



## Synthesis, Characterization, Kinetic, Thermodynamic and Antimicrobial Activity Studies of Complexes of Cd(II), Cr(III), and Zr(IV) derived from Benzaldehyde and Ethylenediamine

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### ABSTRACT

A Schiff base ligand N,N'-Bis(Benzoin)ethylenediimine (B<sub>2</sub>ED) obtained from benzaldehyde and ethylenediimine and its Cd(II),Cr(III) and Zr(IV) complexes was synthesized and characterized using electronic spectra (ultra-violet visible spectroscopy), infrared spectra (vibrational spectroscopy), molar conductivity measurement, solubility test and melting point determination. The ligand and the metal complexes were insoluble in the solvents distilled water, hexane, aqueous ammonia, potassium hydroxide and sulphuric acid but soluble in acetone, glacial acetate, DMSO and DMF. The conductance data showed that both the ligand and the metal complexes exhibited weak ionic (non-electrolytic character) while the melting point for ligand and decomposition temperature for complex showed they are fairly stable. The IR spectrum of B<sub>2</sub>ED showed characteristic vibrations of C=N and OH groups at 1677 and 3377 cm<sup>-1</sup>, respectively. In the spectra of Cd<sup>2+</sup>, Cr<sup>3+</sup> and Zr<sup>4+</sup> complexes, the azomethine band ( $\nu_{C=N}$ ) was shifted to lower wave number (1630–1634 cm<sup>-1</sup>) whereas, the  $\nu(OH)$  is shifted by 18–48 cm<sup>-1</sup> to lower frequency upon complex formation suggesting involvement of C=N and OH groups in coordination. The electronic spectra showed that the metal complexes exhibited single, double or triple bands which are consistent with the octahedral and tetrahedral geometry for the metal complexes. The Kinetics of the complex formation were studied by determining the amount of complex formed with time at different temperatures of (25, 35, 45, 55 and 65 °C). The kinetic results showed that increase in time and temperature respectively increased the yield of the complexes. The activation energies of the reactions were in the range of 12.288-18.707 kJ mol<sup>-1</sup>. The high values of the activation energies reflect the thermal stability of the complexes. The metal complexes exhibited antimicrobial performances, such as low minimum inhibitory concentration (MIC ≤ 250) and bactericidal effect than the ligand. These could become promising antimicrobial agents with potential applications in pharmaceutical industry for controlling the pathogenic bacteria.

**Keywords:** Antimicrobial studies, kinetics, Schiff base Complexes, thermodynamics

### INTRODUCTION

Annually, millions of people die due to infections caused by microorganisms resistant to current antibiotics (WHO, 2012). When an antibiotic is discovered and commercially available, the appearance of resistant strains begins to reduce its clinical utility after a period of indiscriminate use, leading to future use restriction (Rocha *et al.*, 2011). Microbiologists acknowledge that Gram-negative bacteria own mechanisms specialized in the extrusion of strange substances out of the cell (efflux bomb), limiting the access of antimicrobial agents to its active site. Consequently, it prevents the accumulation of antibiotics in the interior of the cell, and inhibits the action of antimicrobial agents (Reichling *et al.*, 2005). Furthermore, the enzymes are known to be capable of rendering drugs inactive ( $\beta$ -lactamases), making the cell resistant to them in their periplasmic space (Silva *et al.*, 2011). In the search

for new antimicrobes effective in the treatment of infections caused by multi resistant bacteria, due consideration ought to be given for the synthesis of drugs with new activation targets, as well as the potentialization of the activity of compounds with known antimicrobial activity (Schaechter *et al.*, 2002; Masunari and Tavares, 2006). A new strategy in the production of drugs proposes the interaction of metal ions for antibiotics within three study fields: the first one aiming at creating a reversed mechanism of microbial resistance; the second one seeking to promote the development of new drugs with an action mechanism unknown to the pathogenic bacteria; and a third one aiming at reducing the toxicity of the metal ion in the form of a complex (Rocha *et al.*, 2011).

The interaction of metal ions with organic ligands shows better antimicrobial activity compared to free ligands (not coordinated), and as such, it justifies the investigation of new drugs with

unknown mechanism of action against pathogenic bacteria. The use of these new compounds is likely to have great potential against pathogenic bacteria; nonetheless, the need for new methodologies of evaluation of antimicrobial activity cannot be relegated to the background (Mamidala and Gujjeti, 2013; Soniya *et al.*, 2013).

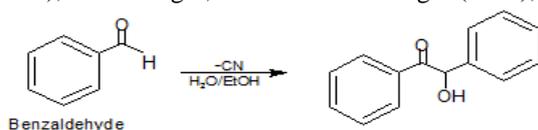
A survey of the literature reveals that some works have been carried out on the synthesis, characterization and antimicrobial activities of complexes of transition metals derived from N,N-Bis(Benzoin)ethylenediimine, other than the ones used here, but few work have been done on their kinetic and thermodynamics which is the study of their rate of formation and stability (Mamidala and Gujjeti, 2013; Soniya *et al.*, 2013).

This research study is limited to the synthesis, characterization, kinetic, thermodynamic and the antimicrobial activity studies of complexes of  $Cd^{2+}$ ,  $Cr^{3+}$ , and  $Zr^{4+}$  derived from N,N-bis(benzoin)ethylenediimine.

## MATERIALS AND METHODS

### Reagents and solvents

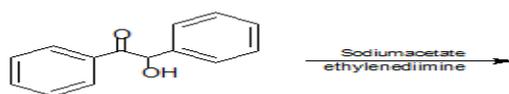
The following reagents and solvents used were: ethyl alcohol, distilled water, benzaldehyde, sodium cyanide, ethylenediimine, Cadmium (II) chloride hemihydrate ( $CdCl_2 \cdot 5/2H_2O$ ) [BDH], Chromium (III) chloride heptahydrate ( $CrCl_3 \cdot 7H_2O$ ) [BDH], Hydrated Zirconyl Nitrate ( $ZrO(NO_3)_2 \cdot 3H_2O$ ) [BDH], sodium hydroxide, methanol, sodium acetate, ammonia, ether, dimethylsulphoxide (DMSO), dimethylformamide (DMF), nutrient agar, Potato Dextrose Agar (PDA),



Scheme 1: Synthesis of Benzoin

### Synthesis of N, N'-Bis(benzoin)ethylenediimine Ligand

To a methanolic solution of benzoin (8.48 g) and ethylenediimine (2.40 mL) was added anhydrous sodium acetate (16.00 g) and the mixture refluxed for 3600 seconds. The hot solution was poured into



Scheme 2: Synthesis of N,N-bis(benzoin)ethylenediimine

## CHARACTERIZATION OF LIGAND AND COMPLEX

### Synthesis of N, N'-Bis(benzoin)ethylenediiminato (B<sub>2</sub>ED) Metal Complexes

Exactly 0.002 mol of the hydrated metal salts were dissolved in 25 mL of hot absolute ethanol and 1.79 g (0.004 mol) of N, N'-

Acetone, Salmonella shigella Agar (SSA). All the chemicals were of analytical grade with sigma Aldrich. These chemical were used immediately as they arrived.

### Apparatus and instrument

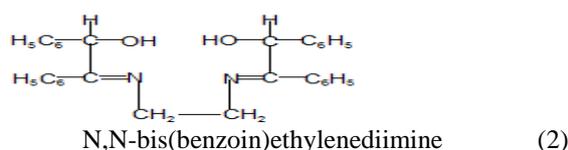
The apparatus and instruments used include: Barnstead electro thermal BI9100 melting point apparatus with digital thermometer, pH/Conductivity series 510 conductivity meters, ProRaman-L-785-B1S FT-IR spectrophotometer, Carry 630 FT-IR spectrophotometer, Shimadzu 1800UV-Visible spectrophotometer, SB160 heat-stirrer, PW 184 weighing balance, 98-1-B temperature regulating heating mantle, and OV/100/F ovum.

