# PROJECT REPORT

**On**

**"SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL STUDIES OF SCHIFF BASE AND ITS METAL COMPLEXES"**


Submitted by

**R.AMRUTHA(AB16CHE018)**

**ATHULYA P. R(AB16CHE013)**

**CHAITHANYA BOSE (AB16CHE004)**


***In partial fulfillment for the award of the Bachelor's Degree in Chemistry***


**POST GRADUATE AND RESEARCH DEPARTMENT OF CHEMISTRY**

**ST. TERESA'S COLLEGE (AUTONOMOUS) ERNAKULAM**


**2018-2019**

**POST GRADUATE AND RESEARCH**

**DEPARTMENT OF CHEMISTRY**

**ST. TERESA'S COLLEGE (AUTONOMOUS)**

**ERNAKULAM**

**B.Sc. CHEMISTRY PROJECT REPORT**

| | |
|---|---|
| **Name** | **: R. Amrutha,Athulya P.R, Chaithanya Bose** |
| **Register Number** | **: AB16CHE018 ,AB16CHE013, AB16CHE004** |
| **Year of Work** | **: 2018-2019** |

This is to certify that the project "SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL STUDIES OF SCHIFF BASE AND ITS METAL COMPLEXES" is the work done by R .Amrutha,Athulya P.R,Chaithanya Bose**.**

**Dr. Ushamani M**
**Head of the Department**

**Sicily Rilu Joseph**
**Staff-member in charge**

**Submitted to the Examination of Bachelor's Degree in Chemistry**

**Date:**……………

**Examiners:**………………………………………

# DECLARATION

We hereby declare that the project work entitled "SYNTHESIS, CHARACTERISATION AND ANTIMIOCROBIAL STUDIES OF SCHIFF BASE AND ITS METAL COMPLEXES" submitted to Department of Chemistry, St. Teresa's College (Autonomous) affiliated to Mahatma Gandhi University, Kottayam, is a record of an original work done by us under the guidance of Sicily Rilu Joseph,Assistant Professor(guest) , Department of Chemistry, St. Teresa's College (Autonomous), Ernakulam and this project work is submitted in the partial fulfillment of the requirements for the award of the degree of Bachelor of Science in Chemistry.

**R. Amrutha,**
**Athulya P.R,**
**Chaithanya Bose**

Acknowledgements

The omniscient God's benevolence makes anything what it is. Thank you my Lord God for being with me throughout all the days and helping me complete the project successfully.

We would like to express our sincere gratitude to our project guide miss Sicily Rilu Joseph,Assistant Professor(guest) , Department of Chemistry, St. Teresa's College (Autonomous),Ernakulam for her invaluable guidance, support and suggestions which helped us in completion of our project work.

We would also like to thank Mrs.Anitta Antony, research scholar, St. Teresa's College (Autonomous), Ernakulam for her encouragement and moreover her timely support and guidance till the completion of our project work.

We extend our sincere gratitude to Dr. Ushamani M, Head of the Department of Chemistry, St. Teresa's College (Autonomous), Ernakulam for providing us with all the facilities and support to meet our project requirements.We also express our sincere gratitude to Dr.Sr.Vinitha CSST ,Director and Dr.Sajimol Augustine M,Principal of St.Teresa's College (Autonomous),Ernakulam for their extended support and co-operation during our project work.

We also like to extend our sincere thanks to Sophisticated Test and Instrumentation Centre (STIC), CUSAT and Unibiosys Lab, South

Kalamassery for their assistance in various stages of completion of the project.

We would like to express our gratitude towards our parents and friends.

We thank all the teachers and non- teaching staff of the department of chemistry, St. Teresa's College(Autonomous), Ernakulam for their support and cooperation during our entire project work.

**CHAITHANYA BOSE,**

**ATHULYA P R,**

**R AMRUTHA**

# *Contents*

## Chapter 1

# INTRODUCTION

## 1.1 SCHIFF BASE

Schiff base (also known as imine or azomethine) is a nitrogen analogue of an aldehyde or ketone in which carbonyl group (C=O) has been replaced by an imine or azomethine group.

