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Synthesis, Characterization and Biological Profiles of Schiff Base Derived from Benzaladehyde and Phenylalanine and M^{3+} Complexes using Conventional Method

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Abstract

Schiff base ligand was synthesized by a condensation reaction between Benzaldehyde and Phenylalanine, using solid state method. The reaction was done in the ratio of 1:1 Metal : Schiff base. The Solid state method produced yield of ranges from 74-93%, and the complexes were found to have low molar conductance (4.30-15.20 $\Omega^{-1}cm^{-2}$) and the Schiff base have higher melting point than the metal complexes indicating higher stability. The ligand and its metal complexes were characterized by Fourier Transform-Infrared Spectroscopy (FT-IR), Ultraviolet-Visible Spectroscopy (UV-Vis), X-Ray Diffraction (XRD) and Thermogravimetric Analysis (TGA). Spectral results which had Ligand-Cr(III) (1639 cm^{-1}), ligand-Fe(III) (1618 cm^{-1}) and ligand -Co(III) (1685 cm^{-1}) revealed complexation arising from the Nitrogen of the azomethine group, Crystallographic pattern exhibited broader and less intense peaks which indicates reduced crystallinity and potentially smaller crystalline sizes. The thermal behaviour of the ligand and its metal complexes was studied at different temperatures. The Schiff base showed weight loss around 200°C and began decomposition at a slightly higher temperature (~302°C) and a two-step process. Antimicrobial activity was also observed with Schiff base and its M^{3+} Complexes on two bacterial and two fungal species. The Schiff base showed activity on Escherichia coli and Candida albica, while M^{3+} Complexes showed activity against bacteria and Candida albica but no effect on Mucor specie. In conclusion, the solid-state method offers more advantages including more convenience, higher yield, shorter reaction time, milder conditions, no pollution generation, and safer analyst experience.

Keywords: Antimicrobial, Benzaldehyde, Characterization, Ligand and Phenylalanine.

Introduction

The coordination chemistry of transition metal complexes has gained attention in recent years because of their wide range of applications in various fields of chemical and medical sciences and comprises a large body of bio-inorganic chemistry research [1,2]. It has motivated chemical researchers to design and synthesis novel metal complexes all over the world. Pharmaceutical research has expanded after the Second World War to include massive screening of microorganisms for new antibiotics because of the discovery of penicillin [2]. Schiff base are the compounds containing azomethine group (>C=N-). They are condensation products of ketones or aldehydes with primary amines and were first reported by Hugo Schiff in 1864 [3].

Nowadays, Schiff bases are used as intermediates for the synthesis of metal complexes having a series of different structure [4]. Schiff bases with aryl substituent are more stable and readily synthesized, whereas those containing alkyl substituent are relatively unstable [5]. Following the recommendation of IUPAC, Schiff bases are defined as chemical compounds (imines) bearing a hydrocarbonyl group on the nitrogen atom $R_2C = NR'$ ($R' \neq H$) they are considered by many to be synonymous with azomethines. Schiff base is the most familiar discovery of Hugo Schiff, after the novel work of Schiff in 1864, many researchers were involved in this research field and got success in the synthesis and structural design of new class of compounds.

The first metal-based drug that emerged in the late 19th century was cisplatin, whose discovery as a potent anticancer drug unlocked the search of metal-based chemotherapeutic agents. It was the most effective anticancer drug in the market [6]. The resounding therapeutic success of cisplatin and its analogs has triggered tremendous effort in the search for alternative metal-based chemotherapeutic agents in the past few decades. Since then the metal-drug interactions in the field of coordination chemistry have been focused well and considered as an active field of research [6]. Nowadays, the research field dealing with Schiff base coordination chemistry has been expanded enormously. The importance of Schiff base complexes for bioinorganic chemistry, biomedical application, supramolecular chemistry, catalysis and material science, separation and encapsulation processes, and formation of compounds with unusual properties and structures has been well-recognized and reviewed [7].

Their metal complexes have widely studied because they have anticancer and herbicidal applications [8]. They serve as models for biologically important species. Schiff bases are also used as versatile components in nucleophilic addition with organometallic reagents and in cycloaddition reactions [9]. In the present work, we report a synthesis, characterization, antibacterial and antifungal studies of a Schiff base derived from N-Benzylidene-Phenylalanine and its metal(III) Complexes using conventional method.

Materials and Methods

All the reagents used are of analytical grade and was used as purchased without purification. Glass wares were washed and dry in an oven at 110 ^{0}C overnight before used.

Distilled Water, Ethanol (JHD), Methanol (99.9 % Fisher Chemical^{AR}), Petroleum Ether (JHD), Chloroform, Acetone(JHD), DMF, DMSO (LOBA^{AR} CHEMIE PVT Ltd), $CrCl_3.6H_2O$ (LOBA^{AR} CHEMIE PVT Ltd),, $CoCl_3.6H_2O$

(LOBA^{AR} CHEMIE PVT Ltd), $FeCl_3.6H_2O$ (LOBA^{AR} CHEMIE PVT Ltd),, Benzaldehyde (JHD 98.5%), Phenylalanine (LOBA^{AR} CHEMIE PVT Ltd), H_2SO_4 (97 – 99 % assay, JHD^{AR}),, HNO_3, (LOBA^{AR} CHEMIE PVT Ltd), FTIR (Cary 630 Agilant Technolology, United State), XRD, TGA, UV-Vis (UnicoR 2800P, USA), Conductivity Meter (Jenway, model 4010,UK) Weighing Balance (aeADAM PW 184, AE 437531) Measuring Cylinder, Test Tube, Magnetic stirrer, Spatula, Dropper, Filter Paper, Funnel, Beakers Mortar and Pestle.

Preparation of Schiff Bases (Solid Method/Grind Stone)

Phenylalanine (8 g) was weighed and placed in a mortar and 10 ml of 0.1 M NaOH was added and grounded continually until the mixture became sticky. Then, benzaldehyde (10 ml) was added too and ground continuously; until the colour of the reactants turned into yellow, and a loose solid powder was obtained after about 30 mins. The reaction was carried out at room temperature [10]

Synthesis of the Complexes (Solid Method/Grindstone)

Grindstone preparation of the complexes was carried out by the equimolar (1:1) ratio (1.36 g, 5 mol) Schiff base ligands with 5 mol of $MCl_3.nH_2O$ where M= Cr^{3+}, Co^{3+} or Fe^{3+}) was ground in a mortar for about 10-12 minutes continuously, an alteration in the color of the reaction mixture was obtained. A coloured compound appeared indicating the formation of the product [10].

