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Heteroleptic metal chellates of *m*-nitrobenzaldehyde benzoylhydrazone and nicotinamide: synthesis, characterization and antibacterial studies

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Abstract

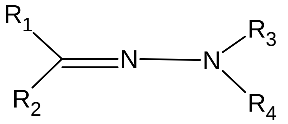
Mixed ligand complexes of m-nitrobenzaldehydebenzoylhydrazone (*m*-NBH) and nicotinamide (NA), were synthesized and their elemental, magnetic moment, molar conductivity, Fourier Transform Infra-Red (FT-IR)(UV) spectroscopic characterization were carried out. The IR spectra revealed that the *V*C=O band of the hydrazone shifted in wavenumber from 1654 cm^{-1} to 1624,1674, 1625, 1622 cm^{-1} in the Fe-, Co-, Ni-, Cu- complexes, respectively, while the intensity of the *V*C=N band at 1542 cm^{-1} decreased. Nicotinamide coordinated via the –N of pyridine, and not through the $-NH_2$ or –C=O groups. The vibrations of M-N and M-O bonds were identified by sharp bands in the range of 426 – 582 cm^{-1} and 526 – 688 cm^{-1}, respectively. Intra-ligand $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ electronic transitions shifted to lower wavenumbers between 214 - 224 nm and 245 - 262 nm, respectively. For the metal-centered bands, Cu-complex had a broad band around 647 nm. Co-complex had bands at 505, 645, and 761 nm, and Ni-complex at 654, 721, and 876 nm. Weak bands were observed for the Fe-complex. The molar conductivities of the complexes range from 259 to 331 Ω^{-1}cm^{2}mol^{-1}, indicating their electrolytic nature and the magnetic moment (5.83, 4.30, 2.73, 1.74 MB) showed that the complexes (Fe, Co, Ni, Cu, respectively) were paramagnetic. The stoichiometry ratio of the complexes was 1:1:2 (*m*-NBH**:M:**NA). The antibacterial activity of the complexes and ligands was evaluated against fifteen bacteria isolates. There was improved activity by the metal complexes compared to the ligands, except for the Cu-complex.

Keywords: Mixed ligands complexes, Nicotinamide, m-Nitrobenzaldehydebenzoylhydrazone, Antimicrobial activity

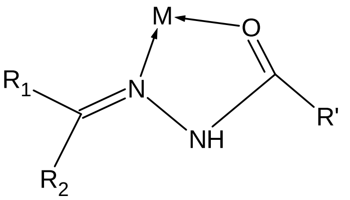
Introduction

Some transition metal complexes are known to have biological activities, and bioactive compounds, particularly those with chelating ligands whose donor atoms are oxygen, nitrogen, and sulphur, have shown increased activity when complexed to metal (Canpolat *et* *al.*, 2004; Cozzi, 2004; Ferrari *et al.*, 1999; Singh *et al.*, 2007; Yildiz *et al.*, 2004). Mixed ligand/heteroleptic complexes have different ligands bonded to the same metal. The study into the bioactivity of mixed ligands of know drugs or bioactive compounds is of great interest because mixed ligand systems increase the probability of discovering better drugs (Ahmad *et al.*, 2017; El-Sonbati *et al.*, 2019; Jurca *et al.*, 2017; Karem *et al.*, 2020; Khan *et al.*, 2021). For example, drugs to which bacteria have developed resistance, can be modified, drug reactions and negative interactions can be reduced or prevented, and new drugs can be created from mixed ligand complexes of known bioactive molecules.

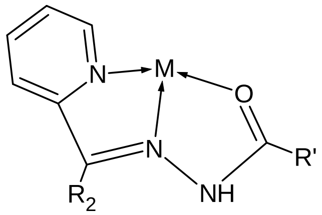
Hydrazones are one of the ligands whose biological activities have been investigated (Aly and Fathalla, 2020; Ibrahim *et al.*, 2016; Hollo *et al.*, 2016; Kucukoglu *et al.*, 2019; Megger *et al.*, 2017; Recio Despaigne *et al.*, 2014; Secci *et al.*, 2019). These are organic compounds with the general formula $\mathrm{R_2R_1C = N - NR_3}R_4$ (where $\mathrm{R_2}$ and $\mathrm{R_4}$ may be hydrogen). Hydrazones are synthesized via the condensation reaction of a hydrazide $\mathrm{R_3NH - NH_2}$ and an aldehyde $(\mathrm{R_1CH = O})$ or a ketone $(\mathrm{R_2R_1C = O})$. The two nitrogen atoms are nucleophilic; however, the azomethine nitrogen more readily coordinates with metal ions (Wood *et al.*, 2004). In addition, the denticity of these ligands increases to two when an acylhydrazone ($R_3 = R^{'}CO$) is used. Therefore, high stability is attained due to the formation of chelates (Clarke *et al.*, 1998; Spin *et al.*, 2008; Brehme *et al.*, 2007; Mangalam *et al.*, 2010; Mangalam, 2014; Shallaby *et al.*, 1986).



Hydrazone



acylhydrazone (bidentate ligand)



acylhydrazone (tridentate ligand)

Nicotinamide, one of the forms of Vitamin B3, is found in foods milk, vegetables, milk, and yeast. In the body, it is converted to nicotinamide adenine dinucleotide (NAD) and is used in treating pellagra, and added in the cream for treating acne (Stipanuk and Caudill, 2016). Complexes of nicotinamide have been reported (Allan *et al.*, 1979, Sun and Kirschenbaum, 2018, Yurdakul and Kose, 2015) and nicotinamide is often one of the ligands in mixed ligand complexes or is chemically modified, mostly for biological activity studies (Abed and Ahmed, 2021, Akinyele *et al.*, 2021; Al-Noor *et al.*, 2013; Dilip *et al.*, 2016; El-Shiwiniy, *et al.*, 2021; Fazary *et al.*, 2016; Osowole and Odutemu, 2016, Sarker and Ehsan, 2017). The structure of nicotinamide consists of pyridine with the amide group at the meta-position and most studies have reported that the coordinating atom is the nitrogen in pyridine. In this work, we report the synthesis and characterization of *m*-

nitrobenzaldehydebenzoylhydrazone, its mixed ligand metal complexes with nicotinamide, and the antimicrobial activities of these compounds.