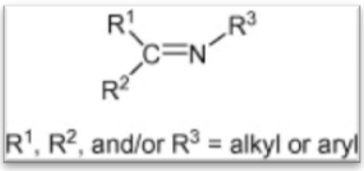



Fig 1.1: Schiff base

Schiff base is formed when a primary amine reacts with an aldehyde or a ketone under specific conditions. It is named after Hugo Schiff because he was responsible for research in to aldehyde leading to his development of Schiff test.[1]

The electrophilic carbon atoms of aldehydes and ketones can be targets of nucleophilic attack by amines.The end result of this reaction is a compound in which the C=O is replaced by a C=N bond.

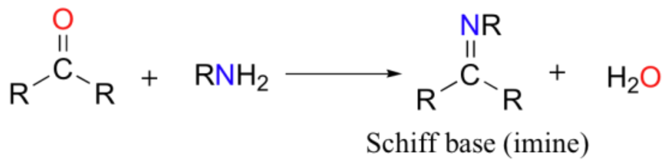



Fig 1.2:Scheme of formation of Schiff base

The term Schiff base is normally used to name those compounds which are being used as ligands to form coordination complexes with metal ions .Some complexes occur naturally,such as Corrin,but the majority of Schiff bases are artificial and are used to form many important catalyst such as Jacobsen's catalyst.[2]. A number of special naming systems exist for these compound. The schiff base derived from an aniline,where $R^3$ is a phenyl or substituted phenyl is called an anil,while bis-compounds are often refered to as salene-type compound.

## 1.2 DENTICITY AND BASICITY OF SCHIFF BASES

A Schiff base coordinates through the O atom of the deprotonated phenolic group and the N atom of azomethine group.Ligands are classified according to the number of donor atoms contained in them such as uni,di,tri or quadridentate ligands. When donor sites of a ligand occupy two or more coordination positions on the same central metal ion, a complex possessing a closed ring is formed and this phenomena of ring formation is called Chelation .The ring so formed is called Chelate ring. Schiff Bases primarily possess nitrogen donor atoms, though many can act as bi-,tri-,tetra- or polydentate mixed donor capabilities as shown in fig 3 . In general ,the donor nature of the ligands depends both on the type of aldehyde/ketone used and the nature of primary amine / diamine.

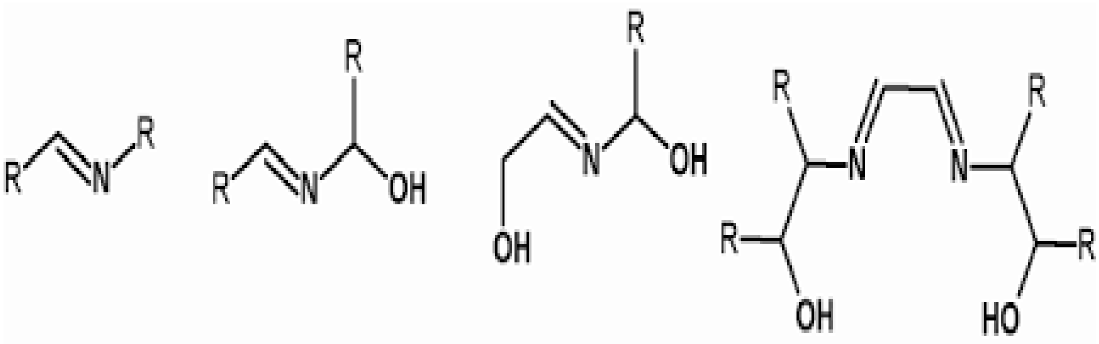
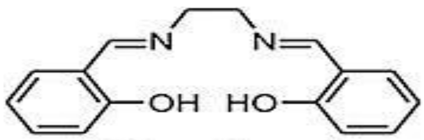
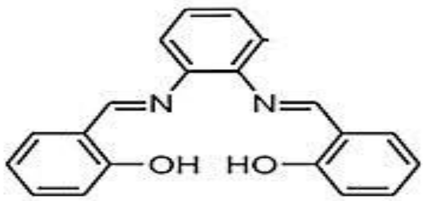



Fig 1.3 : Schiff Bases of varying denticity ; monodentate to tetradentate R groups may be variously substituted .