Antimicrobial Activity

The antimicrobial activity of the Schiff base and its complexes were carried out using two bacterial isolates: *Staphylococcus aureus* (gram positive) and *Escherichia coli* (gram negative) and two fungi: *Mucor specie* and *Candida albicans* using Agar well diffusion method. The solidified culture media was inoculated with the test micro-organisms and well was dug in the nutrient agar (NA) medium for bacteria and potato dextrose agar (PDA) medium for fungi with the help of a sterile cork borer [11]. The compound was dissolved (0.06 g) in DMSO (1.0 mL) to give a stock solution (500,000 µg/mL). Various concentrations of the test compounds was made (60, 30 and 15µg/mL) and introduced into the well by means of a micro syringe and incubated for 24 hours at 35 ^{0}C. The zone of inhibition was measured in mm [12].

Bacterial and fungal isolates was obtained from College of Agriculture Science and Technology and identified at Microbiology Laboratory, College of Agriculture Science and Technology, Jalingo, Taraba State. Nutrient Agar and Potato Dextrose Agar were used as media for the bacterial and fungal isolates respectively and the results are shown in Table 5 and 6.

Results and Discussion

Table 1: Physical properties of the Schiff base and the complexes

Compound	Method Used	Color	Percentage Yield %	Melting point	Conductive $\Omega^{-1}cm^{-2}$
Solid Method (a)					4.30
$C_{16}H_{14}NO_2$	Solid	Pale Yellow	93%	261^0C	
$[Cr(C_{16}H_{14}NO_2)]$	Solid	Mint green	75%	240^0C	10.30
$[Fe(C_{16}H_{14}NO_2)]$	Solid	Brown	77%	182^0C	13.60
$[Co(C_{16}H_{14}NO_2)]$	Solid	Dark green	74%	160^0C	15.20

Table 1: shows the physical properties of the Schiff base (N-Benzylidene – Phenylalanine, M.F $C_{16}H_{14}NO_2$) and their corresponding metal complexes. The reaction between phenylalanine and benzaldehyde produced a crystalline pale yellow colored Schiff base ligands with 93% yield, Cr (III) complex appeared mint green with 75% yield, Fe(III) complex shows brown color with 77% yield and Co(III) complex has 74% yield and appeared dark green. Both the synthesized ligands and metal (III) complexes shows excellent percentage yield in solid state method similar report by [13].

Table 2: UV- Visible spectra of the Schiff base

Ligand/ Metal Complexes	ƛmax (nm)	Assignment
Solid Method (a)		
$C_{16}H_{14}NO_2$	242.18	$\pi - \pi^*$
$[Cr(C_{16}H_{14}NO_2)]$	245.88	$\pi - \pi^*$
$[Fe(C_{16}H_{14}NO_2)]$	261.79	$\pi - \pi^*$
$[Co(C_{16}H_{14}NO_2)]$	233.8	$\pi - \pi^*$