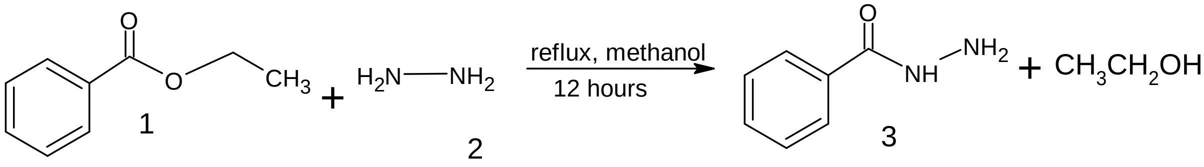
Materials and Methods

Synthesis and Characterization

All the reagents, solvents, and metal salts were of analytical grade (BDH Chemical, (London, UK) except for nicotinamide (Bond Chemical, Nigeria). The infrared analyses were carried out using Shimadzu FT-IR 8000 Spectrometer (KBr disc). The electronic spectra were determined using Shimadzu UV-Vis Spectrophotometer. Conductivity measurement using HANNA instrument (TDS Conductivity meter). Gallenkamp apparatus was used to determine the melting points of the ligands and complexes. Magnetic susceptibility measurements were done using Guoy Balance, Magnetic Susceptometer (Sherwood Scientific Company, Cambridge, UK).

Synthesis of benzoylhydrazide (3)

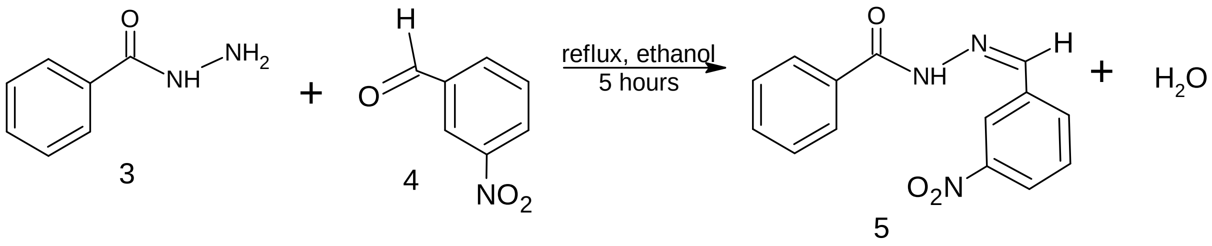
The synthesis of benzoylhydrazide (3) was done following the procedure of Shrestha and Maharjan (2013). Solution of ethylbenzoate (1) (38 mL, 0.2663 mol in methanol) and hydrazine hydrate (2) (39 mL, 0.2663 mol in methanol) were poured into a round bottom flask, refluxed for 12 hours and the precipitate formed was filtered and washed with ethanol.



Scheme 1: Reaction equation for the synthesis of benzoylhydrazide

Preparation of m-nitrobenzaldehydebenzoylhydrazone (5)

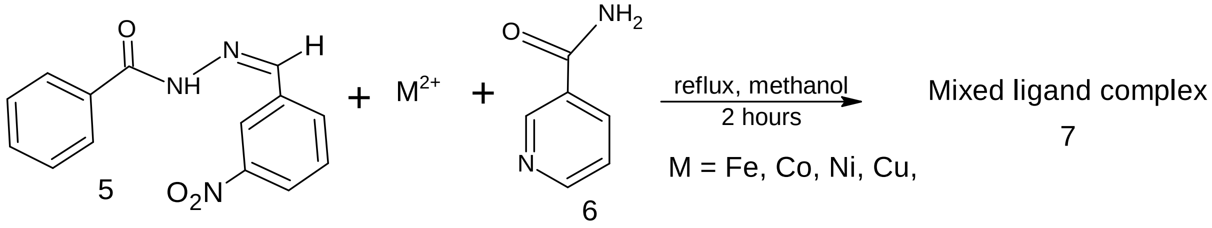
The synthetic route of this compound is shown in Scheme 2. Benzoylhydrazide (3) (2.06 g. 0.0152 mol) and *m*-nitrobenzaldehyde (4) (2.229 g, 0.0152 mol) were each dissolved in 20 mL of absolute ethanol, the solutions were mixed together and refluxed for 5 hours. This was left in a beaker overnight, and the precipitate formed was filtered under suction and washed with ethanol. The crystal obtained were left to dried in a desiccator containing calcium chloride (Shrestha and Maharjar, 2013).



Scheme 2: Reaction equation for the synthesis of m-nitrobenzaldehydebenzoylhdrazone

Synthesis of mixed ligand metal complexes of m-nitrobenzaldehydebenzoylhydrazone (5) and nicotinamide (6)

Solutions of metal salt (M^{2+}), (0.35 g, 1.5 mmol) in 10 mL of methanol were added into stirring homogenous solutions of *m*-nitrobenzaldehydebenzoylhydrazone (5), (0.40 g, 1.5 mmol) and nicotinamide (6), (0.36 g, 3.0 mmol) in 20 mL of hot methanol. During a two-hour reflux and stirring process (Scheme 3), a precipitate was formed. The products were then filtered under suction, washed with methanol, and dried in a desiccator containing calcium chloride (Osowole, *et al.* 2015).



Scheme 3: Reaction equation for the synthesis of the mixed ligand complexes

The ligands and the complexes were characterized using melting point, UV-Vis spectroscopy, and FTIR. For the UV-Vis analysis, all the samples were dissolved in methanol.

Metal Analysis of the Complexes

Complexometric titration was used to determine the percentage composition of the metal in the complexes. This was done by preparing solutions of a known amount of the digested complexes and then titrating them against the standardized EDTA solution and using murexide as indicator. 0.05 M EDTA was standardized using 0.005 M, $ZnSO_4$ and the indicator used was solochrome black T at pH ~ 10 using 2 mL NH_3/NH_4Cl buffer solution. Metal complexes (0.0141 gram) were weighed and a few drops of nitric acid and perchloric acid (1:1) were added to digest the metal complexes to dryness. It was allowed to cool before a few drops of distilled water was added and then heated to almost dryness and cooled. Distilled water was added, just enough to dissolve the residue. This solution was transferred with care into a 100 mL standard flask, and the flask was filled with distilled water to reach the marked level. 20 mL of this solution was titrated against the standardized EDTA (0.047 M) and this was done thrice and an average volume was taken. Blank determination was also carried out to minimize errors in the analysis.