A large number of tetradentate Schiff Base ligands are derived from Salicylaldehyde and 1,2-diamines . The ONNO donor Schiff Bases form a family of compounds, salen or salophen, which causes a wide variety of applications. The tridentate ligands can tune the formation of complexes and these complexes have found various applications in medicine such as antibacterial agents, local anaesthetics, antiviral agents and antisplasmodics.



Salen ligand



Salophen ligand

Fig 1.4 : Tetradentate Schiff Base Family

Schiff Bases have been found to be the most convenient ligands for forming complexes. Its steric and electronic effects around the metal core can be finely tuned by an appropriate selection of bulky and / or electron withdrawing or donating substituents incorporated into the Schiff Bases . The second donor atoms , N and O, of the chelated Schiff Base exert two opposite electronic effects: the phenolate oxygen stabilizes the higher oxidation state of the atom, whereas the imine nitrogen stabilizes the lower oxidation state of the metal ion.Schiff bases are currently prepared in high yield through one step procedures via condensation of common aldehydes with amines, in practically quantitative yields.

The basicity of Schiff bases contributes to the stability and formation of complexes. The –OH or –SH groups present in Schiff Bases can induce tautomerism in compounds which leads to complexes with different structures.

## 1.3 APPLICATIONS OF SCHIFF BASES AND THEIR COMPLEXES

The Schiff Bases can form metal complexes with p and d- block elements and these complexes are efficient catalysts in various reactions. Example: many Ruthenium and Palladium complexes are used as catalysts in the synthesis of polymers. Schiff Base metal complexes of metals are calaytic in ring opening polymerization processes at low temperature. These complexes also catalyse the oxidation of sulfides,phenol , aldehydes etc. They also act as a catalysts in decomposition of hydrogen peroxide, isomerisation etc. The high thermal stabilities of many complexes are responsible for their applications as catalysts at high temperature.
Almost all the Schiff base metal complexes show antiviral ,antibacterial, antimalarial, antitubercular activity. The biological activity of these complexes is due to the presence of nitrogen atom with lone pair of electron in it as a result of which the nitrogen atom can form hydrogen bonding with the –NH or –OH groups present in DNA or RNA, proteins,aminoacids. Investegations in interactions of DNA with the Schiff base metal complexes are important in pharmaceutical area. Many Schiff Base complexes are found to be good corrosion inhibitors. Chromium and Cobalt Schiff Base complexes are used as dyes which gives fast colours to leather,wools and food packages. Tetradentate Schiff Bases like salens and salophens acts as chromogenic reagent for determination of nickel in some natural food samples.
Their chemical and physical properties such as preparative uses, identification or protection and determination of aldehydes or ketone , purification of carbonyl and amino compounds have been studied by various scientists.

Photo and thermochromic properties of Schiff bases as well as their biological activity make them applicable in modern technology.Among others,they are used in optical computers,to measure and control the intensity of the radiation,in imaging systems,as wellas in the molecular memory storage,as organic materials in reversible optical memorise and photodetectors in biological systems.Due to photochromic properties,Schiff compounds could behave as photostabilizers,dyes for solar collectors,solar filters.They are also exerted in optical sound recording technology. Because of its thermal stability Schiff bases can be used as stationary phase in gas chromatography.