### Synthesis of Benzoin

To a 500 mL round bottom flask was added 65 mL of rectified spirit, 47.5 mL of benzaldehyde and a solution of 5 g of sodium cyanide in 50 mL of water. Few anti-bumping granules were introduced into the flask. A condenser was then attached and the mixture was refluxed on a steam bath for 1800 seconds. The round bottom flask and its contents were cooled in an ice-bath and pale yellow crystals were observed. These were filtered, washed with cold water, dried and then recrystallized from 40 mL of hot ethanol. The crystals were dried at 50 °C and weighed (Soniya *et al.*, 2013). The equation for the reaction is as given in Scheme 1:

(1)

ice-cold water where upon yellow precipitate of the schiff's base separated: it was filtered, washed with water, dried and recrystallised from ethanol (Soniya *et al.*, 2013). The equation for the reaction is as given in Scheme 2:



(2)

Bis(benzoin)ethylenediimine the Schiff base dissolved in 10 mL of hot absolute ethanol. The metal salt solutions were added with constant stirring to the solution of the N, N'-Bis(benzoin)ethylenediimine the Schiff base and heated on SB160 heat-stirrer for about 7200 seconds using magnetic stirrer. The mixture was poured into a beaker and left overnight to

crystallise. The resulting crystals were recrystallized with a minimum quantity of ethanol. The final crystals obtained were dried over OV/100/F ovum at 50 °C and weighed (Waeel, 2013).

### Solubility Test

The solubility test of the ligand and the metal complexes was carried out in different solvents such as water, ethanol, methanol, acetone dimethylsulfoxide, dimethylformamide and other common organic and inorganic solvents by shaking a small amount of each compound in a test tube containing 10 mL of the solvent. The entire solute dissolved to give a homogenous mixture after shaking the sample (S). However, some samples were slightly soluble (SS) and some were not soluble (NS).

### Melting Point Determination

The Schiff base with the metal complexes was put in separate capillary tubes and inserted into the heating block of the Barnstead Electrotherma (9100) melting point apparatus and was heated separately and the temperature at which each of the samples melted was read from the digital screen.

### The Molar Conductivity Measurement

The molar conductivities of the complexes were obtained from DMSO using pH/Conductivity series 510 conductivity meters.

### Infrared spectra studies for the ligand and their complexes

The infrared spectra data of the synthesized Schiff base alongside with the complexes was obtained by using carry 630 FT-IR spectrophotometer for the Schiff base ligand while proRamman-L-785-B1S FT-IR spectrophotometer was used for the complexes.

### Spectroscopic electronic spectra studies for the ligand and their complexes

The electronic spectra of the ligand and the complexes was obtained using Shimadzu UV-1800PC series Spectrophotometer in solution state in a 190 to 800 nm wavelength range.

### Kinetics and Thermodynamic Studies

The kinetic study of the complex was done by mixing an accurate amount of 4.0 mmole of N, N'-Bis(benzoin)ethylenediimine the Schiff base dissolved in 20 mL of absolute ethanol with 4.0 mmole of the salts treated separately in 20 mL of ethanol in 100 mL beaker. The mixture was placed on a SB160 heat-stirrer. The stirrer was set at the required rpm and the mixture was heated at temperatures of 25, 35, 45, 55 and 65 °C, this was maintained for the required time of 10, 20, 30, 40 and 50 minutes for each of the different temperatures used. The precipitates obtained were filtered off, washed with hot ethanol, ether and

dried under vacuum at 80°C and weighed. The various mass obtained were analysed to ascertain the yield in mol/dm<sup>3</sup>. A graph of concentration against time was plotted to obtain the observed rate constant of each of the various temperatures.

The data from this experiment was used to create a plot of  $\ln k_{obs}$  vs.  $1/T$ , to obtain activation energy ( $E_a$ ) and  $\ln(K_{obs}/T)$  vs.  $1/T$  to obtain thermodynamic parameters.

### Antimicrobial Studies

The antimicrobial studies were done in the microbiology laboratory of University of Agriculture, Makurdi using the Agar-well diffusion technique.

### Antibacterial Susceptibility Testing

The bacterial cultures were incubated at  $30 \pm 0.1$  °C for 24 hours by inoculation into nutrient agar. The ciprofloxacin (standard), Schiff base and metal complexes were stored dry at room temperature and dissolved 100 mg/mL in dimethylsulfoxide (DMSO). Antibacterial activities of each compound were evaluated by Agar-well diffusion technique described by Aliyu *et al.*, (2012). Mueller Hinton Agar Media (15 mL) kept at 45 °C was poured in the sterile petri dishes and one mL of the broth inoculums (microbial species inoculated in normal saline and incubated 24 hrs), dispensed into a sterile Muller Hinton Agar (Himedia, India) was poured into the plate and mixed properly (pour-plate method) and allowed to solidify/gel. A sterile cork borer, 6 mm in diameter was used to bore wells on the plate and 20  $\mu$ L of the stock antimicrobial agent was dispensed into the wells and labelled properly. The plates were allowed to stand for 30 minutes to allow the antimicrobial agent diffuse into the agar. Poured Petri plates (9 cm) were incubated with 20  $\mu$ L of normal saline solution of above culture media ( $10^5$ - $10^6$  bacteria per mL). The Petri plates were placed at 37 °C for 24 hours. At the end of period, the inhibition zones formed on media were measured with a zone reader in millimetres and recorded.

### Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal and Fungicidal Concentrations (MBC/MFC)

The MIC of an antimicrobial agent against a microbe (bacteria, fungi etc) is the minimum concentration of the antimicrobial agent that will prevent (inhibit) replication of the microbes while the MBC and MFC were defined as the lowest concentration of the compound capable of preventing the microbial growth in culture medium.

The procedures to determine the MBC and MFC were initiated concomitant with the procedures for the determination of the MIC. The MIC was carried-out based on the method described by Aliyu *et al.*, (2012) using Agar dilution technique. Varying concentrations of the antimicrobial agents were prepared in an agar by

mixing different proportions of the Mueller Hinton agar and the stock solutions of the antimicrobial agents. The following concentrations were done; 6 mL : 6 mL, 3 mL : 9 mL, 1.5 mL : 10.5 mL and 0.75 mL : 11.25 mL of antimicrobial agent and Mueller Hinton agar respectively, thereby, giving the following percentage concentration; 50 %, 25 %, 12.5 % and 6.25 % respectively. The test organisms were spot inoculated on all the concentrations and incubated. After 24 hours and 72 hours for bacteria and fungi incubated respectively, the plates were checked for growth. The minimum concentration at which the organism died was recorded as the minimum bacteriocidal or fungicidal concentration but the minimum concentration at which the organism did not die but did not expand beyond spot of inoculation was recorded as MIC.

## RESULTS AND DISCUSSION

### Physical Properties Results of Ligand and its Complexes

The reaction between N,N-Bis(Benzoin)ethylenediimine and Cadmium(II), Chromium(III) and Zirconium(IV) ions produce metal complexes which are crystalline and coloured (Table 1). The molar conductivity of the complexes was found to be the range of 7.8-8.5 showing their non-electrolytic nature of the complexes (Table 2). The complexes were found to melt in the temperature range of 130.5-138 °C showing a fairly stable complex compounds (Table 3). The complex compounds are insoluble in water and common organic solvents but are soluble in acetone, dimethylsulphoxide (D.M.S.O) and dimethylformaldehyde (D.M.F) (Table 4).