% Metal in the complex was calculated as follows:

Weight of metal complex $= w_1$

The average volume of EDTA used (titre value) $= V_1$

Volume of M^{2+} solution $= V_2$

Reaction equation: mole ratio of Metal ion to EDTA = 1:1

$$M^{2+} + EDTA^{4-} \rightarrow [M(EDTA)]^{2-}$$

Molarity of EDTA solution $= M_1$

Molarity of M^{2+} $= M_2$

The molarity of the metal ion, and hence its mass, w_2 in 100 mL solution was determined and with this, the percentage of each metal in the complex was calculated.

$$\%M^{2+} \text{ in the complex} = \frac{w_2}{w_1} \times 100$$

Conductivity Measurement

For this analysis, each of the complexes (1.0×10^{-3} g) was dissolved in about 10 mL of distilled water and their specific conductivity was measured at 28 °C. From the specific conductivity the molar conductivity was determined using the expression:

$$\text{molar conductivity} = \frac{\text{specific conductivity } (\Omega/\text{cm})}{\text{concentration } (\text{mol/cm}^3)}$$

Magnetic Susceptibility Measurement

The magnetic susceptibility was determined from the value of the effective magnetic moment (μ_{eff}) using the expression (Bain and Berry, 2008):

$$\mu_{eff} = (8\chi_A T)^{1/2} \ldots\ldots\ldots (1)$$

$$\text{where } \chi_A = \chi_M - \chi_D \ldots\ldots\ldots (2)$$

$$\text{and } \chi_M = \chi_g \times M \ldots\ldots\ldots (3)$$

$\chi_A =$ Corrected value of molar susceptibility

$\chi_M =$ Molar susceptibility ($ergG^{-2}mol^{-1}$)

$\chi_g =$ Diamagnetic correction of the complex

M = Molar mass of the complex

T = 298 K

The diamagnetic correction of each complex was determined by adding the diamagnetic correction of each atom in the complex (Bain and Berry, 2008).

Microbial Studies

Test Organisms

Initially, the microorganisms were maintained on agar slants in screw-capped bottles and subsequently, were subcultured and stored on Muller-Hinton (MH) agar plates at 4 °C. They were then grown at 37 °C as when needed. The strain used were obtained from the National Collection of Industrial Bacteria (NCIB) and Locally Isolated Organisms (LIO) from clinical samples. The locally isolated organisms were obtained from the Department of Microbiology, Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Nigeria. The organisms include: *Micrococcus luteus* (NCIB 196), *Pseudomonas aeruginosa* (NCIB 950), *Bacillus cereus* (NCIB 6349), *Pseudomonas fluorescens* (LIO), *Bacillus stearothermophillus* (NCIB 8222), *Escherichia coli* (NCIB 86), *Bacillus subtilis* (NCIB 3610), *Staphylococcus aureus* (NCIB 8588), *Bacillus polymyxa* (LIO), *Corynebacterium pyogenes* (LIO), *Klebsiella pneumoniae* (NCIB 418), *Proteus vulgaris* (LIO), *Proteus mirabilis* (LIO), *Pseudomonas aeuruginosa* (LIO), *Shigella dysenteriea* (LIO), *Salmonella typhi* (LIO). The viability and purity of organisms were checked periodically by regular plating. Test cultures were prepared by aseptic transfer and grown at 37 °C.

Culture Media

- Rapid Labs Mueller Hinton Agar (CM-MHA 135) from the United Kingdom, comprising per Litre: 17.5 g Peptone, 1.5 g Starch, 4.0 g Beef Infusion Solids, and 15.0 g Agar.
- Himedia Nutrient Agar (M001-500G) from India, comprising per Litre: 5.0 g Peptone, 15.0 g Agar, 5.0 g NaCl, 1.5 g Meat Extract, 1.5 g Yeast Extract.
- Oxoid Nutrient Broth (CM-0001) from England, comprising per Litre: 5.0 g Peptone, 5.0 g NaCl, 1.0 g Lab-Lemco Powder. These culture media were purchased from Iyke Diagnostics England.

Antimicrobial activity

The synthesized compounds were evaluated for their antimicrobial activity against a diverse group of 15 microorganisms, including both Gram-negative and Gram-positive bacteria, using 40 mg/mL of the compounds. As a comparison, the commonly used antibiotic, ampicillin, was also tested against these organisms. Sterile water was used to dissolve the ligand and the complexes before the testing was conducted.

Results and Discussion

Physicochemical parameters for the ligands and metal complexes

The physicochemical properties of the ligands m-nitrobenzaldehydebenzoylhydrazone (*m*-NBH) and nicotinamide (NA) and the complexes are presented in Table 1. *m*-NBH and NA were pale yellow and white, respectively while the complexes were all coloured. This shows that the electronic transition in the complexes occurred within the visible region, which could be d – d or charge transfer transitions. The melting point of *m*-NBH was between 200 - 202 °C, NA was between 130 – 132 °C, and those of the mixed ligand complexes were above 290 °C. However, the nickel complex did not melt within the limits of the thermometer. The high melting points of the metal complexes show that they are stable.

From the results of the molar conductivity, the complexes are electrolytes having values ranging between 259 - 331 $\Omega^{-1}cm^2mol^{-1}$ (Geary, 1971). It has been reported that such high values of molar conductivity were as a result of the partial dissociation of the complexes to ions within the solution (Geary, 1971). From the values of their molar conductivity, the cobalt, nickel, and copper complexes have electrolytic ratios of 1:2 while that of iron is 1:3

Table 1: Physicochemical Properties of the Ligand and the Mixed Ligand Complexes

Compound	Colour	Melting point (°C)	%Yield	Λ_m ($\Omega^{-1}cm^2mol^{-1}$)
NA	White	130 – 132	–	–
m-NBH	Yellow	202.2	85	–
[Fe(*m*-NBH)(NA)$_2$(H$_2$O)$_2$]Cl$_2$	Green	301 – 302	79	331.03
[Co(*m*-NBH)(NA)$_2$(H$_2$O)$_2$]Cl$_2$	Lilac	345 – 347	68	295.49
[Ni(*m*-NBH)(NA)$_2$(H$_2$O)$_2$]Cl$_2$	Green	>360	72	269.36

Magnetic properties

The room temperature effective magnetic moment shows the number of unpaired electrons in the synthesized compounds. The results obtained which are presented in Table 2, are similar to previous findings documented in the literature (Akinyele *et al.,* 2019, 2021; Ekennia *et al.,* 2015; Osowole *et al.,* 2014). The Cu-complex displayed a magnetic moment of 1.74 B.M, indicating its mononuclear nature with one unpaired electron. The observed magnetic moment in cobalt-complex at 4.30 BM is typical for complexes having three unpaired electrons. That of the Ni-complex was 2.73 BM which is consistent with two unpaired electrons and hence has an octahedral geometry. The high magnetic moment of 5.83 BM observed for iron-complex indicates high-spin of five electrons.

