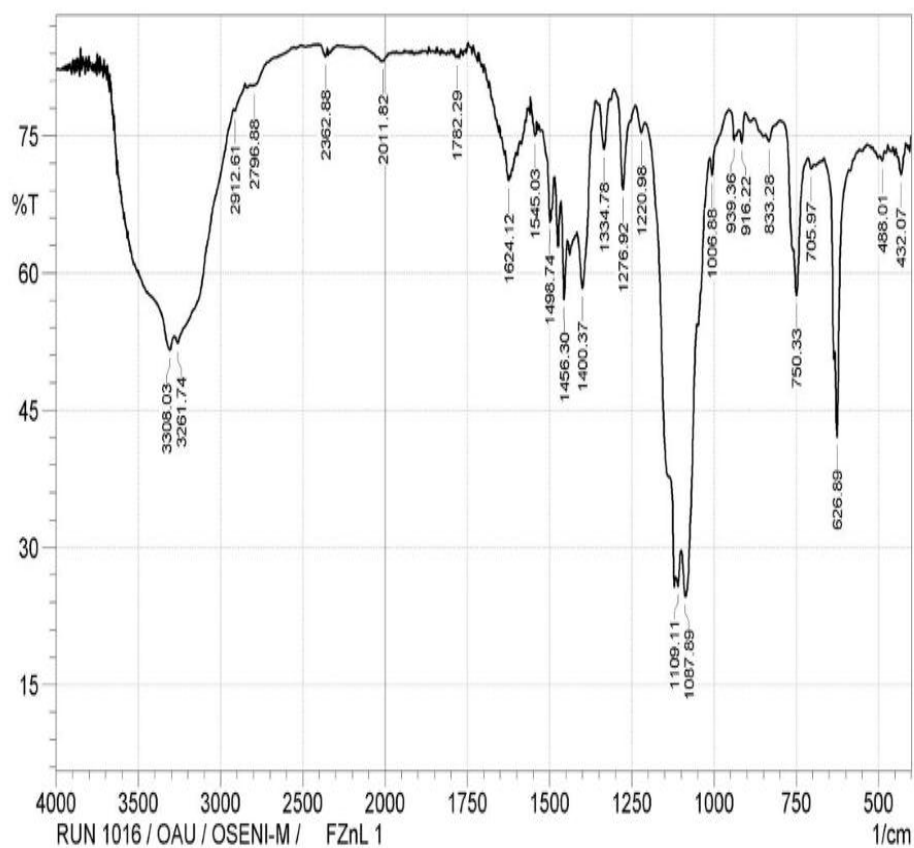


Figure 13: Infrared Spectrum of $[Zn(L1)ClO_4(H_2O)_2]ClO_4$

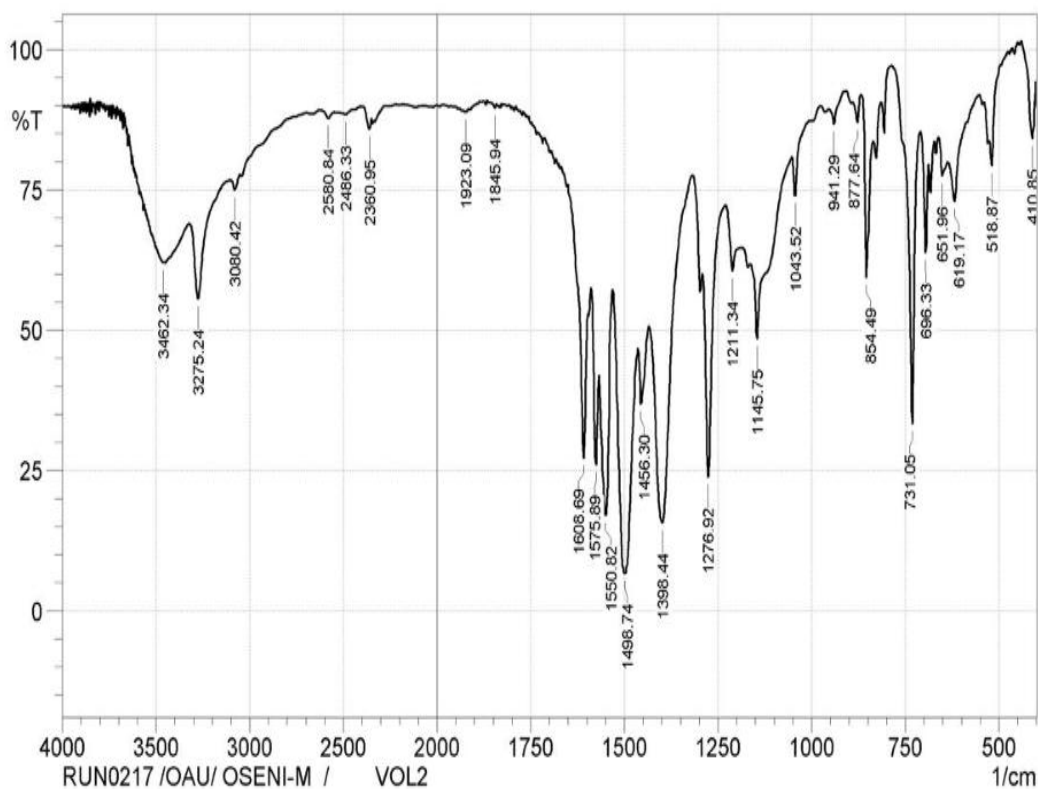