**Table 1: Some Physical Constants for the Ligand and the Metal Complexes**

Ligand/complexes	Molecular Formula	Molecular weight	Yield(%)	Colour
B <sub>2</sub> ED	C <sub>30</sub> H <sub>28</sub> N <sub>2</sub> O <sub>2</sub>	448	59.4	Yellow
[Zr(B <sub>2</sub> ED)(H <sub>2</sub> O)(NO <sub>3</sub> )]O(NO <sub>3</sub> )(H <sub>2</sub> O)	C <sub>30</sub> H <sub>30</sub> N <sub>4</sub> O <sub>11</sub> Zr	713.22	83.9	Red
[CrCl(B <sub>2</sub> ED)(H <sub>2</sub> O)]Cl <sub>2</sub> (H <sub>2</sub> O)	C <sub>30</sub> H <sub>30</sub> N <sub>2</sub> O <sub>4</sub> Cl <sub>3</sub> Cr	640.50	66.1	Green
[Cd(B <sub>2</sub> ED)(H <sub>2</sub> O) <sub>2</sub> ]Cl <sub>2</sub>	C <sub>30</sub> H <sub>30</sub> N <sub>2</sub> O <sub>4</sub> Cl <sub>2</sub> Cd	665.4	80.9	White

**Table 2: Molar conductivity data of 1x10<sup>-3</sup>M Ligand/complexes in acetone**

Compound	Specific Conductance (K)	Molar Conductance (ohm <sup>-1</sup> cm <sup>2</sup> mol <sup>-1</sup> )	Remark
B <sub>2</sub> ED	8.2 x 10 <sup>-6</sup>	8.2	Non electrolyte
[Zr(B <sub>2</sub> ED)(H <sub>2</sub> O)(NO <sub>3</sub> )]O(NO <sub>3</sub> )(H <sub>2</sub> O)	8.5 x 10 <sup>-6</sup>	8.5	Non electrolyte
[Cd(B <sub>2</sub> ED)(H <sub>2</sub> O) <sub>2</sub> ]Cl <sub>2</sub>	7.8 x 10 <sup>-6</sup>	7.8	Non electrolyte
[CrCl(B <sub>2</sub> ED)(H <sub>2</sub> O)]Cl <sub>2</sub> (H <sub>2</sub> O)	8.5 x 10 <sup>-6</sup>	8.5	Non electrolyte

**Table 3: Melting Point Data for the Ligand/Metal Complexes**

Complex	Melting Point/Decomposition Temperature
B <sub>2</sub> ED	136.0
[Zr(B <sub>2</sub> ED)(H <sub>2</sub> O)(NO <sub>3</sub> )]O(NO <sub>3</sub> )(H <sub>2</sub> O)	133.0
[Cd(B <sub>2</sub> ED)(H <sub>2</sub> O) <sub>2</sub> ]Cl <sub>2</sub>	130.5
[CrCl(B <sub>2</sub> ED)(H <sub>2</sub> O)]Cl <sub>2</sub> (H <sub>2</sub> O)	138.0

**Table 4; Solubility of the Ligand and Complexes in Different Solvent**

Ligand/ Complex	Water	Methanol	Ethanol	Acetone	Ether	Glacial acetate	DMSO	DMF
<b>B<sub>2</sub>ED</b>	NS	SS	SS	S	NS	S	S	S
<b>Cd<sup>2+</sup></b>	NS	SS	SS	S	NS	S	S	S
<b>Complex</b>								
<b>Cr<sup>3+</sup></b>	NS	SS	SS	S	NS	S	S	S
<b>Complex</b>								
<b>Zr<sup>4+</sup></b>	NS	SS	SS	S	S	S	S	S
<b>Complex</b>								

**Key:** S = Soluble, SS = Slightly soluble and NS = Not soluble

### Infrared Spectra of the Metal Complexes

The absorption of energy in the infrared region arises from changes in the vibrational energy of the molecules (Cooke, 2005). The relevant IR spectra assignment is shown on Table 5. The IR spectrum of the ligand was compared with that of the metal complexes so as to identify the coordination sites of the ligand (Rathakrishnan *et al.*, 2014). The broad bands above 3200 cm<sup>-1</sup> for the ligand and metal complexes are probably associated with the hydroxyl group that is,  $\nu(\text{O-H})$  (Ragab *et al.*, 2015). Thus, broad bands in the region of 3206 – 3377 cm<sup>-1</sup> is assigned to  $\nu(\text{O-H})$  stretching vibrations which is indicative of the presence of water molecules in the complexes and supporting bands in the range of 872-874 cm<sup>-1</sup> attest for the distorted vibration of  $\nu(\text{O-H})$  band and this implies that water molecules held by a crystal lattice is present in the complexes (Walaa *et al.*, 2014).

The bands at 1630-1677 are assigned to  $\nu(\text{C=N})$  stretching vibration mode, a fundamental feature of azomethine group (Boutcher and Day, 1977). The complex compounds show bands in the regions 2874 – 2881 cm<sup>-1</sup>, 3206 – 3377 cm<sup>-1</sup> and 1140 – 1162 cm<sup>-1</sup>, which were assigned to  $\nu(\text{C-H})$  asymmetric and symmetric stretching vibration modes,  $\nu(\text{O-H})$  stretching vibrations for coordinated water and  $\nu(\text{C-C})$  stretching vibration, respectively. The bands within 532 – 556 cm<sup>-1</sup> and

at 422 cm<sup>-1</sup> were assigned to  $\nu(\text{M-O})$  and  $\nu(\text{M-N})$  stretching vibrations, respectively (Nakamoto, 1963; Patel and Agwara, 1990). The bands due to M-O and M-N established the coordination of the Schiff base to the respective metal ions in each complex compound.

The two bands in the range 322–328 cm<sup>-1</sup> in the spectra of [Cd(B<sub>2</sub>ED)(H<sub>2</sub>O)<sub>2</sub>]Cl<sub>2</sub>, and [CrCl(B<sub>2</sub>ED)(H<sub>2</sub>O)]Cl<sub>2</sub> are assigned to  $\nu(\text{M-Cl})$  vibration (Nakamoto, 1971).

The bands at 692-702 cm<sup>-1</sup> are assigned to  $\nu(\text{Ar-C})$  stretching vibration mode, a fundamental feature of aromatic ring group.

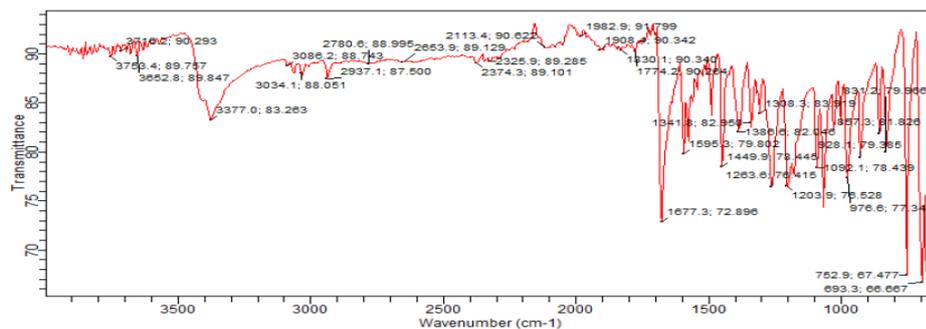
The  $\nu(\text{M-N})$  stretching bands are not observed in the spectrum of the ligand but are observed in the spectrum of the complexes because, there is no metal coordinated to the ligand (Waeel, 2013). Similarly, there is no  $\nu(\text{M-O})$  stretching bands because, there is no metal coordinated to oxygen (Waeel, 2013).

The strong band at 1380-1383 cm<sup>-1</sup> is attributed to the vibration of uncoordinated nitrate ion (Waeel, 2013)

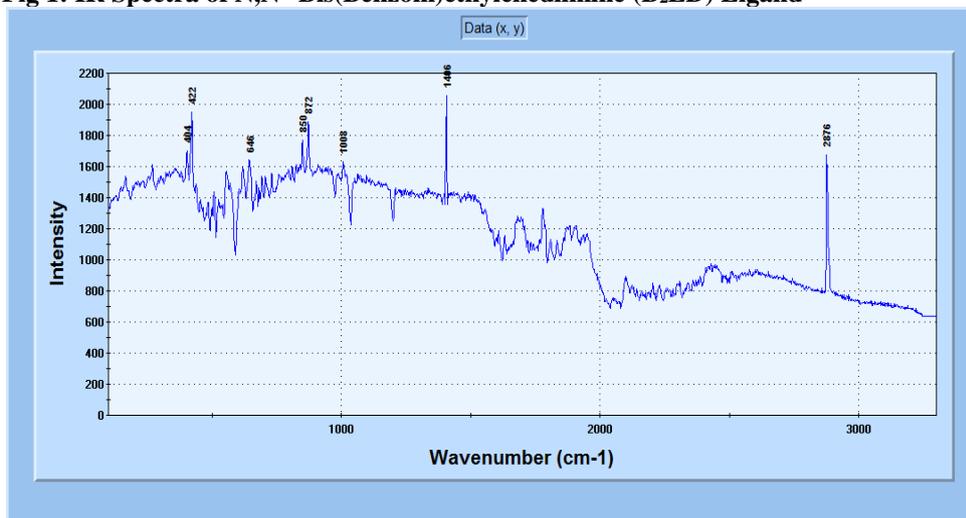
Finally, the ether group,  $\nu(\text{C-O})$  has bands around 1008-1010 cm<sup>-1</sup> and has a shift to lower frequencies probably due to the conjugation with the bond and partly because of resonance effects and this also confirms the participation of the ether group or the aromatic ring in the coordination of the metal and the ligand.