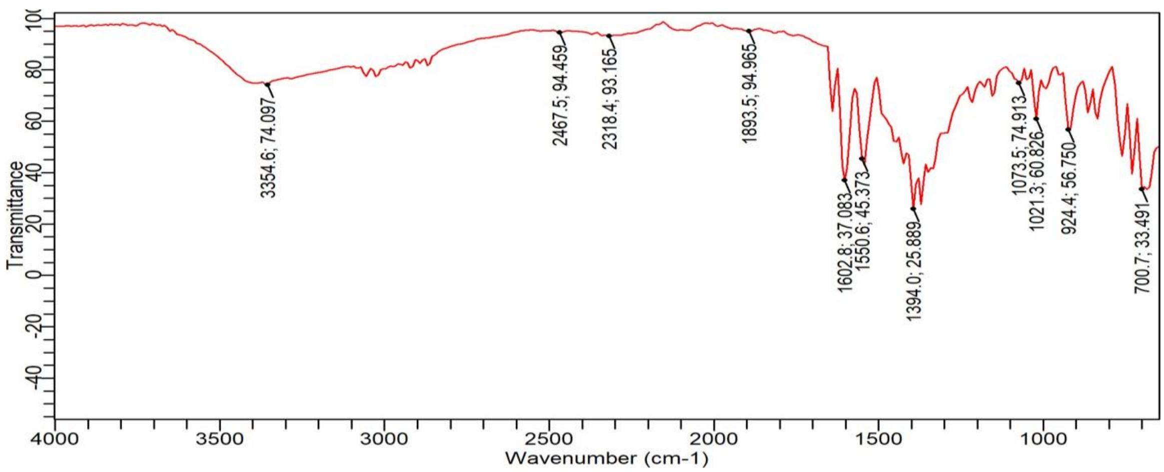

Figure 1: Schiff Base Solid State Method

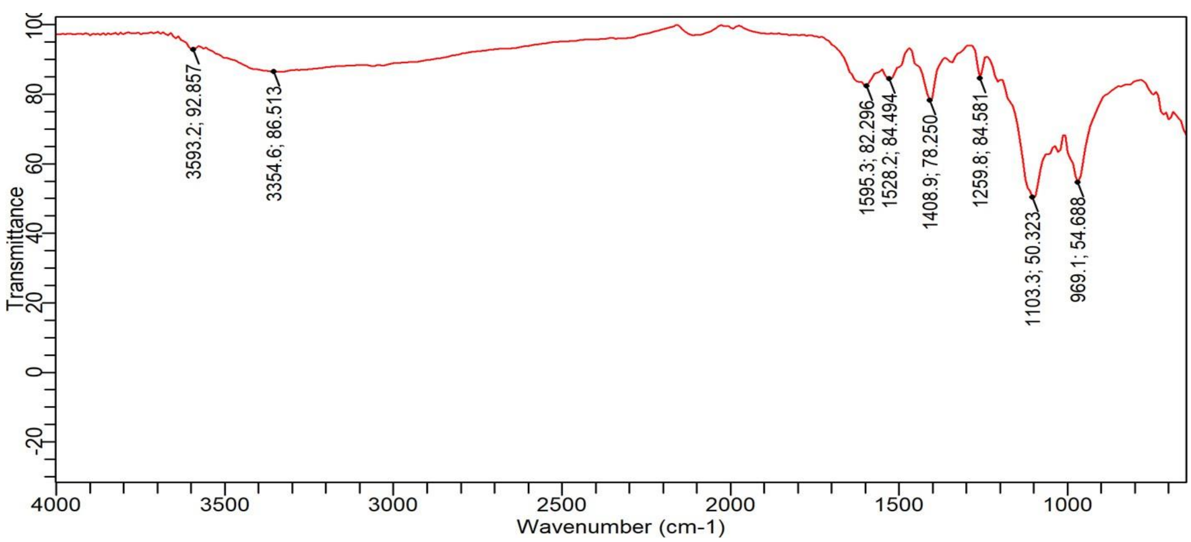

Figure 2: Chromium (III) Complex solid state method

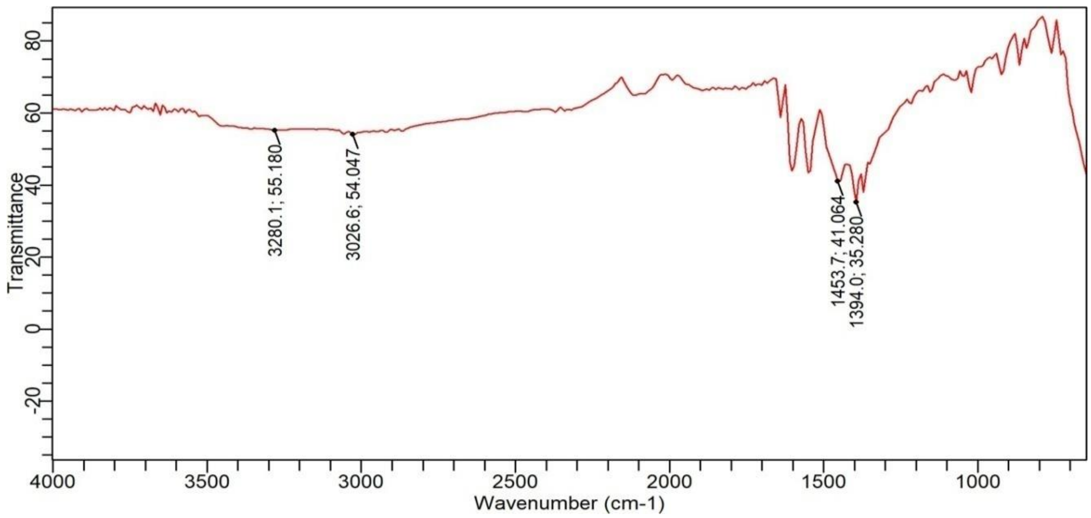

Figure 3: Iron(III) Complex solid state method

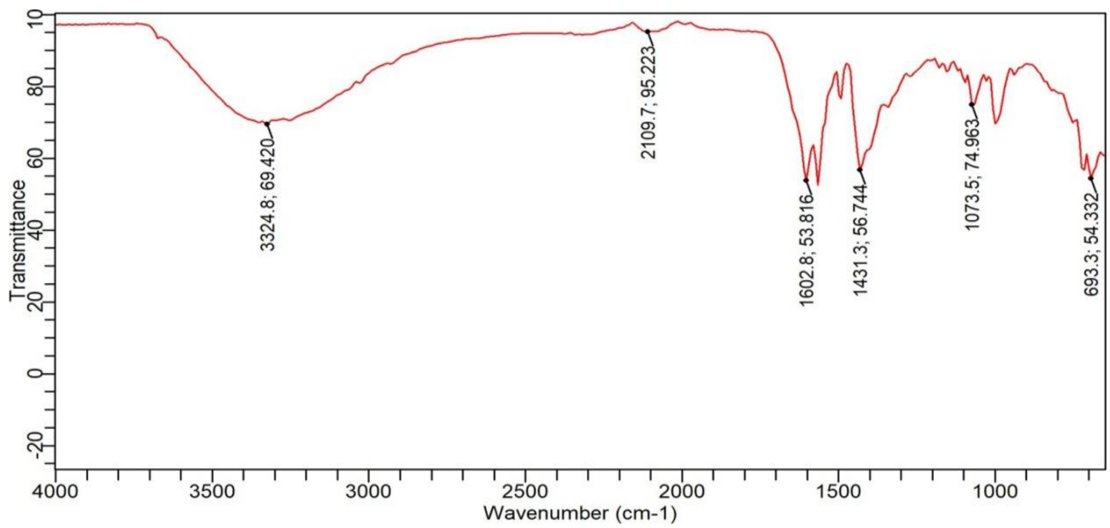

Figure 4: Cobalt(III) Complex solid state method

The UV – visible spectral of the synthesized Schiff base and metal complexes were taking within 200nm – 400nm the absorption region assignment of the Schiff base showed a broad band at 237.92 which is assigned to π – π* transition of the C=N Chromophore. On complexation, this band was shifted to higher wavelength of 245.88 nm for Cr (III) 261.79 nm for Fe(III) and lower wavelength of 233.81 nm for Co(III). The Schiff base also show a spectrum band at 242.18 nm, suggesting a coordination of imine nitrogen with central metal ions, this is in accordance to [14].

Figure 1-4 shows the absorption region assignment of the Schiff base and metal complex ions using liquid state method, the spectra of the ligand a sharp band appeared at 245.93 nm corresponding to π-π* transition of the C=N chromophore. Furthermore, on complexation this band was shifted to higher wavelength of 332.00 nm for Cr(III) and lower wavelength of 244.85 nm for Co(III). Suggesting a coordination of imine nitrogen with the central metal ion. In the spectra of Schiff base (Figure 1) band at 1686 cm^{-1} indicate the presence of the C=N I mine group this wavelength is typical for Schiff bases with an aromatic backline. The relatively high wavelength suggests a strong C=N bond, a similar report was recorded by [15].