Figure 14: Infrared Spectrum of $[VO(L2)_2](SO_4)$

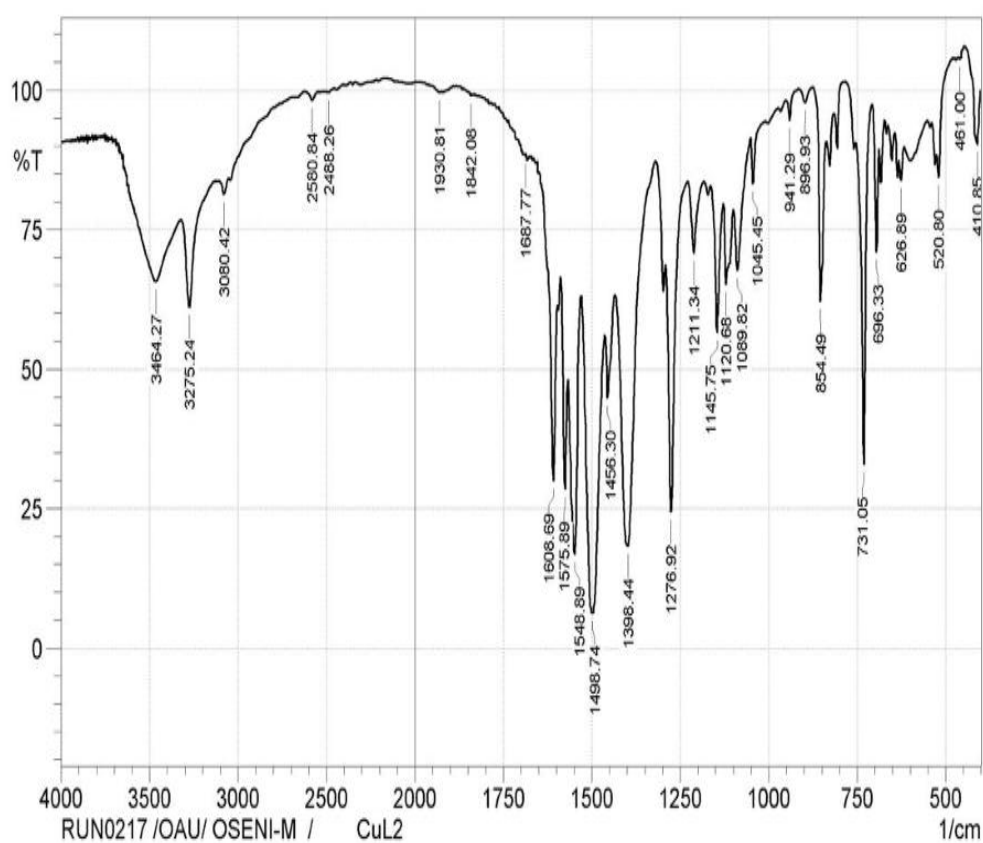


Figure 15: Infrared Spectrum of $[\text{Cu}(\text{L}2)_2\text{ClO}_4]\text{ClO}_4$