**Table 5: The Relevant Infrared Spectra Data for the Ligand and Metal Complexes in (cm<sup>-1</sup>)**

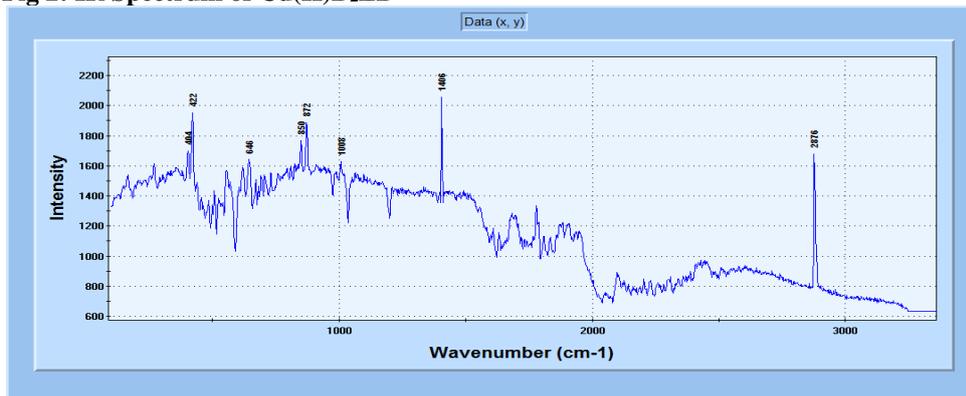
Metal complexes	C-H	C-C	C-O	C=N	H-O	M-N	M-O	M-NO <sub>3</sub>	M-Cl
B <sub>2</sub> ED	2937	1162	1010	1677	3377				-
Zr(B <sub>2</sub> ED)	2876	1156	1008	1630	-	422	532	1383	-
Cr(B <sub>2</sub> ED)	2876	1140	1008	1632	-	422	556	-	322
Cd(B <sub>2</sub> ED)	2876	1150	1010	1634	-	422	556	-	328



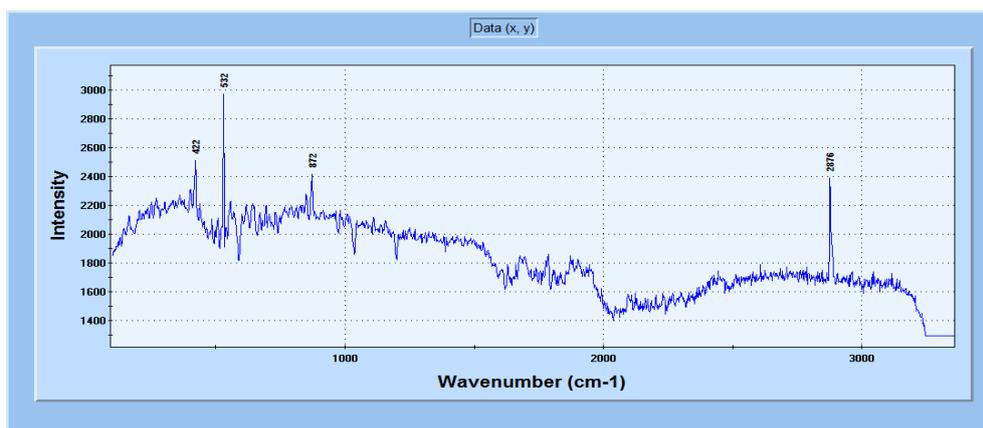
**Fig 1: IR Spectra of  $N,N'$ -Bis(Benzoin)ethylenediimine ( $B_2ED$ ) Ligand**



**Fig 2: IR Spectrum of  $Cd(II)B_2ED$**



**Fig 3: IR Spectrum of  $Cr(III)B_2ED$**



**Fig 4: IR Spectrum of  $Zr(IV)B_2ED$**

**Electronic spectra of the metal complexes**

A useful tool for the evaluation of results provided by other methods of structural investigation is the electronic spectra also known as the ultra-violet visible spectra. It is used to designate or assign the stereochemistry of metal ions in a complex as shown by the positions and numbers of d-d transition peaks. The ligand and the metal complexes absorb in the visible region due to charge transfer processes from the energy in the visible light and hence, provide evidence that they are all coloured compounds (Harvey, 2000).

The structure of the complexes was elucidated by the absorption bands they exhibited. The electronic spectra are represented in Table 6. The most useful transitions for analysis are the intense  $\pi \rightarrow \pi^*$  transitions and the weaker, but lower energy,  $n \rightarrow \pi^*$  transitions.

The electronic spectrum of the ligand B<sub>2</sub>ED shows two bands at 325.50 nm (30,722 cm<sup>-1</sup>) due to the  $\pi \rightarrow \pi^*$  transition of the chromophore (-C=N-) and at 269 nm (37,175 cm<sup>-1</sup>) due to the  $n \rightarrow \pi^*$  transition of the auxochrome (Phenyl). This transition is also found in the spectra of the complexes, but shifted towards lower frequencies, confirming the coordination of the ligand to the metal ions.

The electronic spectrum for the Cadmium (II) N, N'-Bis(benzoin)ethylenediimine [Cd(B<sub>2</sub>ED)(H<sub>2</sub>O)<sub>2</sub>]Cl<sub>2</sub> complex showed three bands at 273.00 nm (36,630 cm<sup>-1</sup>), 325.00 nm (30,769 cm<sup>-1</sup>), and 760.50 nm (13,149 cm<sup>-1</sup>), respectively. The bands are assigned to <sup>1</sup>A<sub>1</sub> transitions respectively since it is the only spin-allowed transition possible for a d<sup>10</sup> ion and they are not symmetrical with respect to a centre of symmetry (Geoffrey, 1980). Both bands are suggestive of a tetrahedral geometry. The first band at 273.00 nm is due to  $\sigma \rightarrow \sigma^*$  transitions as a result of the presence of either C-C or C-H groups, the second band at 325.00 nm is due to  $\pi \rightarrow \pi^*$  transitions as a result of the presence of C=N group and finally, the third band at 760.50 nm is due to  $n \rightarrow \pi^*$  transitions as a result of the

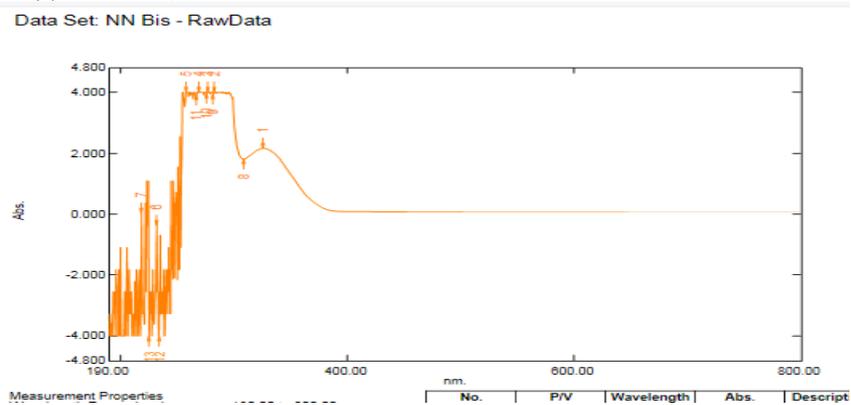
presence of H<sub>2</sub>O molecules, suggestive of a tetrahedral geometry.