Table 2: Electronic Spectra and Magnetic Moments of the Complexes

Compounds	Intra-ligand transitions (nm)	Ligand field transitions (nm)	μ_{eff} (BM)	Proposed geometry
NA	218, 280	-	-	-
m-NBH	229, 295	-	-	-
[Fe(*m*-NBH)(NA)$_2$(H$_2$O)$_2$]Cl$_2$	224, 261	Weak bands	5.83	Octahedral
[Co(*m*-NBH)(NA)$_2$(H$_2$O)$_2$]Cl$_2$	262	506, 645, 761	4.30	Octahedral

$[Ni(m\text{-}NBH)(NA)_2(H_2O)_2]Cl_2$	261	654, 721, 876	2.73	Octahedral
$[Cu(m\text{-}NBH)(NA)_2(H_2O)_2]Cl_2$	245, 262	647	1.74	Octahedral

Electronic spectra

Table 2 provides a summary of the electronic spectral data for the ligands and complexes, and the UV-Vis spectra are contained in the Supplementary Information. In the coordination of metal ions to ligands, certain spectra changes are observed. For instance, an increase in the intensity of the sharp bands (σ→π*, π→π*, and n→π* transitions) arising from the ligand, might be accompanied by a slight shift in wavelength relative to the free ligands (Refat and El-Metwaly, 2012). The NBH ligand had a sharp band at 229 nm assigned to π→π* and a broad band at 295 nm due to n→π* transition. Nicotinamide also shows two transitions as a result of π→π*, and n→π* transitions at 218 and 280 nm, respectively. Upon coordination to the metal ion, the intra-ligand electronic transitions (π→π* and n→π*) in the free ligands and nicotinamide were shifted to wavelengths between 214 - 224 nm and 245 - 262 nm.

In most cases, the metal-centered bands are observed, which are usually broad d – d transitions and, or charge transfer bands. The copper(II) complex showed a broad band around 647 nm. This is can be attributed to the $^2E_g \rightarrow ^2T_{2g}$ transition in an octahedral complex, and the broadening was due to Jahn-Teller distortion (Lee, 1991). In the UV-Vis spectra of the Co(II) complex, three d-d bands (505, 645, 761 nm) were observed. The lowest energy band at 761 nm can be assigned to the ^4T_1g (F)→^4T_2g transition. The ^4T_1g(F)→ ^4T_1g(P) and ^4T_1g(F)→^4A_2g transitions gave rise to the other bands at 645 and 505, respectively, which are common in octahedral fields for Co(II) complexes. The visible spectra of Ni(II) complex had three bands at 876 nm, 721 nm, and 654 nm as a result of $^3A_2g \rightarrow ^3T_2g$, $^3A_2g \rightarrow ^3T_1g$(F), and $^3A_2g \rightarrow ^3T_1g$(P) transitions. Weak bands in Fe-complex are because Fe is in the +3 oxidation state (d^5) having both symmetry and spin-forbidden transitions.

Infrared Data

The wavenumbers of the IR bands of interest are presented in Table 3. The coordination of a hydrazine compound to a metal is indicated by the shift in four of its IR absorption bands. These are bands are the N-H, C=N, C=O, and N-N vibrational frequencies. The observation of additional bands in the spectra of the metal complexes, which are due to metal-oxygen and metal-nitrogen bonds that were not present in the spectra of the ligands, which are further evidence of coordination (Neelamma *et al.*, 2011). In the IR spectrum of *m*-NBH), the vibrational frequencies of the key functional groups, N-N, C=N, C=O, and N-H, occurred at 3390, 1654, 155, and 1024 cm^{-1}, respectively. Symmetric and asymmetric stretching vibrations also occurred at 1353 and 1518 cm^{-1}, respectively for NO_2. On coordination, the carbonyl group shifted to either lower or higher frequency between 1622 - 1674 cm^{-1}. The participation of the azomethine group in coordination was responsible for the decrease in the intensity of the C=N band at 1542 cm^{-1}. Sharp bands around 426 – 582 cm^{-1} and 526 – 688 cm^{-1} were assigned to metal-to-nitrogen (M – N) and metal-to-oxygen (M – O) vibrations, respectively.

Nicotinamide molecule has three possible donor sites; the nitrogen of pyridine, the nitrogen of $–NH_2$, and the C=O. Coordination through the oxygen in the carbonyl group often results in a shift of the C=O bands to lower wavenumbers (Paul and Chadha, 1986). This is also applicable to the N-H groups in amides. In this study, the IR bands of $\nu_{C=O}$ and ν_{N-H} in the complexes shifted to higher wavenumbers than that of nicotinamide. The C=O vibration bands appeared as strong bands between 1666 and 1708 cm^{-1}, and the N-H vibration bands were between 3402 and 3419 cm^{-1} in all the complexes. This indicates that the NH_2 and C=O groups were not involved in the coordination, and therefore, coordination of the nicotinamide to the metal was through the pyridine nitrogen only (Akinyele *et al.*, 2021). [See Supplementary Information for all FTIR spectra]

Table 3: Infra-red spectra data for the ligands and the metal complexes

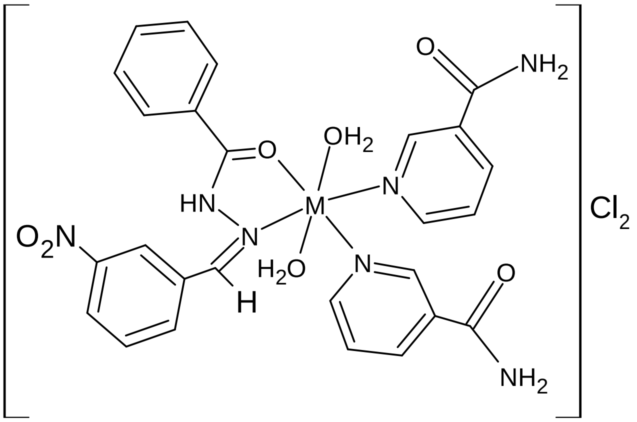
Compounds	$\nu_{(O-H)}$	$\nu_{(N-H)}$	$\nu_{(NH_2)}$	$\nu_{(C=O)}$	$\nu_{(C=N)}$	$\nu_{(M-N)}$	$\nu_{(M-N)}$
NA	-	-	3351	1685	1381	-	-
m-NBH	-	3390	-	1654	1559	-	-
$[Fe(m\text{-}NBH)(NA)_2(H_2O)_2]Cl_2$	3596	3403	3303 3196	1667 1624	1481	500	626
$[Co(m\text{-}NBH)(NA)_2(H_2O)_2]Cl_2$	3420	3419	3298 3203	1708 1674	1481	426	526
$[Ni(m\text{-}NBH)(NA)_2(H_2O)_2]Cl_2$	3596	3406	3317 3212	1666 1625	1481	582	626
$[Cu(m\text{-}NBH)(NA)_2(H_2O)_2]Cl_2$	3596	3402	3305 3160	1706 1622	1503	549	688

Metal Analysis

The % metal ion was used to estimate the ratio of the metal to ligands. From the IR data, we could ascertain that both ligands were coordinated to the metals, and the atoms that were involved. Based on this, all the possible complexes were derived and the % metal in each was calculated. These values were compared to those obtained from the complexometric titration and those with similar results were selected as the probable molecular formula of the complexes. The ratio of metal to the ligands that had similar % metal was 1:1:2.