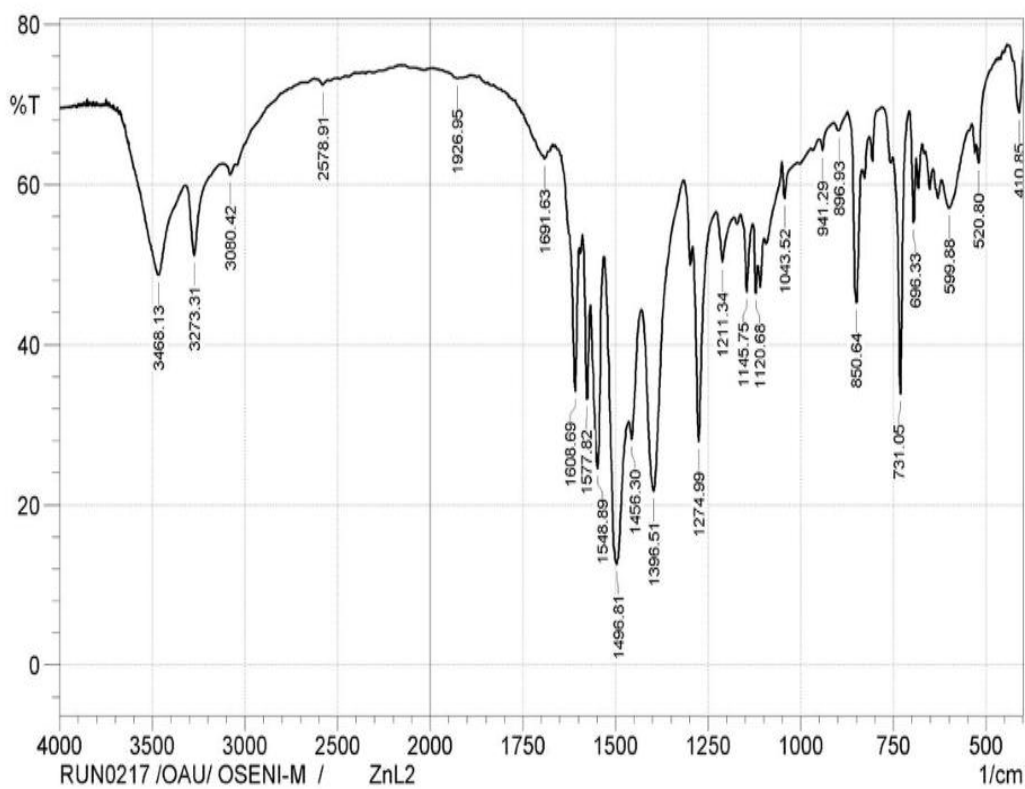


Figure 16: Infrared Spectrum of $[\text{Zn}(\text{L}2)_2\text{ClO}_4]\text{ClO}_4$

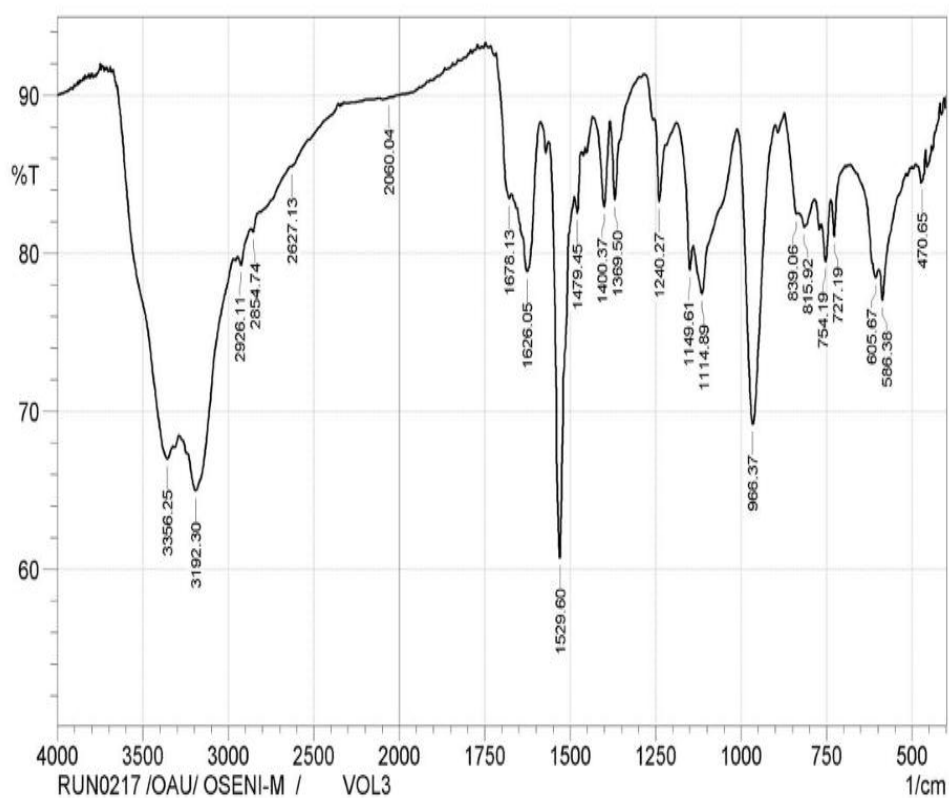


Figure 17: Infrared Spectrum of [VO(L3)(H₂O)₂]