The electronic spectrum of chromium (III) complex shows bands in the region 754.50 nm (13,254 cm<sup>-1</sup>), 324.50 nm (30,817 cm<sup>-1</sup>), 285 nm (35,088 cm<sup>-1</sup>) and 213 nm (46,838 cm<sup>-1</sup>) which are consistent with the octahedral geometry. Thus, an octahedral may be assign to the complex. It displayed four bands at 13,254 (v<sub>1</sub>), 30,817 (v<sub>2</sub>) 35,088 (v<sub>3</sub>) and 46,838 cm<sup>-1</sup> (v<sub>4</sub>). Six coordinate complexes with O<sub>h</sub> symmetry show three spin-allowed bands (Perumareddi 1969). The highest energy band assignable to the <sup>4</sup>A<sub>1g</sub> - <sup>4</sup>A<sub>2g</sub> transition, occurs above 30000 cm<sup>-1</sup>. In the chromium complex, the four transitions observed can be assigned <sup>4</sup>B<sub>1g</sub> → <sup>4</sup>E<sub>g</sub><sup>a</sup> (v<sub>1</sub>), <sup>4</sup>B<sub>1g</sub> → <sup>4</sup>B<sub>2g</sub> (v<sub>2</sub>), <sup>4</sup>B<sub>1g</sub> → <sup>4</sup>E<sub>g</sub><sup>b</sup> (v<sub>3</sub>) and <sup>4</sup>B<sub>1g</sub> → <sup>4</sup>A<sub>1g</sub> (v<sub>4</sub>) transitions arising from the lifting of the degeneracy of the orbital triplet (in octahedral symmetry) in the order of increasing energy and assuming the effective symmetry around the metal ion of D<sub>4h</sub>. In O<sub>h</sub> symmetry (v<sub>1</sub>) and (v<sub>2</sub>) are derived from the <sup>4</sup>T<sub>2g</sub> level, whilst v<sub>3</sub> and v<sub>4</sub> from <sup>4</sup>T<sub>1g</sub>. The C<sub>4v</sub> symmetry has been ruled out because of the greater splitting of the first band.

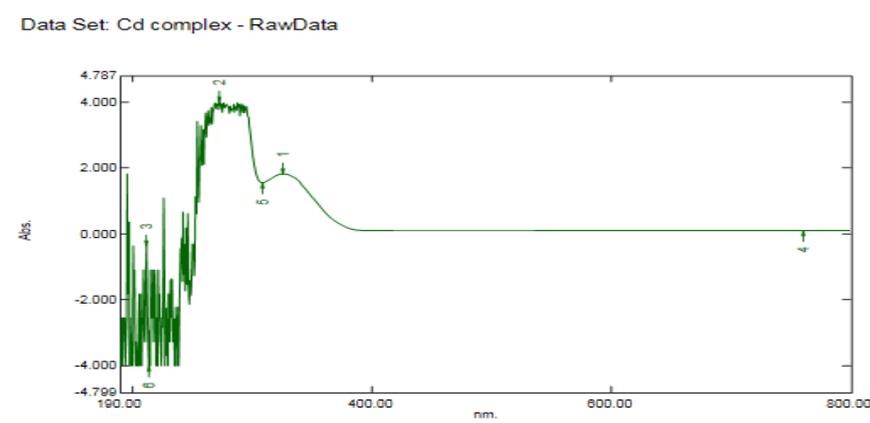
In spectrum of the zirconium complex, three bands are observed at 226.00 nm (44,248 cm<sup>-1</sup>), 262.00 nm (38,168 cm<sup>-1</sup>), and 294.00 nm (34,014 cm<sup>-1</sup>). The bands at 226.00 and 262.00 nm are due to the  $\pi \rightarrow \pi^*$  transitions within the aromatic ring and remain almost unchanged in the spectra of the N,N-Bis(benzoin)ethylenediimine ligand. The other band at 294 nm is due to the  $n \rightarrow \pi^*$  transitions within the >C=N- chromophore that showed a bathochromic shift from the spectrum of the ligand due to the coordination of the azomethine nitrogen to the zirconium atom. This band shifts slightly to the higher-energy region in the spectrum of zirconium complex due to the polarization within the >C=N chromophore caused by the zirconium-ligand electron interaction. The bands both suggest a distorted octahedral geometry due to Jahn-teller effect (unequally populated degenerate orbitals). (Rathakrishnan *et al.*, 2014).

**Table 6: Electronic Spectroscopic Data for the Ligand/Metal Complexes.**

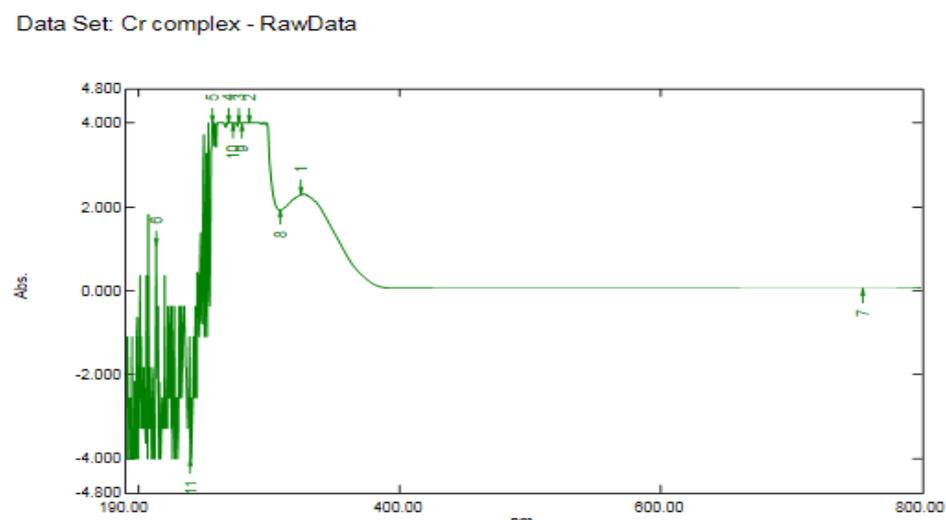
Metal complexes	$\lambda_{\max}$ (nm)	Assignment	Colour	Geometry
B <sub>2</sub> ED	325.50	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$	Yellow	
[ZrO(B <sub>2</sub> ED)](NO <sub>3</sub> ) <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub>	280.00	<sup>3</sup> A <sub>2g</sub> → <sup>3</sup> T <sub>1g</sub>	Red	Octahedral
[CrCl(B <sub>2</sub> ED)(H <sub>2</sub> O)]Cl <sub>2</sub> (H <sub>2</sub> O)	754.50	<sup>4</sup> A <sub>2g</sub> → <sup>4</sup> T <sub>2g</sub>	Green	Octahedral
[Cd(B <sub>2</sub> ED)(H <sub>2</sub> O) <sub>2</sub> ]Cl <sub>2</sub>	760.50	<sup>1</sup> A <sub>1</sub>	white	Tetrahedral