Table 4: Data from the Metal Analysis Based On The Experimental Data Obtained, We Propose the Structure Below.

Chemical formula	Molar mass	Calculated %metal	Complexometric % metal
[Cu(*m*-NBH)(NA)$_2$(H$_2$O)$_2$]Cl$_2$	683.77	9.46	9.29
[Co(*m*-NBH)(NA)$_2$(H$_2$O)$_2$]Cl$_2$	679.16	8.76	8.67
[Ni(*m*-NBH)(NA)$_2$(H$_2$O)$_2$] Cl$_2$	678.92	8.93	8.65
[Fe(*m*-NBH)(NA)$_2$(H$_2$O)$_2$]Cl$_3$	675.45	8.46	8.27



Antimicrobial Activity

From the results obtained, the ligands were not effective at inhibiting the growth of the bacteria except against the *Coryne bacterium*, which was inhibited by *m*-NBH. The synergistic effect involving the metal ions and the ligands in the complexes was observed with improved antibacterial activities except in the case of the Cu-complexes. Co- Ni- and Fe-complexes showed favourable antibacterial activities. Ampicillin was used as standard. The zones of inhibition of the ligands and their complexes are indicated in Table 5.

Compound [Ni(*m*-NBH)(NA)$_2$(H$_2$O)$_2$]Cl$_2$ showed the highest level of inhibition against all the tested microorganisms except for *Shigella dysentrae* and *Salmonella typhi*. Co(*m*-NBH)(NA)$_2$(H$_2$O)$_2$]Cl$_2$, [Fe(*m*-NBH)(NA)$_2$(H$_2$O)$_2$]Cl$_3$ also showed high inhibitory effects against some micro-organisms but there are exceptions in some cases.

Metal chelates of these compounds produce higher antibacterial activity as observed in this study. The increase in antibacterial activity is attributed to the decrease in the electron density of the ligand due to coordination. This causes diffusion across the lipid membrane to occur faster, not giving the organism time for adaptation (Dilip *et al.,* 2016). It is possible that in the case of the Cu-complex, the reduction in the polarity of the ligand may not have been enough to allow the ease of its diffusion into the lipid membrane.

Table 5: Zones of Inhibition of Synthesized Complexes

Growth Inhibition Zone in Millimeters (mm)						
	Synthesized complexes					
Cultured Microorganism	*m*-NBH	Cu	Co	Ni	Fe	Ampi
C. bacterium	10	0	25	30	0	21
B. subtilis	0	0	20	15	15	16
M. luteus	0	0	0	10	15	0
B. polymyxa	0	0	20	20	10	16
B. steaerothermophillus	0	0	15	15	0	15
S. dysentrae	0	0	15	0	10	19
E. coli	0	0	15	10	15	16
P. vulgaris	0	0	10	0	10	15
P. mirabilis	0	0	15	10	10	0
P. aurugius	0	0	20	15	15	22
K. pnuemoniae	0	0	15	20	10	20
P. fluorescence	0	0	0	15	0	22
B .cereus	0	0	15	10	10	0
S. aureus	0	0	0	0	0	21
S. typhi	0	0	0	0	0	21

Key:

Cu = [Cu(*m*-NBH)(NA)$_2$(H$_2$O)$_2$]Cl$_2$
Co = [Co(*m*-NBH)(NA)$_2$(H$_2$O)$_2$]Cl$_2$
Ni = [Ni(*m*-NBH)(NA)$_2$(H$_2$O)$_2$]Cl$_2$
Fe = [Fe(*m*-NBH)(NA)$_2$(H$_2$O)$_2$]Cl$_3$
Ampi = Ampicillin

Conclusion

Mixed ligand complexes of nicotinamide and *m*-nitrobenzaldehydebenzoylhydrazone coordinated to Fe^{3+}, Co^{2+}, Ni^{2+}, and Cu^{2+} were synthesized. The complexes were generally water soluble and are thermally stable due their high melting points. Molecular characterization showed that coordination was through the carbonyl oxygen and azomethine nitrogen for NBH, while nicotinamide coordinated through the pyridyl nitrogen. The stoichiometry of the complexes was found to be in the ratio 1:1:2 (*m*-NBH**:M:**NA). The electronic spectra and magnetic properties of the complexes showed that they were all paramagnetic in an octahedral field. The proposed molecular formula is $[M(m\text{-NBH})(NA)_2(H_2O)_2]Cl_2$. The complexes exhibited moderate activities against the tested micro-organisms, but $[Ni(m\text{-NBH})(NA)_2(H_2O)_2]Cl_2$ had the best antimicrobial activities.

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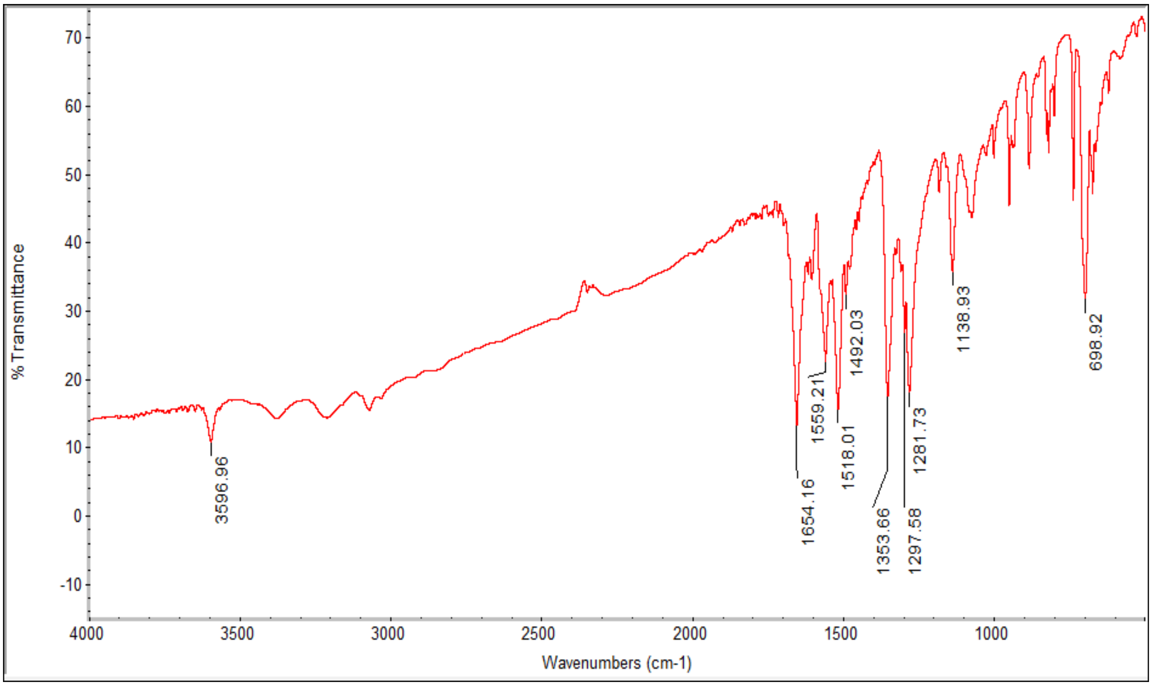
SUPPLEMENTARY INFORMATION (SI)