Table 3: FTIR Spectra of some selected compound

Compound	V(C=N) cm^{-1} 1630-1690	V(C-H) cm^{-1} 3000 – 3100 Aromatic 2800 – 3000 Aliphatic	V(O-H) cm^{-1} 3200-3600	V(C-N) cm^{-1} 1020 – 1350	V(C=O) cm^{-1} 1650-1750
Solid Method (a)					
$C_{16}H_{14}NO_2$	1685	3023	-	1352	1641
[$Cr(C_{16}H_{14}NO_2)$]	1639	3023	-	1026	1676
[$Fe(C_{16}H_{14}NO_2)$]	1618		3417	1026	1734
[$Co(C_{16}H_{14}NO_2)$]	1685	3026	3326	1343	1719

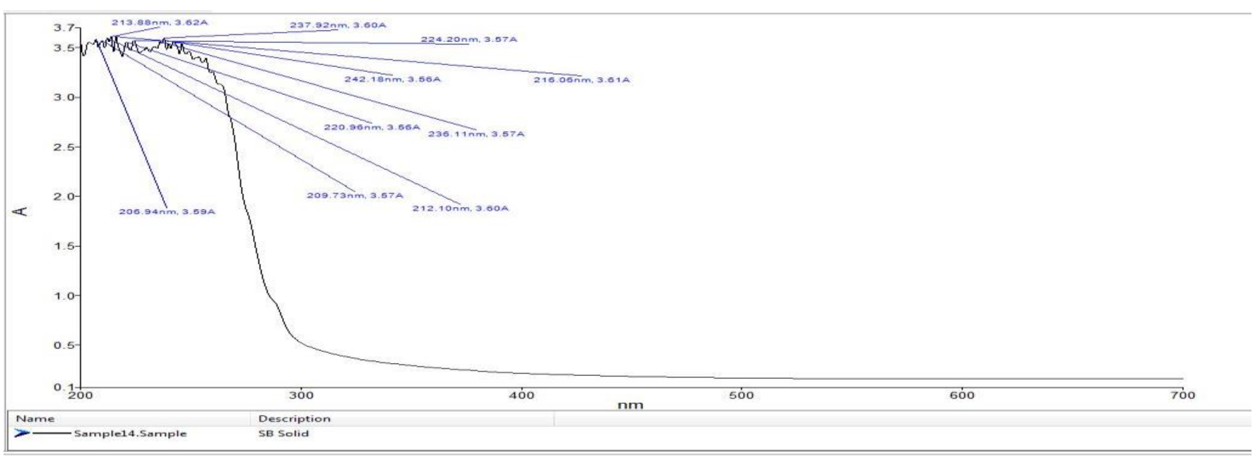

Figure 5: Infrared spectral of Schiff base solid state

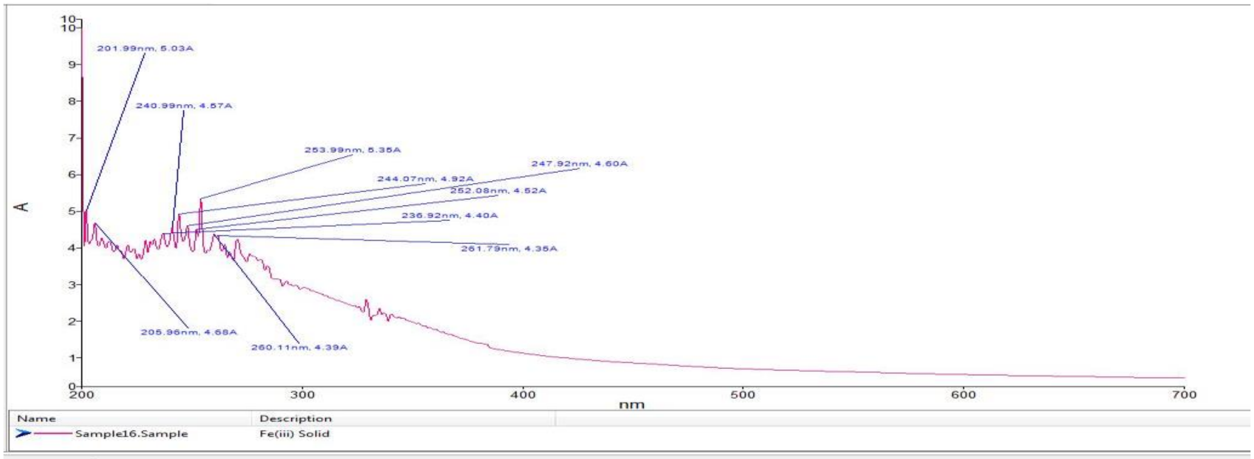

Figure 6: Infrared spectral of Iron(III) Complex solid state

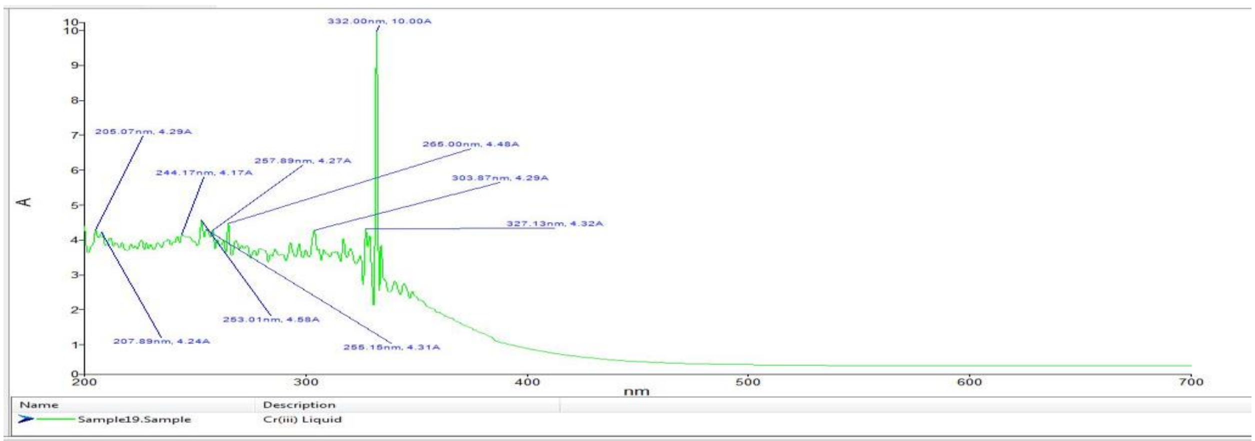

Figure 7: Infrared spectral of Chromium(III) Complex

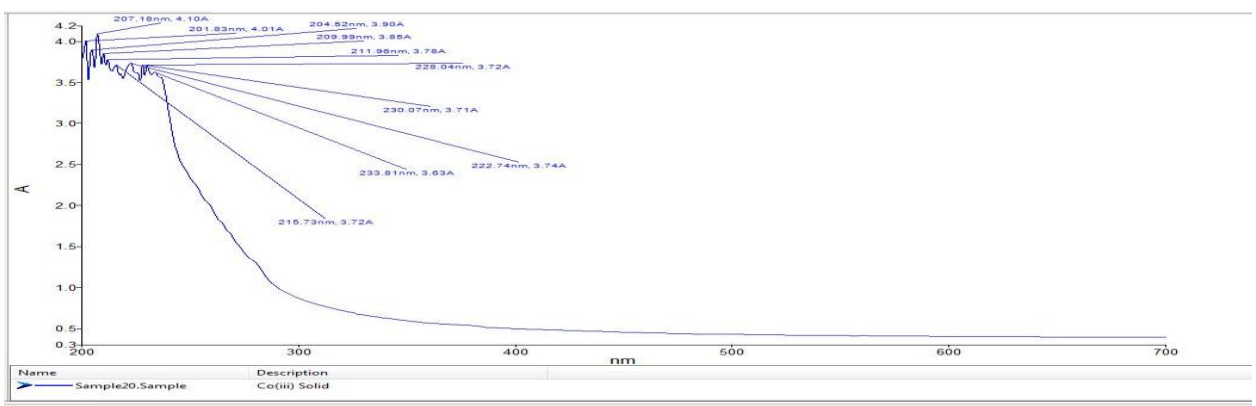

Figure 8: Infrared spectral of Cobalt(III) Complex solid state

The infrared spectral for the ligands and synthesized complexes for solid state (Figure 5 - 8) were recorded using KBr pellets in the range of 4000 – 450 cm^{-1} and provided relevant information with regard to the nature of the functional groups attached to the metal ions. The comparison of the IR spectral of the synthesized ligands and the metal complexes showed the binding mode of the ligands to the metal ions which is confirmed by the shift in the positions of the absorption peaks [15].

In the spectra of Schiff base (Figure 5) band at 1686 cm^{-1} indicate the presence of the C=N imine group, this frequency is typical for Schiff bases with an aromatic backbone. The relatively high frequency suggests a strong C=N bond. A shift to lower frequency for Cr(III) complex, has a high oxidation state, which reduces electron density on the C=N bond leading to a lower frequency. This shift also suggests a strong metal-ligand interaction because Fe(III) complex has a higher oxidation potential than Cr(III) complex, resulting in greater electron withdrawal from the C=N bond, this frequency suggest a more pronounced metal-ligand bonding. Co(III) complex shows a frequency similar to the free Schiff base that band at 1685 cm^{-1} (Figure 8) the complex has a lower oxidation potential than Cr(III) complex and Fe(III) complex, resulting in less electron withdrawal and this frequency suggest a relatively weaker metal-ligand interaction [16].