**Fig 5: Electronic Spectrum of N,N'-Bis(Benzoin)ethylenediimine (B<sub>2</sub>ED) Ligand**

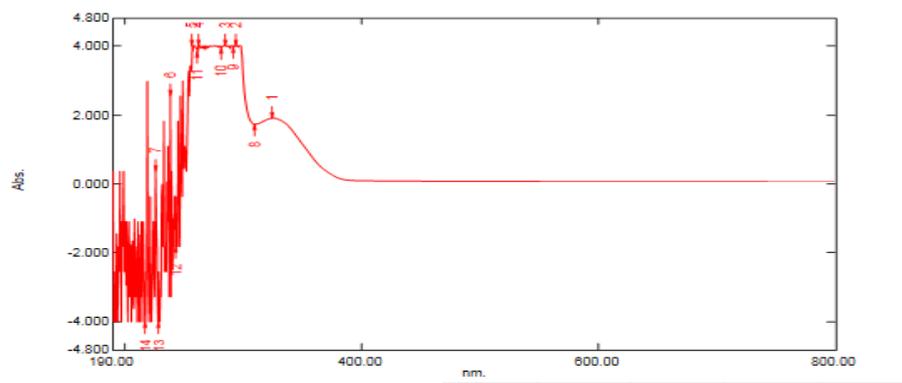


**Fig 6: Electronic Spectrum of Cd(II)(B<sub>2</sub>ED) Complex**



**Fig 7: Electronic Spectrum of Cr(III)(B<sub>2</sub>ED) Complex**

Data Set: Zr complex - RawData



**Fig 8: Electronic Spectrum of Zr(IV)(B<sub>2</sub>ED) Complex**

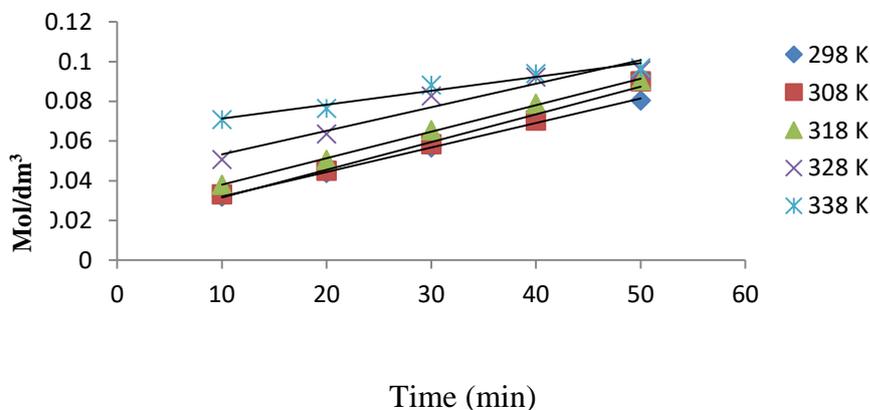
### Kinetic Studies of the Complexes

The kinetic studies investigated the effects of temperature on the amount of metal complex formed at various time. The various mass obtained were analysed to ascertain the yield in mol/dm<sup>3</sup>. A graph of concentration against time was plotted to obtain the observed rate constant of each of the various temperatures. The plots indicated an increase in the concentration of complexes formed as temperature and time increased. As the temperature increases, the molecules of reactants move faster and therefore collide more frequently. The molecules also carry more kinetic energy. Thus, the proportion of collisions that overcome the activation energy for the formation increases with temperature. This, therefore, allows for the complexes to be formed faster (Taon *et al.*, 2011).

Fig 9, 10 and 11 showed the plots of Cd (II), Cr(III) and Zr(IV) complexes yield in concentration (mol/dm<sup>3</sup>) with time (minute) at different temperatures. The three plots showed an increased in observed rate constants of the formation of the three complexes as temperature increases. This means the rate of formation of these

complexes depends on the temperature at which it is run.

The activation energy ( $E_a$ ) of various complexes was determined from the slope of the plots of  $\ln k_{obs}$  versus  $1/T$ . Fig 12 showed the plots of the observed rate constant of Cd(II), Cr(III) and Zr(IV)B<sub>2</sub>ED against temperature inverse ( $\ln k_{obs}$  vs.  $1/T$ ). The values of activation energy ( $E_a$ ) obtained from the plots were 18.707, 14.766 and 12.288 kJoules for Cd(II), Cr(III) and Zr(IV)B<sub>2</sub>ED respectively which were positive activation energy ( $E_a$ ) indicating that, the rate of the formation increases with increasing temperature and the “apparent” rate constant of the overall formation-defined by Arrhenius behaviour will increase as temperature is increased and is a signal that the formation of the complexes has no complex mechanism and the relative higher activation energy of cadmium explained the small yield of cadmium complex at some temperature because only a very small fraction of collision would have enough energy to overcome activation energy (Ochai *et al.*, 2013).



**Fig. 9: Plots of Cd(II)B<sub>2</sub>ED Yield in mol/dm<sup>3</sup> with time at different temperatures**

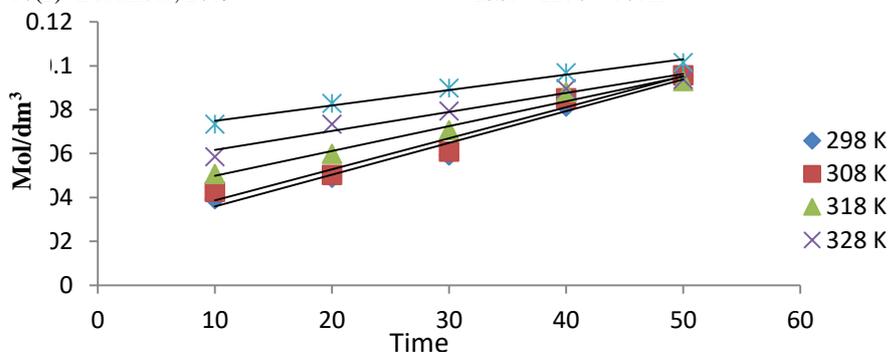


Figure 10: Plots of Cr(III)B<sub>2</sub>ED Yield in mol/dm<sup>3</sup> with time at different temperatures

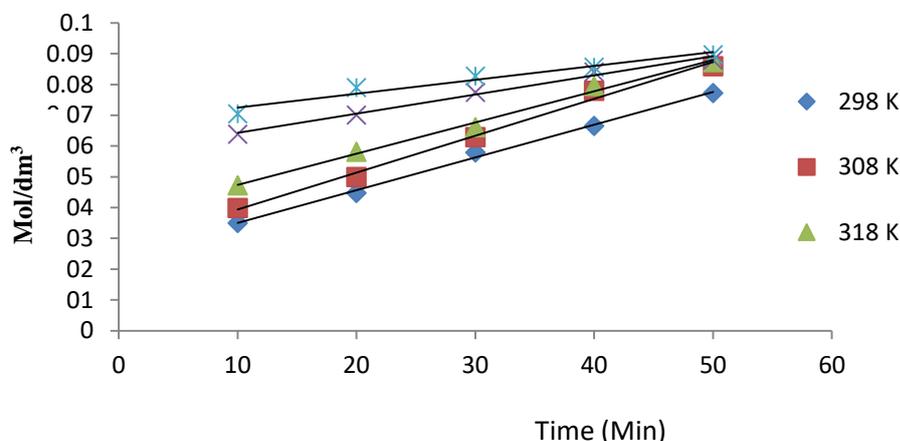


Figure 11: Plots of Zr(II)B<sub>2</sub>ED Yield in mol/dm<sup>3</sup> with time at different temperatures

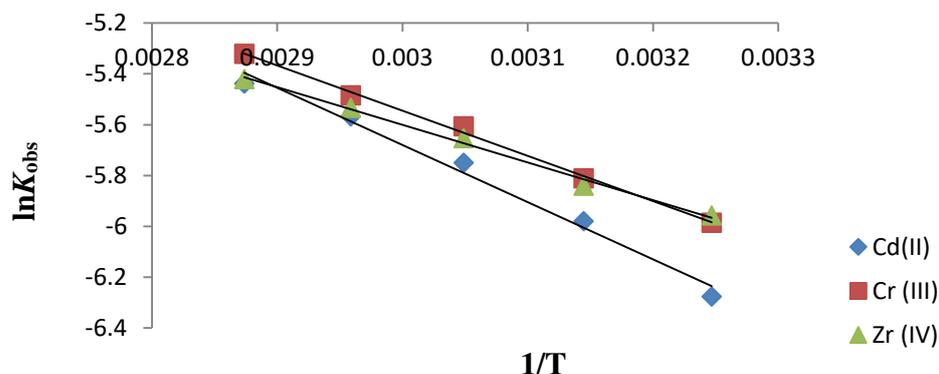


Fig 12: Plots of  $\ln K_{obs}$  versus  $1/T$  for formation of Cd(II), Cr(III) and Zr(IV)B<sub>2</sub>DE complexes