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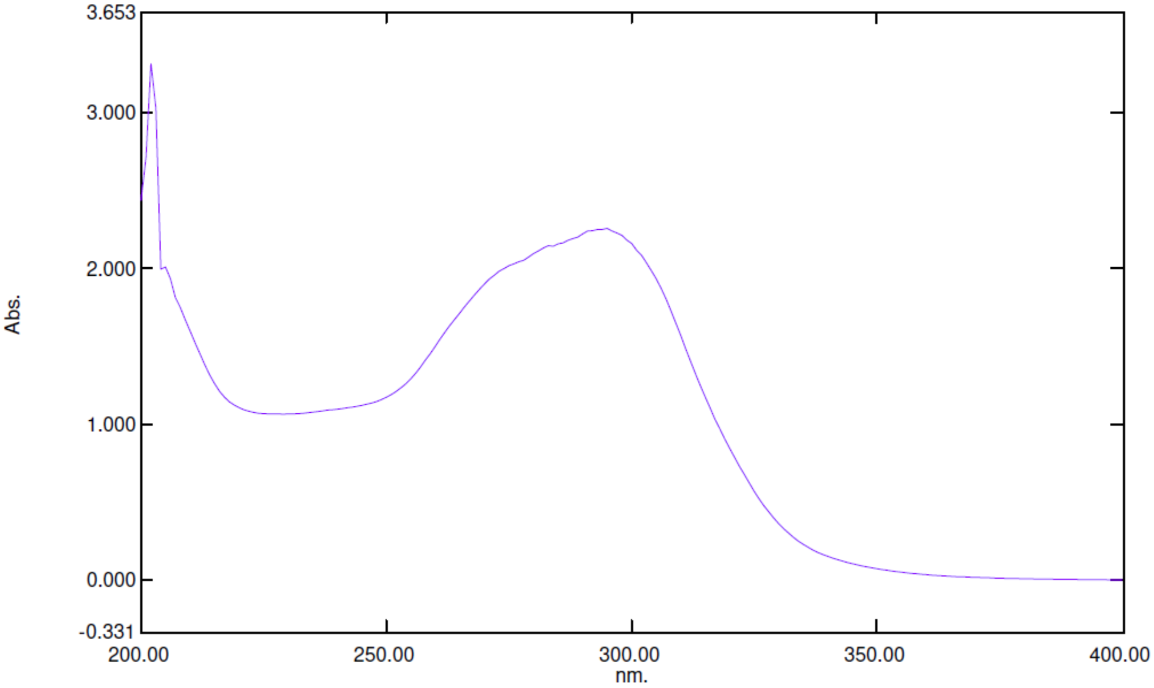

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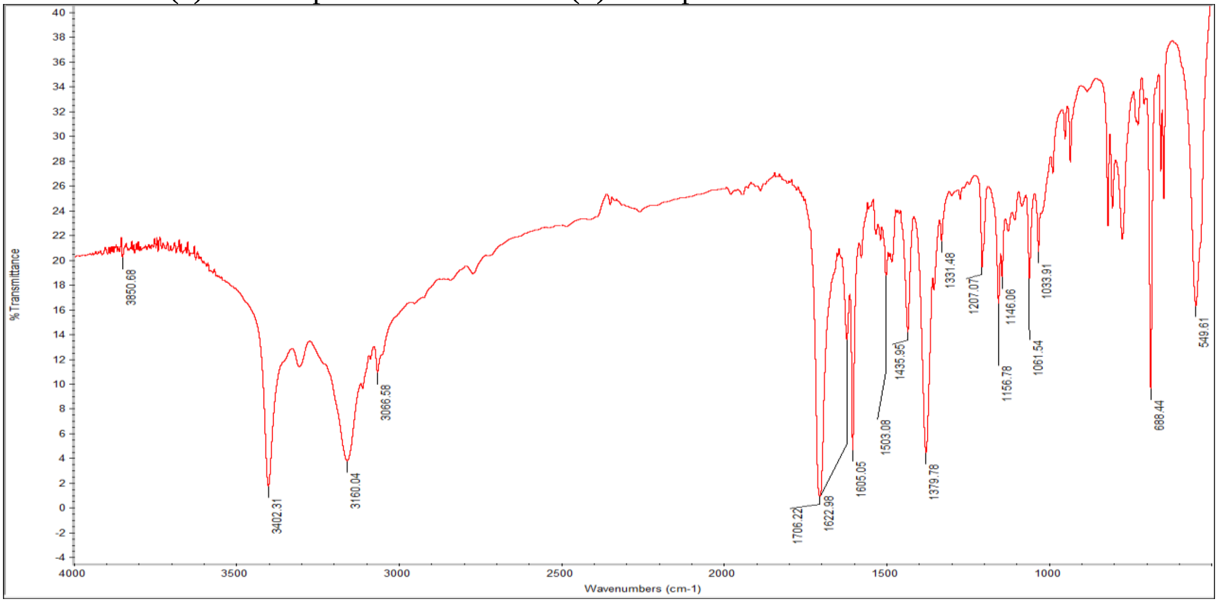
SI 1: (a) FT-IR spectrum of *m*-NBH (b) UV spectrum of MBA