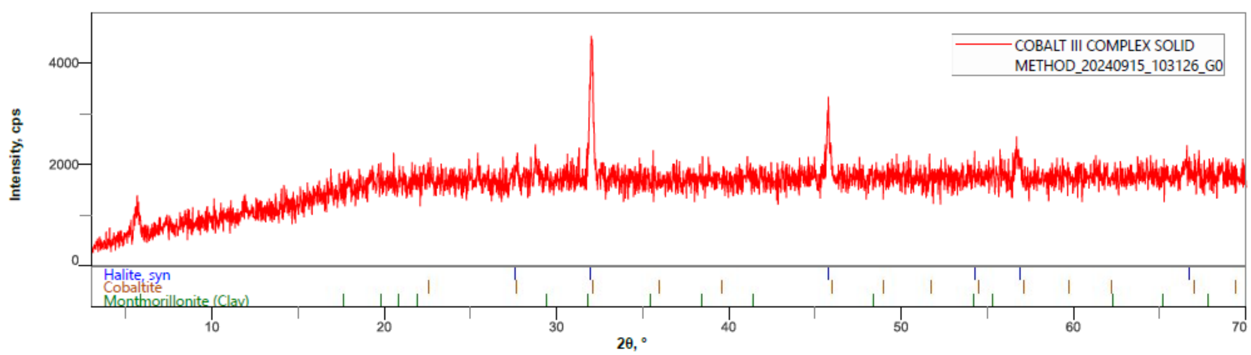

Figure 9: XRD pattern of Cobalt (III) complex prepared by solid method

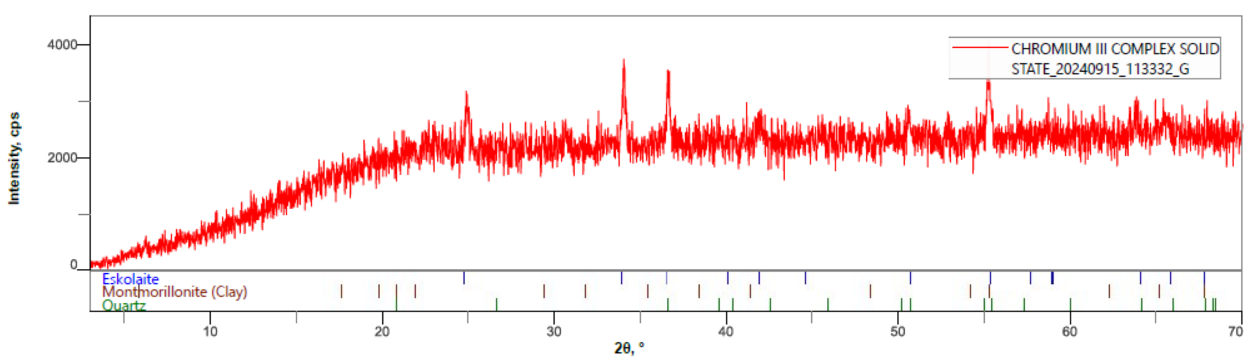

Figure 10: XRD pattern of Chromium (III) complex prepared by solid method

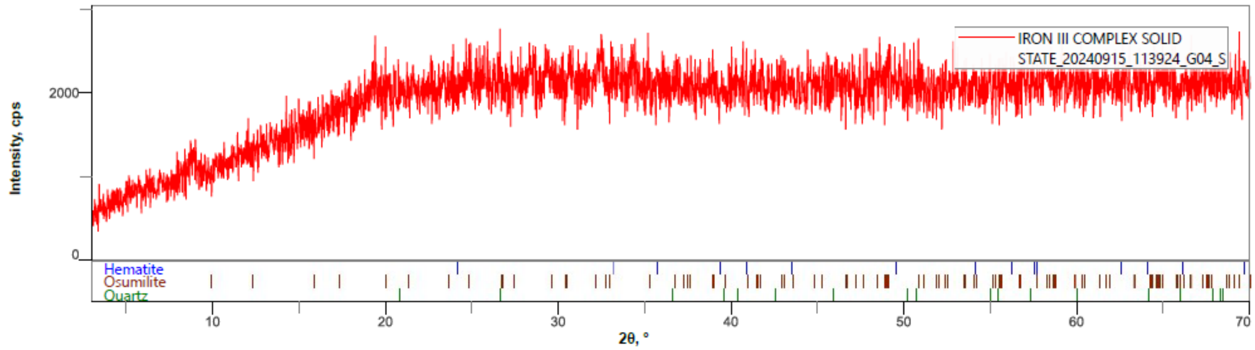

Figure 11: XRD pattern of Iron (III) complex prepared by solid method

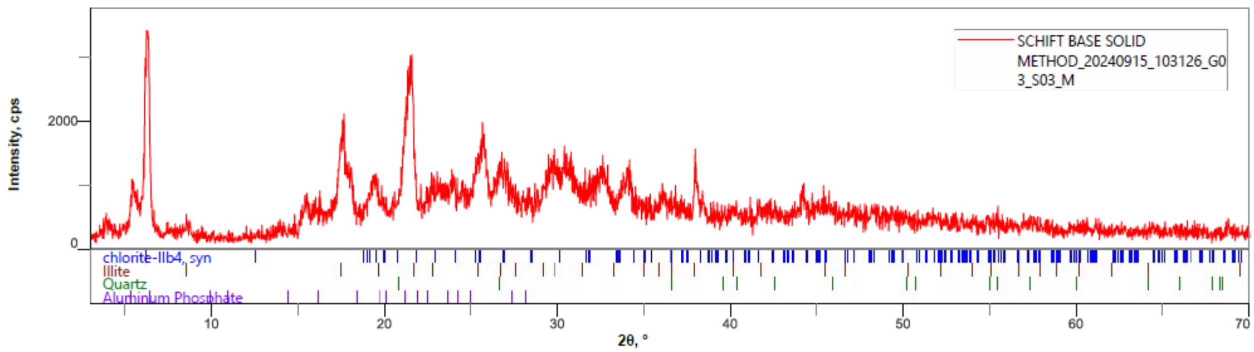

Figure 12: XRD pattern of Schiff base prepared by solid method.

Figures 9 – 12 present the XRD patterns of Co(III), Cr(III), and Fe(III) complexes, along with Schiff bases synthesized using solid-state methods. In Figures 9 – 12, the sharp and intense peaks, particularly at 2θ ≈ 10°, 20°, and 30° (notably in Figures 9 and 10), indicate better crystallinity and a more ordered structure. This suggests the formation of thermodynamically stable products, which are particularly suitable for applications requiring structural uniformity and phase purity. The higher intensity of the peaks in the liquid-phase synthesized samples reflects the dominance of crystalline phases, likely due to the controlled synthesis conditions. In liquid-phase synthesis, the reactants are well-dissolved, allowing for uniform crystal growth and a more ordered product [17].

Conversely, Figures 9 – 12 exhibit broader and less intense peaks, which indicate reduced crystalline and potentially smaller crystallite sizes. The broadening of peaks suggests the presence of structural imperfections or amorphous components, which are characteristic of solid-state reactions. These imperfections arise from kinetic constraints and the absence of solvent-mediated ordering during synthesis. Reduced crystallinity and the presence of mixed phases in the solid-state samples could impact material properties, potentially enhancing reactivity due to defects or smaller crystallite sizes as reported by [17,18].

Thermogravimetric Analysis (TGA)

Thermogravimetric analysis studies of the complexes formed was done to verify the presence of co-ordinated water molecule or water crystallization and relative thermal stability of the Schiff Base and its metal complexes of Cr(III), Fe(III) and Co(III). The formed TGA curves are given below.

Table 4: Thermogravimetric Analysis (TGA)

Samples	**Drying and loss of volatile gases**	**Pyrolysis at:**	**Trends in pyrolysis**	**Decomposition to ash at:**	**% weight loss before Ashing**	**% weight AfterAshing**	**Thermal stability**	**50% weight loss at:**
SBSS	~200ºC (< 2% wt loss)	~300ºC	2 steps decomposition	~540ºC	~82%	~10%	~300ºC	~450ºC
Co(III)CSM	~200ºC (< 5% wt loss)	~320ºC	2 steps decomposition	~560ºC	92%	< 5%	~320ºC	~450 ºC
Cr(III)CSS	~120ºC (< 5% wt loss)	~320ºC	Single step decomposition	~450ºC	~72%	< 10%	~320ºC	~400ºC
Fe(III)CSS	NE	~320ºC	2 steps decomposition	~560ºC	~95%	~5%	~320ºC	~450ºC