### Thermodynamics Studies of the Complexes

Thermodynamics parameter such as enthalpy ( $\Delta H^\circ$ ) and entropy ( $\Delta S^\circ$ ) change of activation during the formation of the complexes according to Ochai *et al.* (2013) can be evaluated from the equations 3 and 4.

$$\ln\left(\frac{K_{obs}}{T}\right) = \ln\left(\frac{R}{Nh}\right) + \frac{\Delta S^\ddagger}{R} - \frac{\Delta H^\ddagger}{RT} \quad (3)$$

$$\Delta G^\circ = \Delta H^\circ - T_a \Delta S^\circ \quad (4)$$

Where  $K_{obs}$  = observed reaction rate constant,  $T$  = Temperature (K),  $T_a$  = the absolute temperature at which reaction can occur,  $N$  = Avogadro's constant,  $h$  = Planck constant,  $R$  = Gas constant ( $Jmol^{-1}K^{-1}$ ),  $\Delta G^\circ$  = The Gibbs free energy of activation ( $kJmol^{-1}$

<sup>1</sup>),  $\Delta H^\circ$  = the enthalpy of activation ( $kJmol^{-1}$ ) and  $\Delta S^\circ$  = the entropy of activation ( $kJmol^{-1}$ ).

The values of  $\Delta H^\circ$  and  $\Delta S^\circ$  were determined from the slope and intercept of the graphs in Fig 5 by plotting  $\ln\left(\frac{K_{obs}}{T}\right)$  against  $\frac{1}{T}$ . From equation 3. While the values of  $\Delta G^\circ$  were calculated from Equation (4).

The values of the enthalpy change of activation ( $\Delta H^\circ$ ), the entropy change of activation ( $\Delta S^\circ$ ) and the Gibbs free energy of activation ( $\Delta G^\circ$ ) found in this work were  $-15.99 kJmol^{-1}$ ,  $1.098 kJmol^{-1}$  and  $-343.347 kJmol^{-1}$  for Cd(II)B<sub>2</sub>ED while  $-12.047 kJmol^{-1}$ ,  $1.097 kJmol^{-1}$  and  $-339.022 kJmol^{-1}$  for Cr(III)B<sub>2</sub>ED and  $-9.569 kJmol^{-1}$ ,  $1.096 kJmol^{-1}$  and  $-336.263 kJmol^{-1}$  for Zr(IV)B<sub>2</sub>ED respectively.

The negative values of  $\Delta G^\circ$  showed the ability of the studied ligand to form a stable complex since the complex had less potential energy and the formation of the complexes is spontaneous in nature. The negative value of enthalpy change of

formation ( $\Delta H^\circ$ ) of the complexes implies that the enthalpy is the driving force for the formation of the complexes. However, the positive values of entropy ( $\Delta S^\circ$ ) shows that entropy is responsible for the complexation process (Ochai *et al.*, 2013).

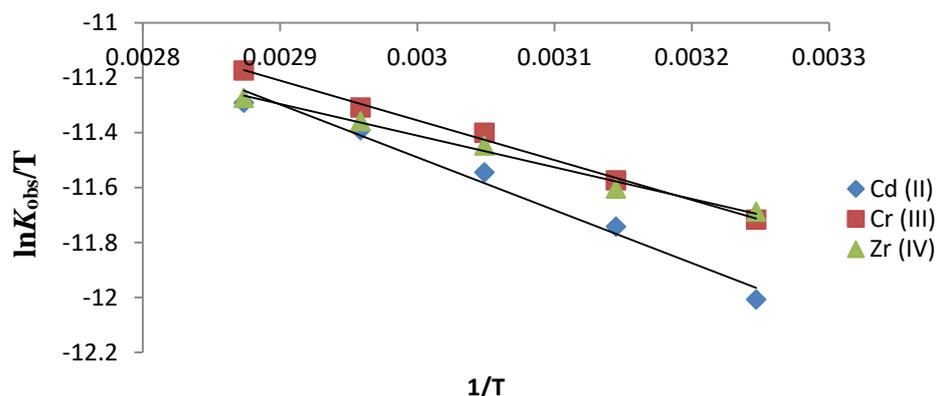


Figure 13: Plots of  $\ln(K_{obs}/T)$  versus  $1/T$  for formation of Cd(II), Cr(III) and Zr(IV)B<sub>2</sub>ED Complexes

Table 7: Thermodynamic Parameters for Formation of Cd(II), Cr(III) and Zr(IV)B<sub>2</sub>ED Complexes

COMPLEXES	A	$\Delta S$ ( $\times 10^3$ )	$\Delta H$ ( $\times 10^3$ )	$\Delta G$ ( $\times 10^5$ )	E <sub>a</sub> ( $\times 10^3$ )
[Cd(B <sub>2</sub> ED)(H <sub>2</sub> O) <sub>2</sub> ]Cl <sub>2</sub>	2.92	+1.099	-15.99	-3.433	18.7
[CrCl(B <sub>2</sub> ED)(H <sub>2</sub> O)]Cl <sub>2</sub> (H <sub>2</sub> O)	0.81	+1.097	-12.05	-3.390	14.8
[Zr(B <sub>2</sub> ED)(H <sub>2</sub> O)(NO <sub>3</sub> )]ONO <sub>3</sub> (H <sub>2</sub> O)	0.31	+1.096	-9.57	-3.363	12.3

### Antimicrobial Susceptibility

The bacteria strains used include *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Klebsiella pneumonia* the strains of fungi were used were *Candida albican*, *Trichophyton rubrum* and *Microsporum canis*. They were obtained and identified from the Department of Microbiology, Federal University of Agriculture, Makurdi – Nigeria.

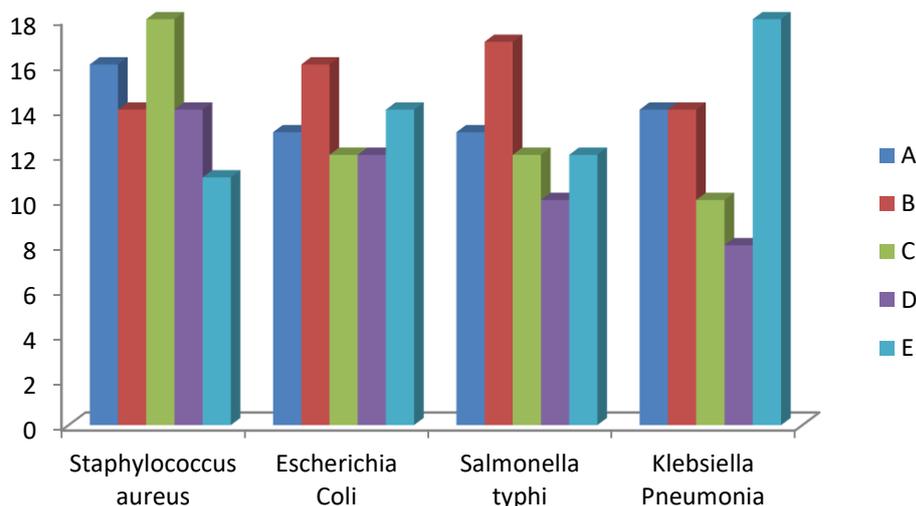
### Zone of Inhibition

The zone of inhibition of the antimicrobial agents (B<sub>2</sub>ED, Cd<sup>2+</sup>, Cr<sup>3+</sup> and Zr<sup>4+</sup> complexes) against the microbial species used, at 100 mg/mL, measured in millimetre is given in Table 8. This was evaluated by Agar-well diffusion technique described by Aliyu *et al.*, (2012).

The zones of inhibition of the antimicrobial agents (the ligand and complexes) against the microbial species are compared with a statistical tool (bar graph) to elucidate the different potent activities of these antimicrobial agents as in Fig. 14.