## 1.4 SCHIFF BASE –METAL COMPLEX

Transition metals are known to form Schiff base complexes and Schiff bases have often been used as chelating ligands. Their metal complexes have been of great interest for many years.It is well known that N,O and S atoms play a key role in the coordination of metals at the active sites of numerous metallobiomolecules .Schiff base is complexed with transition metals because they are widely used for industrial purposes .They exhibit a broad range of biological activities including antifungal, antimalarial, antibacterial ,antiproliferative, antiviral properties. Many Schiff base complexes show excellent catalytic activity in various reactions and in presence of moisture. The influence of certain metals on the biological activity of these compounds and their intrinsic chemical interest as multidentate ligands have promoted a considerable increase in the study of their coordination chemistry. There are certain metallo-elements without which the normal functioning of living organisms is inconceivable. Among the metallo-elements so called, metals of life ,four members form an island. These are Na, Mg , K , Ca ,the transition elements are V, Cr, Mn ,Fe , Co, Ni, Cu and Zn. These elements are present at trace and ultra-trace quantities and play vital role at molecular level in a living system. These transition elements are known to form Schiff base complexes. Schiff base complexes have been known since the mid nineteenth centuary.Schiff base metal complexes have occupied a central place in coordination chemistry after the work of Jorgensen and Werner.Hugo Schiff prepared complexes of metal-salicylate with primary amines .The study of bionuclear and polynuclear complexes of transition metal ions has received a growing attention in recent years. It has been an interesting area for chemists, physicists, biologists,since these complexes form the basis of several research fields such as bioinorganic chemistry, magnetochemistry, material science, catalysis, superconductivity etc. The main reason for the preference of transition metal ions over the other metal ions is ultimately due to their unique features such as flexibility to adopt more than one coordination geometries and the ability to exist in multiple oxidation states.

In this project , the Schiff base which was prepared by the condensation of benzaldehyde and aniline was made to complex with Zn(II) and Mg(II) ions. The Schiff base – Zn complex formed was used to study its antimicrobial activities. Zinc(II) Schiff base complexes are generally found to be effective emitters.They act as visual and fluorescent sensor. The electron transfer of nitrogen atom to the metal ion enhances the internal charge transfer. These fluorescent sensors are used in recognition of chiral organic compounds and drug molecules. Mixed valence Schiff Base complexes are functional materials in optoelectronics and molecular magnets

## 1.5 BENZALDEHYDE - ANILINE SCHIFF BASE

**Benzaldehyde** ($C_6H_5CHO$)

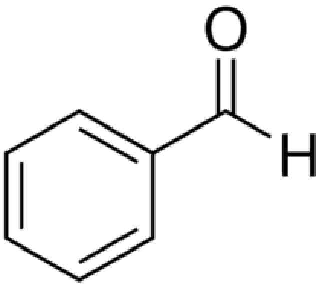



Fig 1.5: Structure of Benzaldehyde

Benzaldehyde is an organic compound consisting of a benzene ring with a formyl substituent. It is the simplest aromatic aldehyde and one of the most industrially useful.It is a colorless liquid with a characteristic almond-like odor. The primary component of bitter almond oil, benzaldehyde can be extracted from a number of other natural sources. Synthetic benzaldehyde is the flavoring agent in imitation almond extract, which is used to flavor cakes and other baked goods.

**Aniline**($C_6H_5NH_2$)

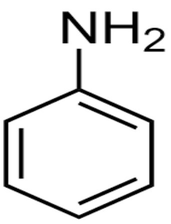



Fig 1.6: Structure of Aniline

Aniline consists of a phenyl group attached to an amino group, aniline is the prototypical aromatic amine. Its main use is in the manufacture of precursors to polyurethane and other industrial chemicals. Like most volatile amines, it has the odour of rotten fish. It ignites readily, burning with a smoky flame characteristic of aromatic compounds.

When benzaldehyde reacts with aniline it will form Benzylidene aniline with elimination of water molecule.