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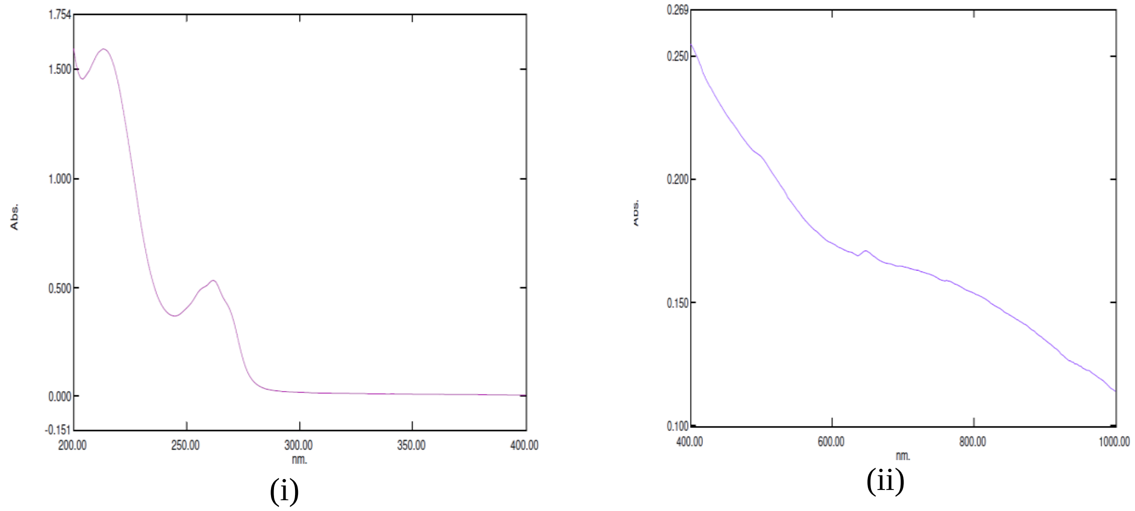

(i)

(ii)

(b)

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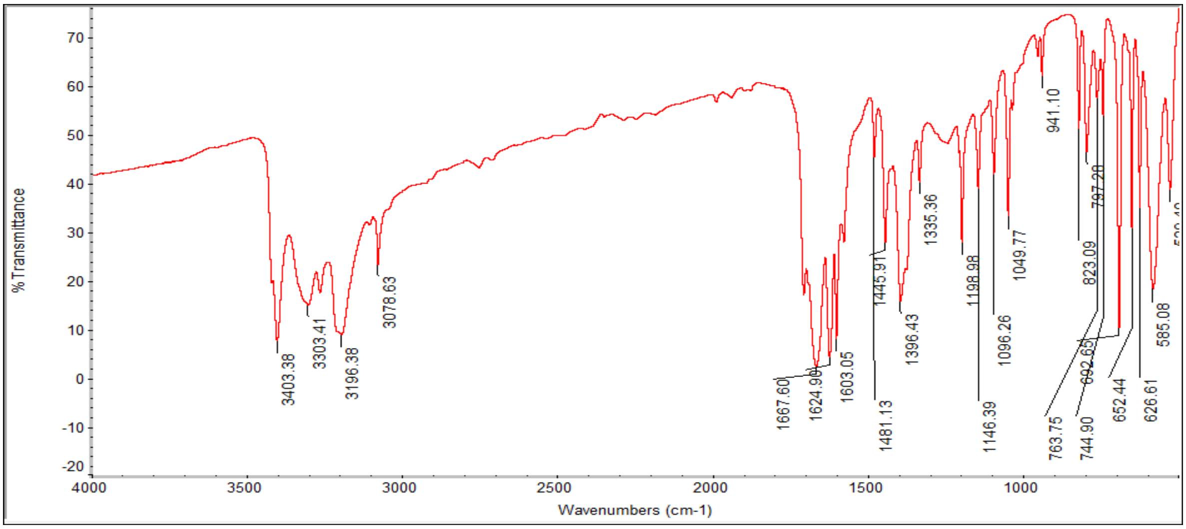

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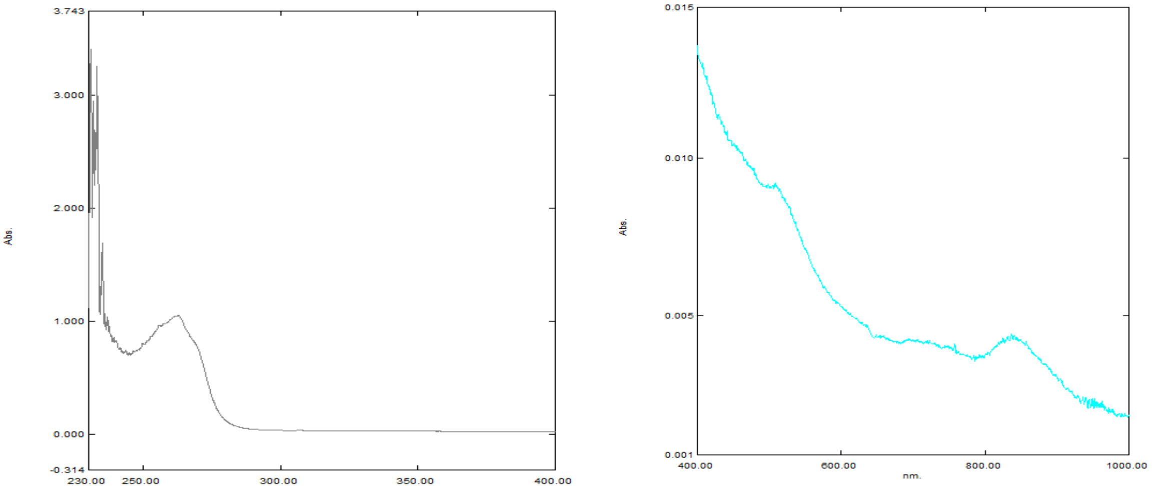

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(ii)