From the graph, it is noticed that all the antimicrobial agents inhibited the growth of the tested bacterial at different extent but were inactive to the tested fungi at 100 mg/mL. This is because in bacteria, the permeability of the cell membrane is associated with the loss of ions as well as the reduction of its potential (Trombeta *et al.*, 2005). It also showed a greater inhibitory effect than their individual ligand, thus, the addition indicated an increase in the antimicrobial activity after the coordination.

[CrCl(B<sub>2</sub>ED)(H<sub>2</sub>O)]Cl<sub>2</sub>(H<sub>2</sub>O) complex shows the highest inhibitory activity against *Escherichia Coli* and *Salmonella typhi* and can be used as closed substitute against these pathogenic bacteria. This shows no action on *Staphylococcus aureus* as its extent of inhibition equals that of the ligand, thus, the metal effect is not evident. So also with [Zr(B<sub>2</sub>ED)(H<sub>2</sub>O)(NO<sub>3</sub>)]O(NO<sub>3</sub>)(H<sub>2</sub>O) which shows no metallic potency on *Escherichia Coli* but gives the highest inhibition against *Staphylococcus aureus*.



**Fig. 14: Zone of inhibition against *Salmonella typhi*, *Klebsiella Pneumonia*, *S.aureus*, and *E. coli* (Bacterial Species)**

$A_i = [Cd(B_2ED)(H_2O)_2]Cl_2$  Complex,  $B = [CrCl(B_2ED)(H_2O)]Cl_2(H_2O)$  Complex,  $C = [Zr(B_2ED)(H_2O)(NO_3)]O(NO_3)(H_2O)$  Complex,  $D = B_2ED$  and  $E = Ciprofloxacin$  (standard for antibacterial)

#### Minimum Inhibitory Concentration

The metal complexes exhibited antimicrobial performances, such as low minimum inhibitory concentration ( $MIC \leq 250$ ), bactericidal effect and a broad activity spectrum. These could become promising antimicrobial agents with potential applications in pharmaceutical industry for controlling the pathogenic bacteria. Thus, qualify these compounds as suitable candidates to the next step of drugs fabrication.

The criteria for categorizing the antimicrobial activity of synthesized ligand and metal complexes used in this work was based on the MIC results obtained. However, several studies have also used this classification to evaluate the

antimicrobial activity of ligands and their complexes, as: good, MIC inferior to 100 mg/mL; moderate: MIC between 100 and 500 mg/mL; weak: MIC between 500 and 1000 mg/mL; and inactive when the MIC is superior to 1000 mg/ml (Holetz *et al.*, 2002; Dalmarco *et al.*, 2010). This way, it was possible to evaluate the antimicrobial activity of both the ligand and the metal complexes as having a moderate antibacterial activity, the complexes as weak antifungal and the ligand as inactive antifungal.

Nevertheless, further studies on the mechanism of growth inhibition and toxicity are needed, in order to evaluate the potential of therapeutic application for safety purposes.

**Table 9: Minimum Inhibitory Concentration of the Ligand, Standards and the Metal Complexes on Selected Microbial Species (mg/mL)**

Test Organisms	A	B	C	D	E	F
<i>Staphylococcus aureus</i>	150	150	150	300	150	
<i>Escherichia Coli</i>	150	150	150	300	75	-
<i>Salmonella typhi</i>	150	150	300	300	150	-
<i>Klebsiella Pneumonia</i>	150	150	300	300	75	-
<i>Candida albican</i>	600	600	600	-	-	150
<i>Trichophyton rubbrum</i>	600	600	600	-	-	150
<i>Microsporium canis</i>	600	600	600	-	-	150

#### Minimum Bactericidal and Fungicidal Concentrations (MBC and MFC)

A wide array of studies have reported that the antimicrobial activity of compounds is likely to be related to their ability to form complexes with soluble extracellular proteins and with the cell wall, for the lipophilic character of these compounds, which may cause the rupture of the cell membrane of microorganisms (Sartori, 2005). Besides, in bacteria, the permeability of the cell membrane is associated with the loss of ions as well as the

reduction of its potential (Trombeta *et al.*, 2005), causing damage that may lead to the extravasations of macromolecules, resulting in a collapse of the cellular functions and, consequently, the bacterial death (Tortora *et al.*, 2003).

The inhibitory effect of the metal complexes was found to be bactericidal, as suggested by Berche *et al.*, 1988 and Konat. *et al.*, 2012; Abou *et al.*, 2013), who proposed that when the MBC/MIC or MFC/MIC ratio is less than or equal to 4.0, the agent will be considered

bactericidal or fungicidal, and when this ratio is over 4.0 it should be considered bacteriostatic or fungi static. The evaluated compounds inhibited the growth of all the bacteria, indicating a broad

spectrum of activity while opening perspectives for their use as future drugs. These compounds are fungi static.

**Table 11: Minimum Bactericidal and Fungicidal Concentrations (MBC and MFC) Measured in mg/mL**

Test Organism	A	B	C	D	E	F
<i>Staphylococcus aureus</i>	300	300	300	600	150	-
<i>Escherichia Coli</i>	300	300	300	600	150	-
<i>Salmonella typhi</i>	300	300	600	600	150	-
<i>Klebsiella Pneumonia</i>	150	150	600	600	150	-
<i>Candida albican</i>	-	-	-	-	-	300
<i>Trichophyton rubbrum</i>	-	-	-	-	-	300
<i>Microsporium canis</i>	-	-	-	-	-	300

## CONCLUSION

The ligand: N,N-Bis(Benzoin)ethylenediimine was synthesized. The ligand as well as the metal complexes showed melting points in the range of 130.5-138 °C. The molar conductance for the ligand and metal complexes indicated their non-electrolytic character or behaviour. The ligand and metal complexes showed high solubility behaviour in acetone, glacial acetate, DMSO and DMF but were insoluble in distilled water and other organic solvents. The significant IR frequencies of most relevant bands of the free Schiff base B<sub>2</sub>ED and its [Cd(B<sub>2</sub>ED)], [Cr(B<sub>2</sub>ED)] and [Zr(B<sub>2</sub>ED)] complexes with their probable assignments are also given in Table 5. The IR spectrum of B<sub>2</sub>ED showed characteristic vibrations of C=N and OH groups at 1677 and 3377 cm<sup>-1</sup>, respectively. In the spectra of Cd<sup>2+</sup>, Cr<sup>3+</sup> and Zr<sup>4+</sup> complexes, the azomethine band (νC=N) was shifted to lower wavenumber (1630–1634 cm<sup>-1</sup>) whereas, the ν(OH) is shifted by 18–48 cm<sup>-1</sup> to lower frequency upon complex formation suggesting involvement of C=N and OH groups in coordination. The electronic spectra showed that the metal complexes exhibited single, double to triple bands which are consistent with the octahedral and tetrahedral geometry for the metal complexes. A graph of concentration against time was plotted to obtain the observed rate constant of each of the various temperatures. The plots indicated an increase in the concentration of complexes formed as temperature and time increased. The thermodynamic parameters of the complexation reactions were calculated by using an Arrhenius-type kinetic model. The data are summarized in Tables 7. The activation energies of the reactions were in the range 12.3-18.7 kJ mol<sup>-1</sup>. The high values of the activation energies reflect the thermal stability of the complexes. The negative values of ΔG<sup>0</sup> showed the ability of the studied ligand to form a stable complex since the complex had less potential energy and the formation of the complexes is spontaneous in nature. The negative value of enthalpy change of formation (ΔH<sup>0</sup>) of the complexes implies that the enthalpy is the driving force for the formation of

the complexes. However, the positive values of entropy (ΔS<sup>0</sup>) shows that entropy is responsible for the complexation process. The antimicrobial agents (ligand and the complexes) inhibited the growth of the tested bacterial at different extent but were inactive to the tested fungi at 100 mg/mL. The metal complexes exhibited antimicrobial performances, such as low minimum inhibitory concentration (MIC ≤ 250), bactericidal effect and a broad activity spectrum. The N,N-Bis(Benzoin)ethylenediimine ligand and the metal complexes exhibited the octahedral geometry in the Chromium and Zirconium complexes, and tetrahedral geometry in the Cadmium complex, based on the physicochemical data provided.

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