Key:
SBSS = Schiff Base Solid State

Co(III)CSM = Cobalt (III) Complex Solid Method

Cr(III)CSM = Chromium (III) Complex Solid State

Fe(III)CSS = Iron (III) Complex Solid State

NE = Not evident

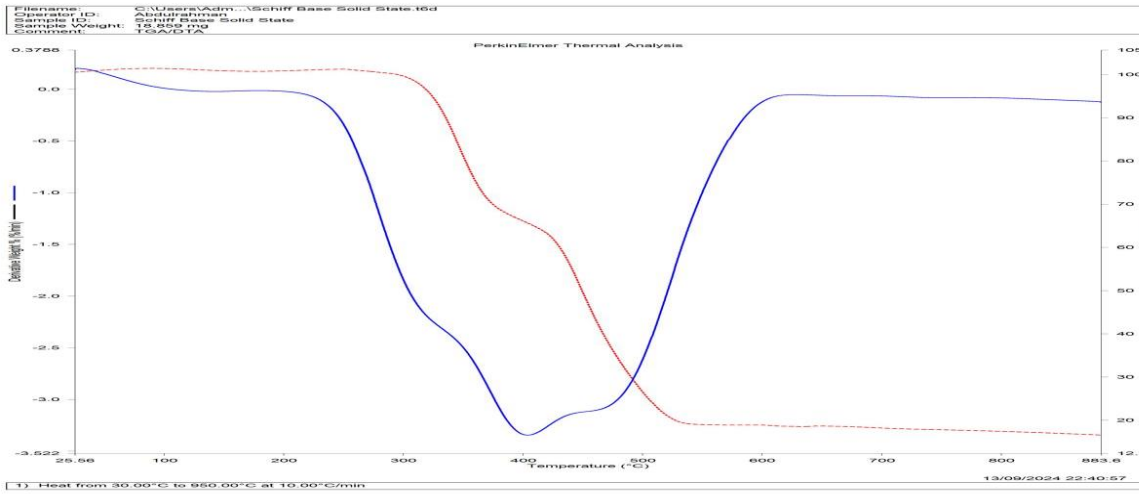

Figure 13: infrared spectra of the Schiff Base Solid State Method

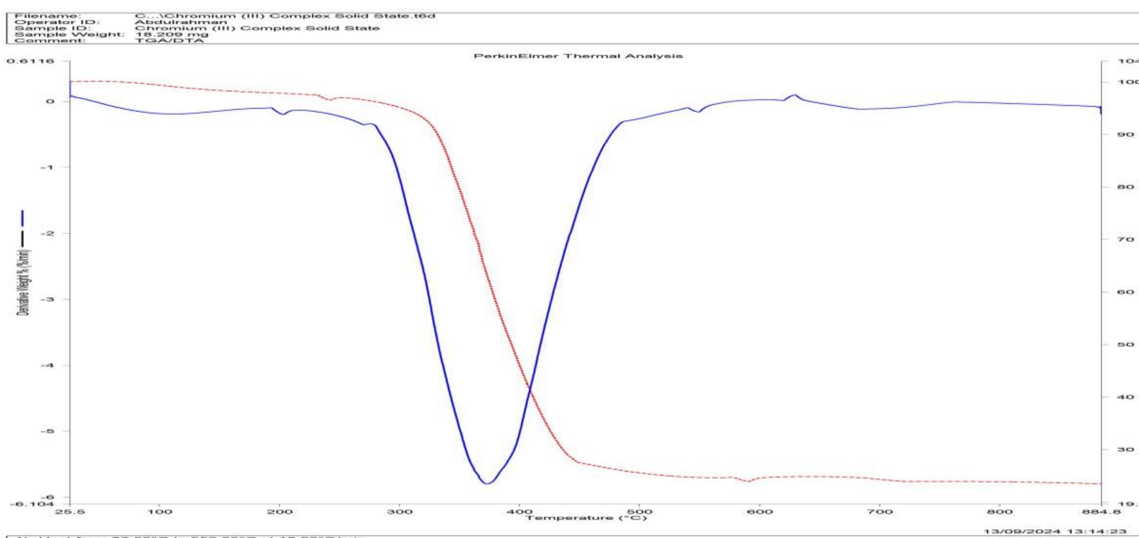

Figure 14: Thermogravimetric analysis for Chromium (III) Complex Solid State Method