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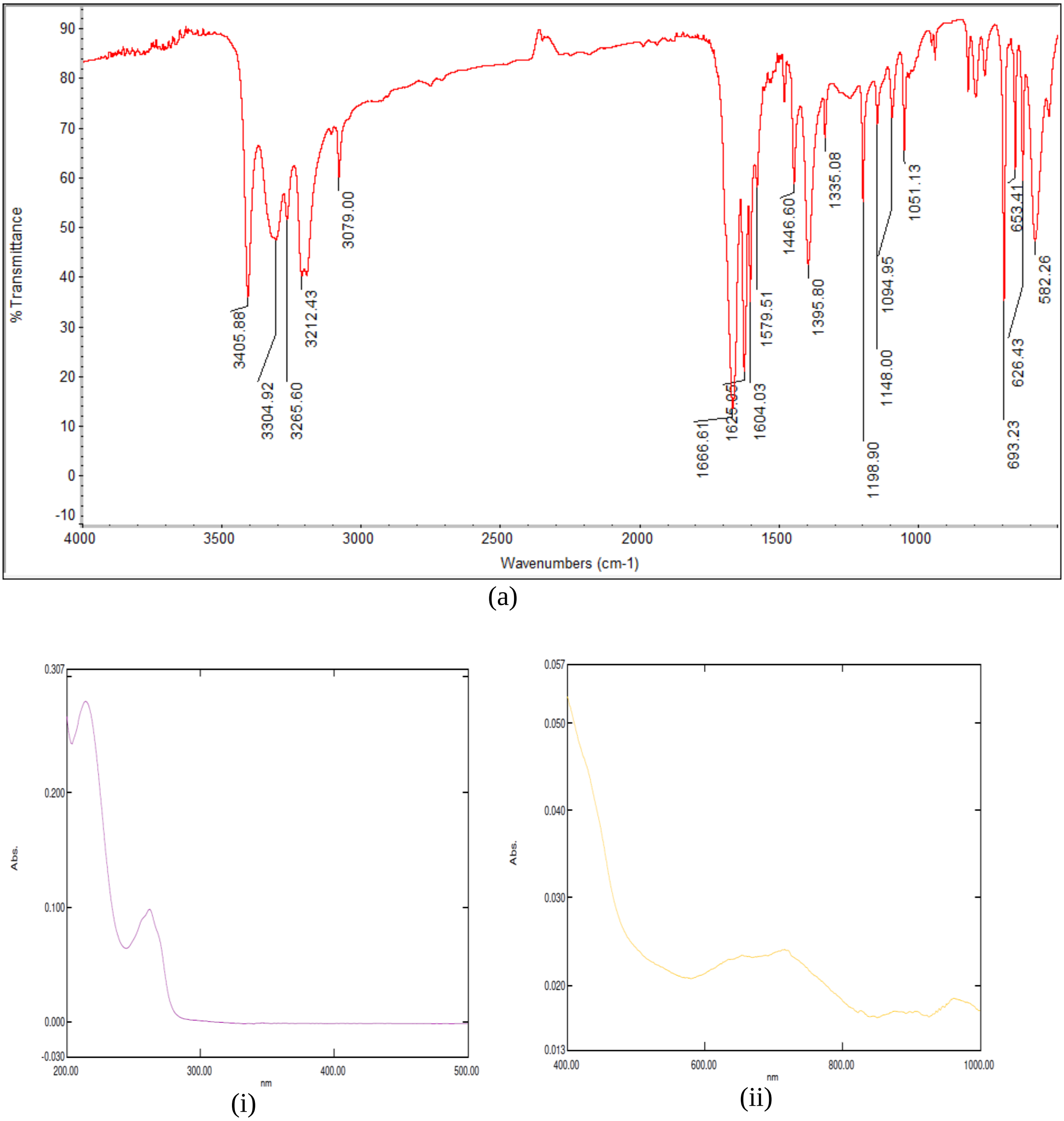

(a)

(i)

(ii)

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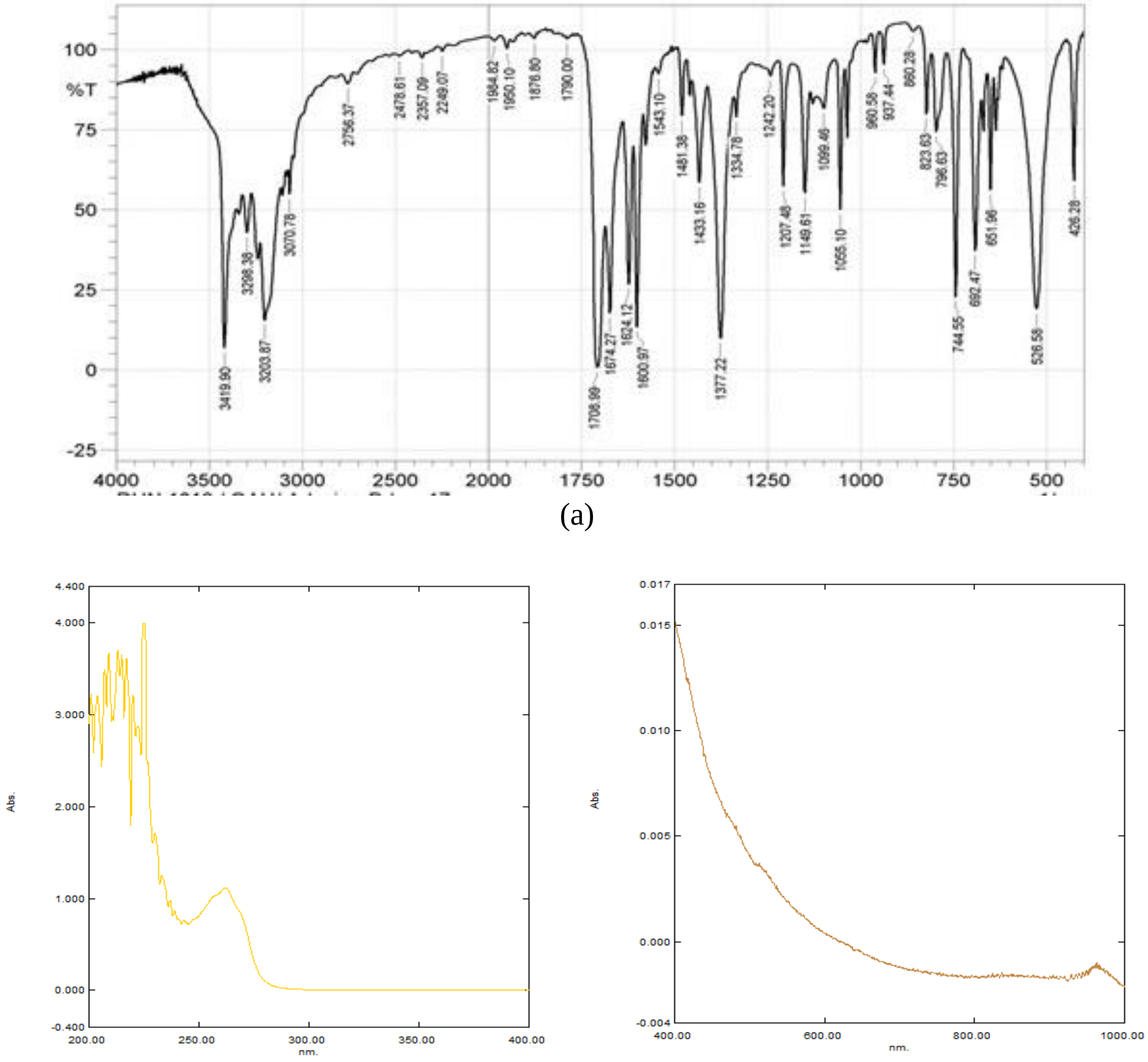

(a)

(i)

(ii)

(b)

SI 5: (a) FT-IR (bi) UV (bii) Visible spectra of [Fe(*m*-NBH)(NA)$_2$(H$_2$O)$_2$]Cl$_2$