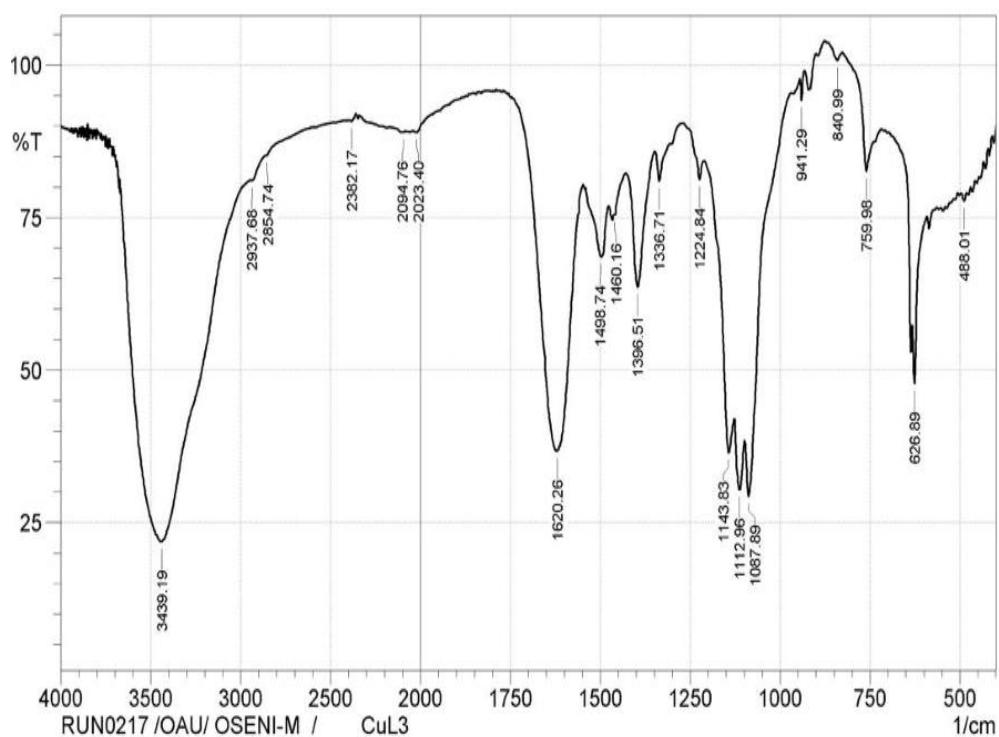


Figure 18: Infrared Spectrum of [Cu(L3)(H₂O)₂]₂H₂O

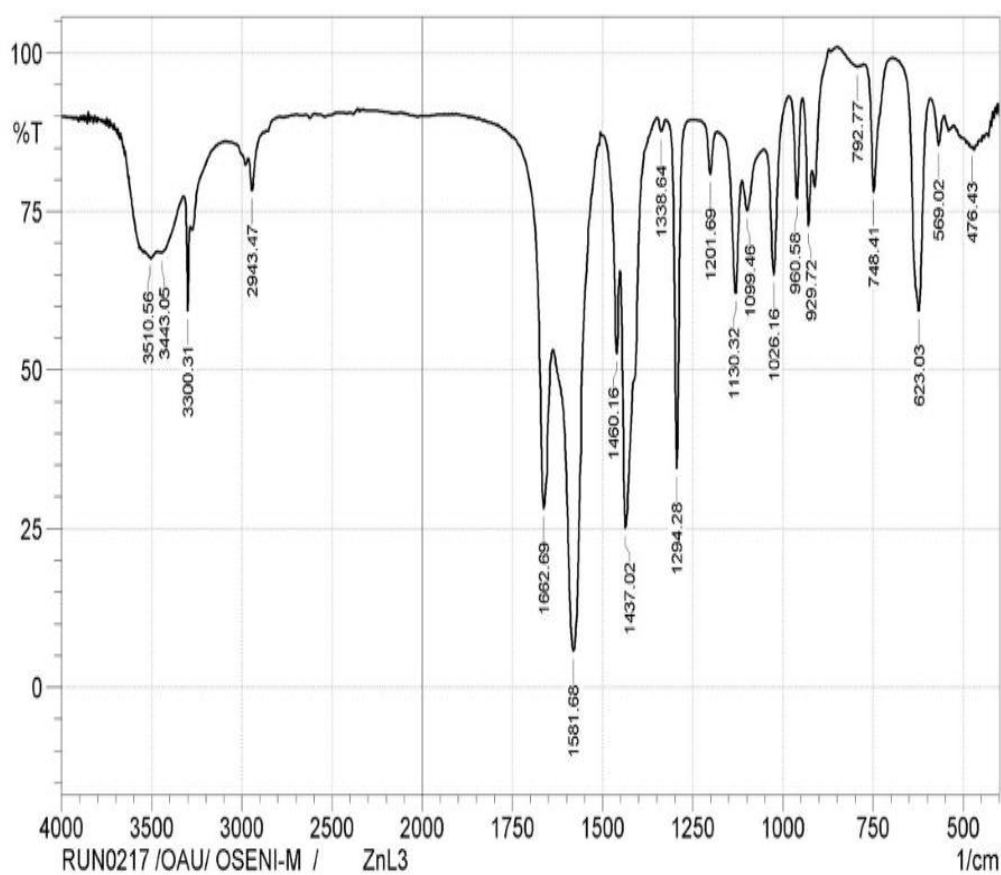


Figure 19: Infrared Spectrum of [Zn(L3)(H₂O)₂]H₂O