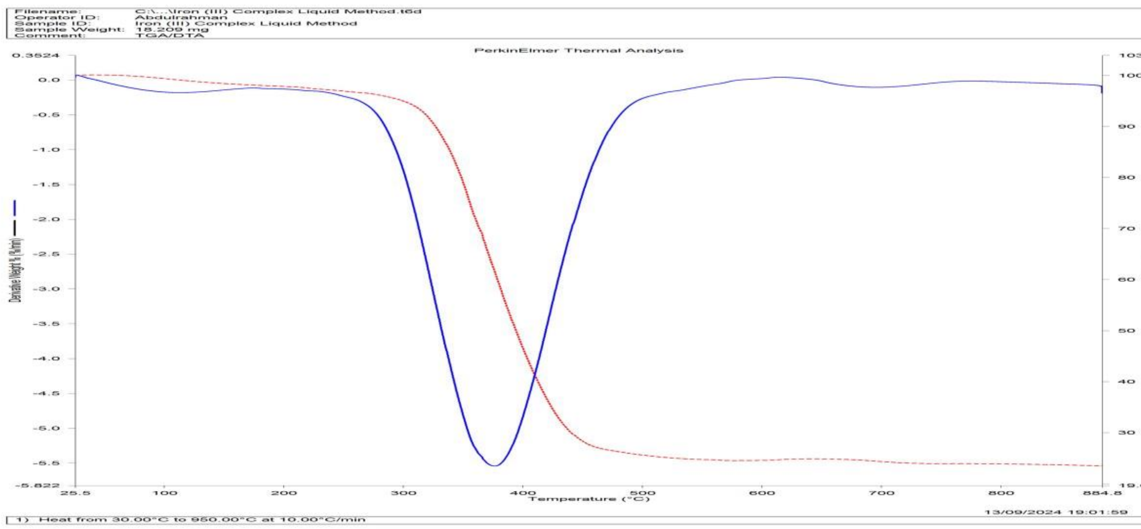

Figure 15: Thermogravimetric analysis for Iron (III) Complex Solid State Method

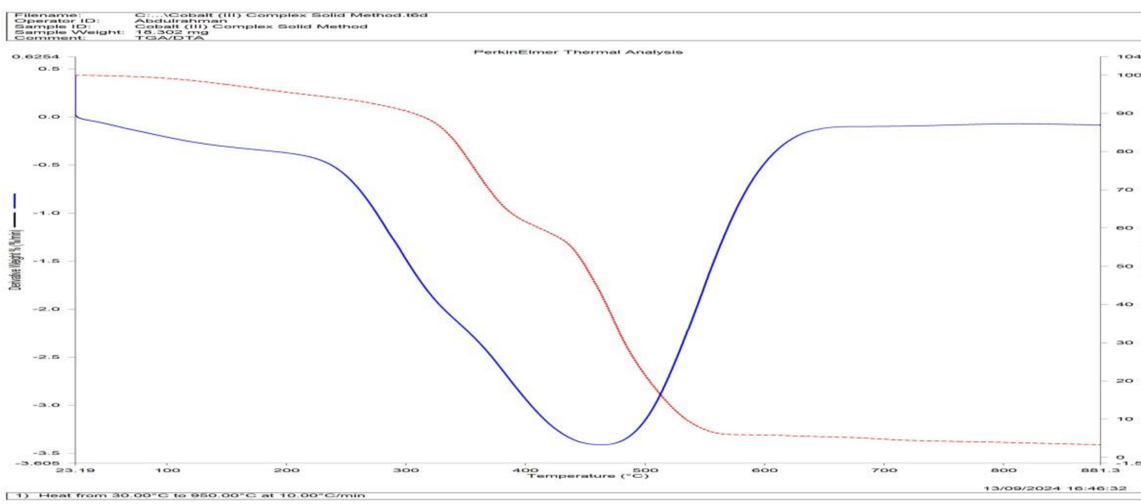

Figure 16: Thermogravimetric analysis for Cobalt (III) Complex Solid State Method

SBSS in Figure 13 starts to lose weight around 200°C, with minimal weight loss (< 2%), and undergoes two steps of decomposition at around 300°C, reaching significant weight loss by 540°C and corresponded to [19]. Figure 14 shows single-step and two-step decompositions, indicating varying thermal stability, this shows similar report by [19].- Fe(III)CSS (Figure 15) has a high weight loss percentage (95%) at elevated temperatures. The % weight loss before ashing and % weight after ashing provide insights into how much of the sample remains after combustion, which is essential for understanding the material's composition and stability. Co(III)CSM (Figure 16) exhibit weight loss at lower temperatures (~200°C) and have different patterns of weight loss and decomposition steps [20].

Thermal stability is evaluated by the temperature at which 50% weight loss occurs, indicating how resistant a sample is to thermal degradation. The data shows varying thermal stabilities across different samples, with some losing significant weight at lower temperatures and others remaining stable until much higher temperatures [21,22].

Antimicrobial Activities

Antibacterial of Schiff Base and its Metal (III) Complexes

Table 5: Antibacterial sensitivity test of the Schiff base and it metal (III) complex

Compound	***Zone of inhibition (mm) / concentration (µg/ml)***					
	Staphylococcus aureus			***Escherichia coli***		
	60	30	15	60	30	15
Ligand/Solid Schiff base	0	0	0	14	13	11
$[Co(C_{16}H_{14}NO_2)]$	16	15	11	14	10	0
$[Cr(C_{16}H_{14}NO_2)]$	23	18	15	16	13	10
$[Fe(C_{16}H_{14}NO_2)]$	20	16	12	15	12	10
Standard (Ciprofloxacin)	31	0	0	27	0	0

Table 5 shows the antibacterial activity of Schiff base and its metal (III) Complexes as carried out against bacterial isolate (*Staphylococcus aureus* and *Escherichia coli*) using ciprofloxacin (500 mg) as control. The Schiff base shows no activity against *Staphylococcus aureus* at all concentration, but shows activity against *Escherichia coli.* The three (3) metal complexes shows activity against the two bacterial isolates. The Cr (III) complex was found to be more active than both Co(III) complex and Fe(III) complex.

Antifungal Sensitivity Test of the Schiff Base and its Metal (III) Complexes

Table 6: Antifungal sensitivity test of the Schiff base and it metal (III) complex

Compound	***Zone of inhibition (mm) / concentration (µg/ml)***					
	Mucor specie			***Candida albican***		
	60	30	15	60	30	15
Solid Schiff base	0	0	0	26	23	13
$[Co(C_{16}H_{14}NO_2)]$	0	0	0	27	20	12
$[Cr(C_{16}H_{14}NO_2)]$	0	0	0	30	27	15
$[Fe(C_{16}H_{14}NO_2)]$	0	0	0	20	16	11
Standard (Ketoconazole)	35	0	0	37	0	0

Table 6 Shows the antifungal activity of Schiff base and its metal (III) Complexes as carried out against fungal isolate (*Mucor specie* and *Candida albica*) using ketoconazole (200 mg) as control, which indicates that the Schiff base ligand and its metal (III) complexes shows no activity against

Mucor specie at all concentration with a control having 35 mm inhibition, but are active against *Candida albica* with control having 37 mm inhibition. The Cr (III) comp

Conclusion

Schiff base ligand was synthesized by a condensation reaction between Benzaldehyde and Phenylalanine, using solid state method. The reaction was done in the ratio of 1:1 Metal : Schiff base. The Solid state method produced yield of ranges from 74-93%, and the complexes were found to have low molar conductance (4.30-15.20 $\Omega^{-1}cm^{-2}$) and the Schiff base have higher melting point than the metal complexes indicating higher stability.

The ligand and its metal complexes were characterized by Fourier Transform-Infrared Spectroscopy (FT-IR), Ultraviolet-Visible Spectroscopy (UV-Vis), X-Ray Diffraction (XRD) and Thermogravimetric Analysis (TGA). Spectral results which had Ligand-Cr(III) (1639 cm^{-1}), ligand-Fe(III) (1618 cm^{-1}) and ligand - Co(III) (1685 cm^{-1}) revealed complexation arising from the Nitrogen of the azomethine group, Crystallographic pattern exhibit broader and less intense peaks which indicates reduced crystallinity and potentially smaller crystalline sizes. The thermal behaviour of the ligand and its metal complexes was studied at different temperatures. The Schiff base showed weight loss around 200°C and began decomposition at a slightly higher temperature (~302°C) and a two-step process. Antimicrobial activity was also observed with Schiff base and its M^{3+} Complexes on two bacterial and two fungal species. The Schiff base showed activity on *E. coli* and *C. albica*, while M^{3+} Complexes showed activity against bacteria and *C. albica* but no effect on M. specie.

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