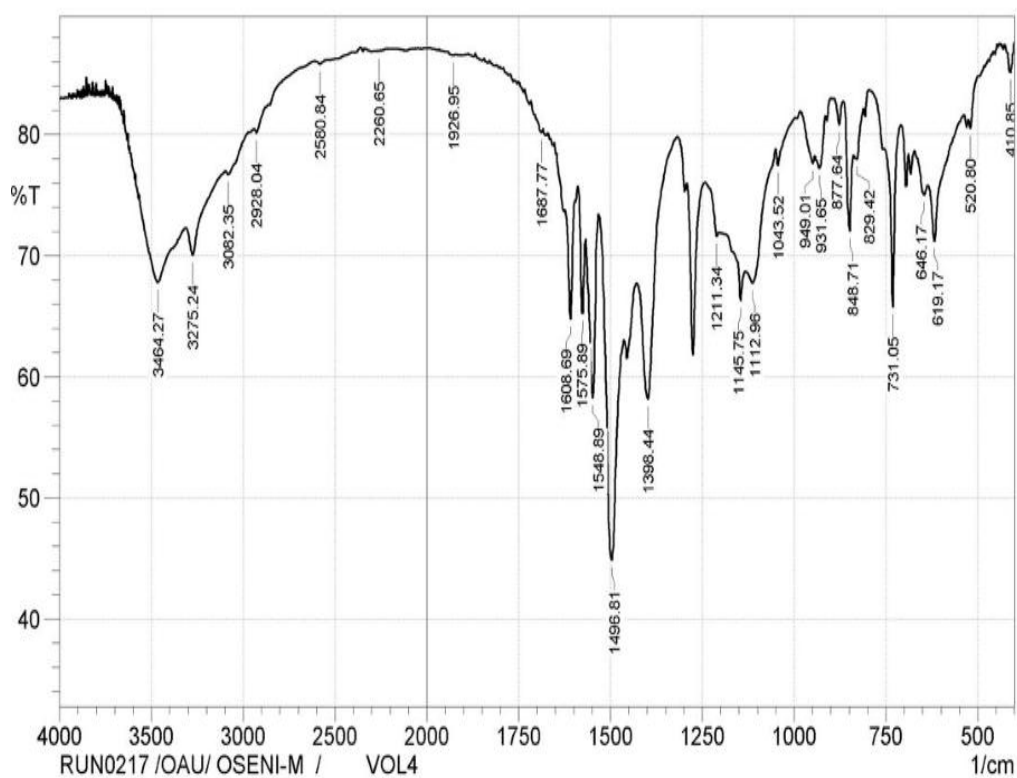


Figure 20: Infrared Spectrum of [VO(L4)₂]H₂O

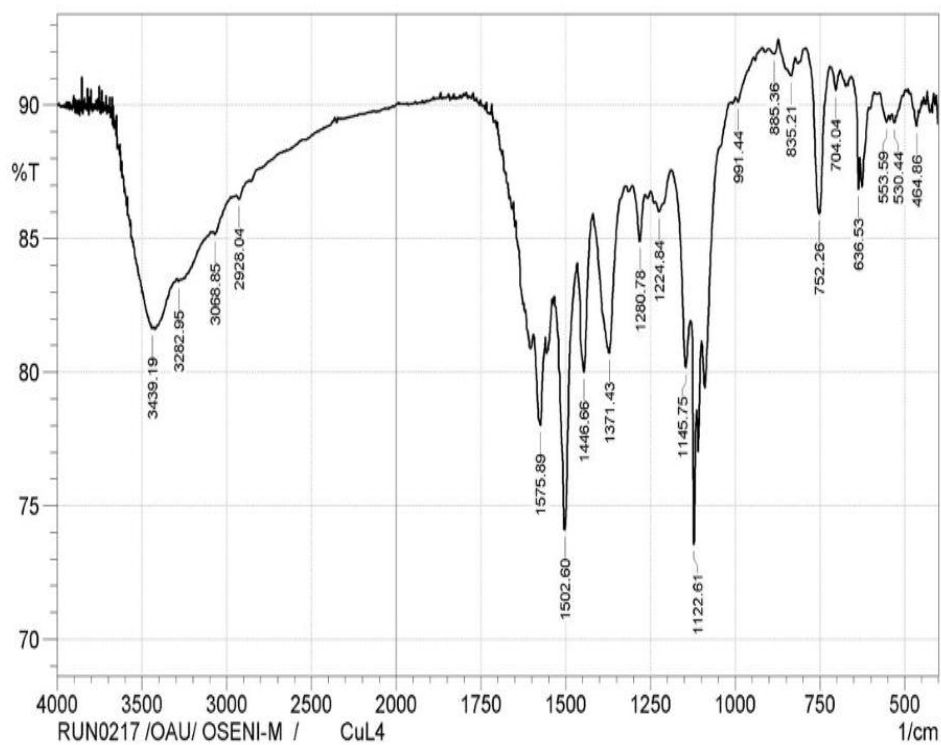


Figure 21: Infrared Spectrum of [Cu(L4)2(H2O)2]

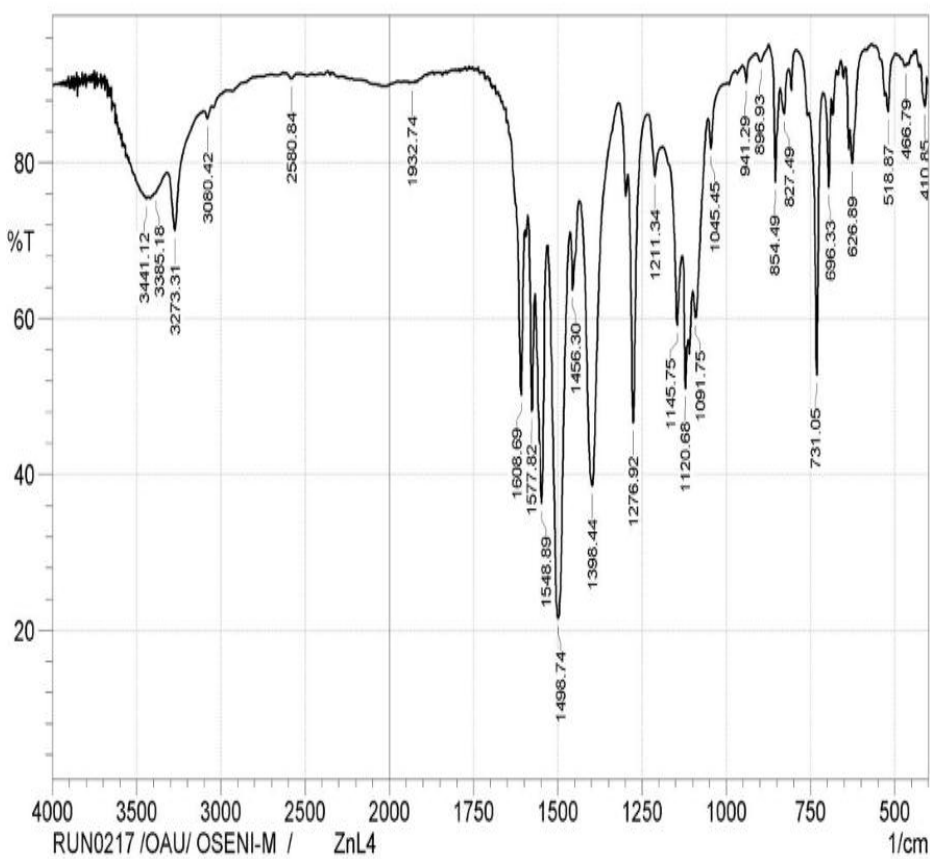


Figure 22: Infrared Spectrum of [Zn(L4)2H2O]

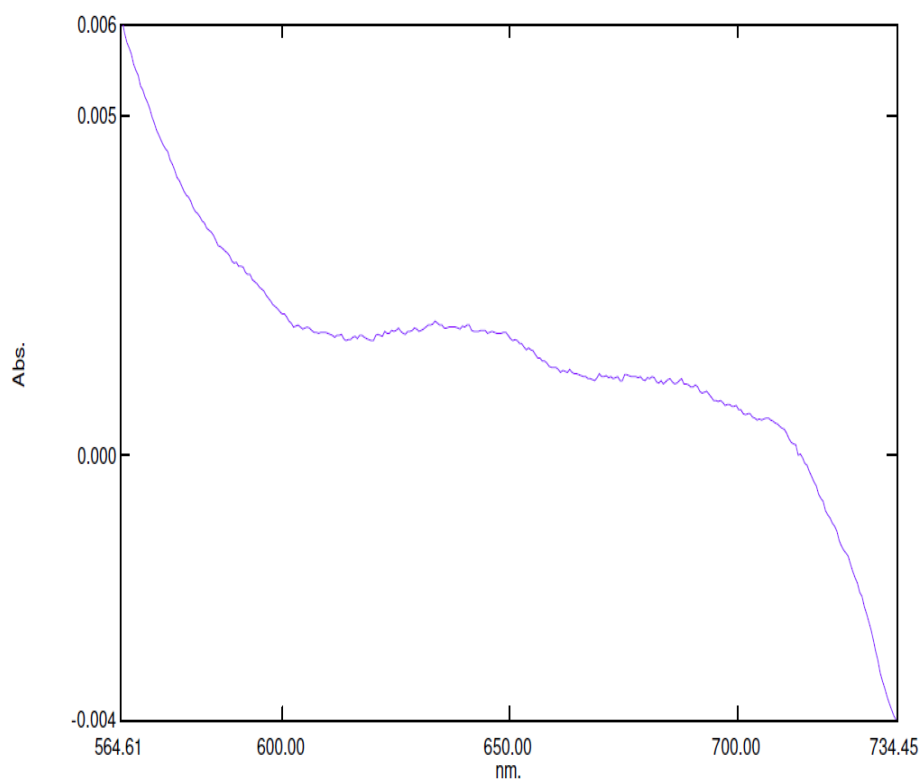


Figure 23: Visible Spectrum of CuL2

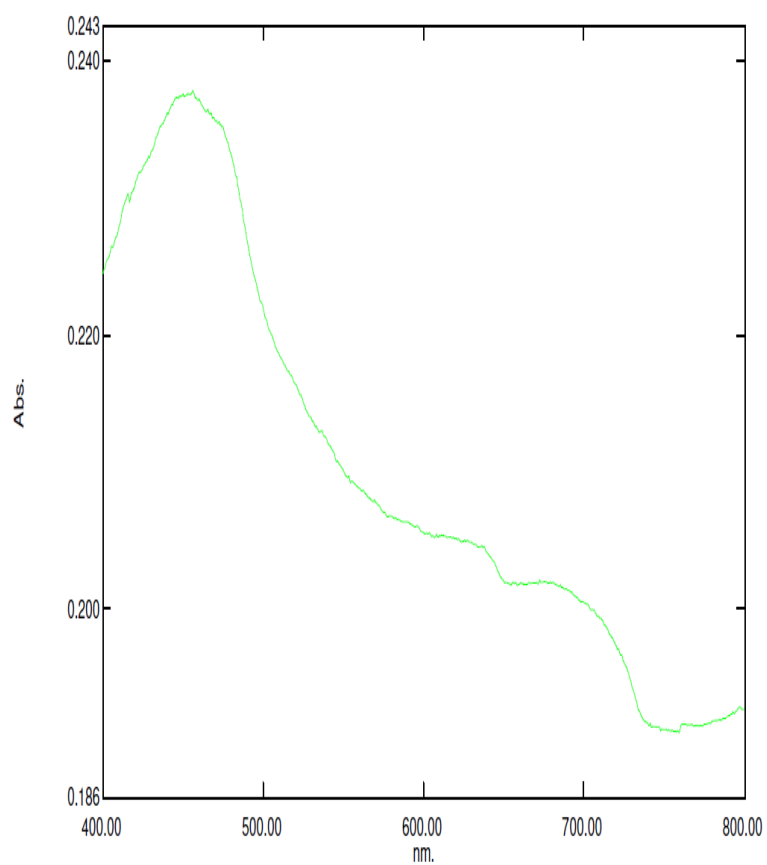


Figure 24: Visible Spectrum of CuL3

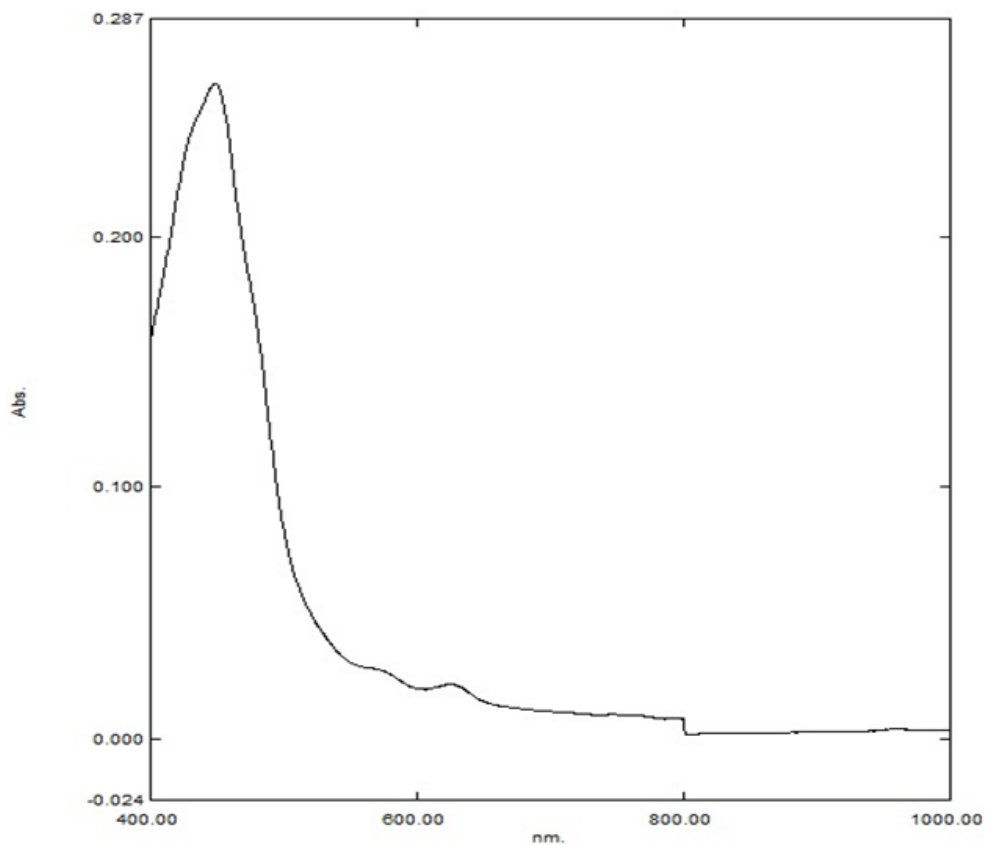


Figure 25: Visible Spectrum of VOL1

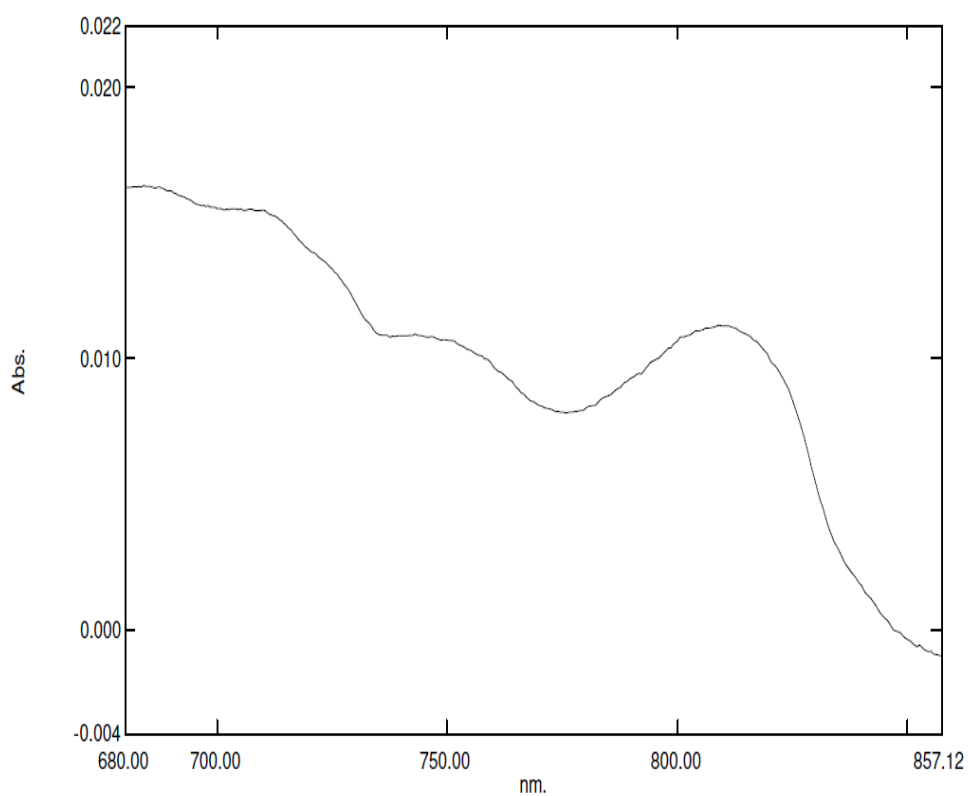


Figure 26: Visible Spectrum of VOL4

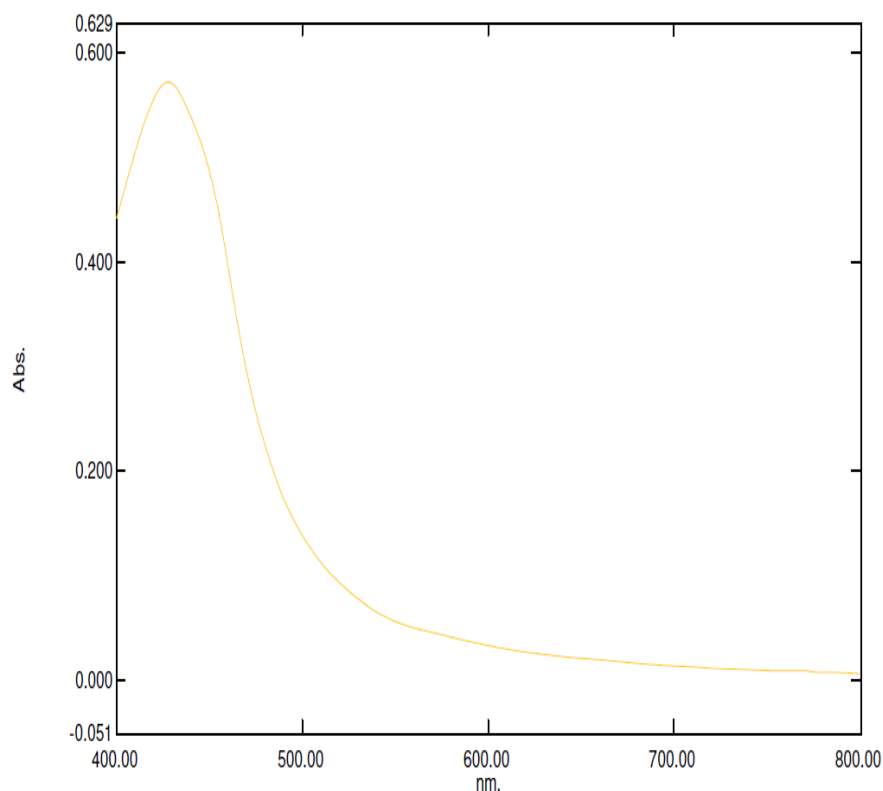


Figure 27: Visible Spectrum of ZnL1

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