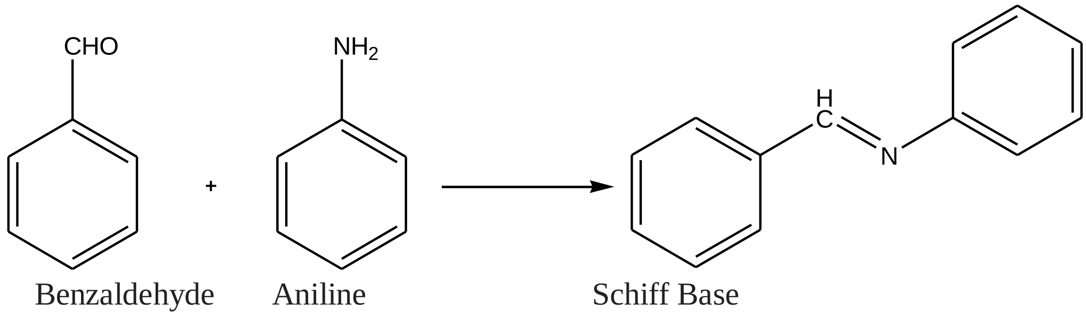



Fig 1.7: Formation of Schiff base from Benzaldehyde and Aniline

Schiff bases derived from an amino and carbonyl compound are an important class of ligands that coordinate to metal ions via azomethine nitrogen and have been studied extensively. In azomethine derivatives, the C=N linkage is essential for biological activity, several azomethine have been reported to possess remarkable antibacterial, antifungal, anticancer and activities. In this project we used Zn(II) and Mg(II) to form complexes with the sciff base.

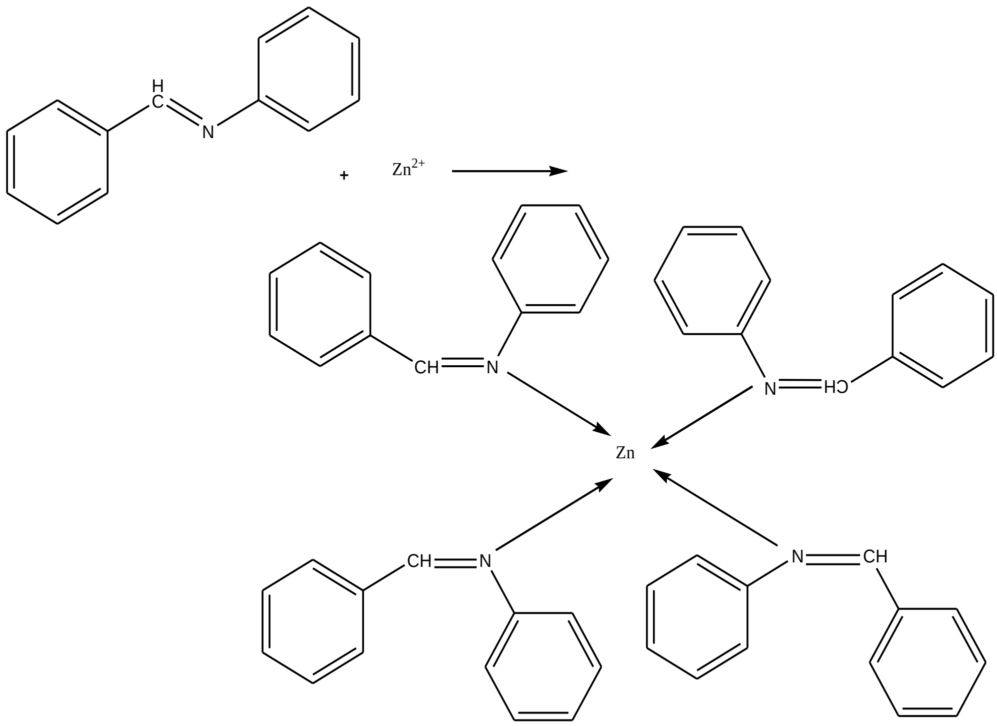



Fig 1.8:Expected structure of Schiff base-Zinc complex

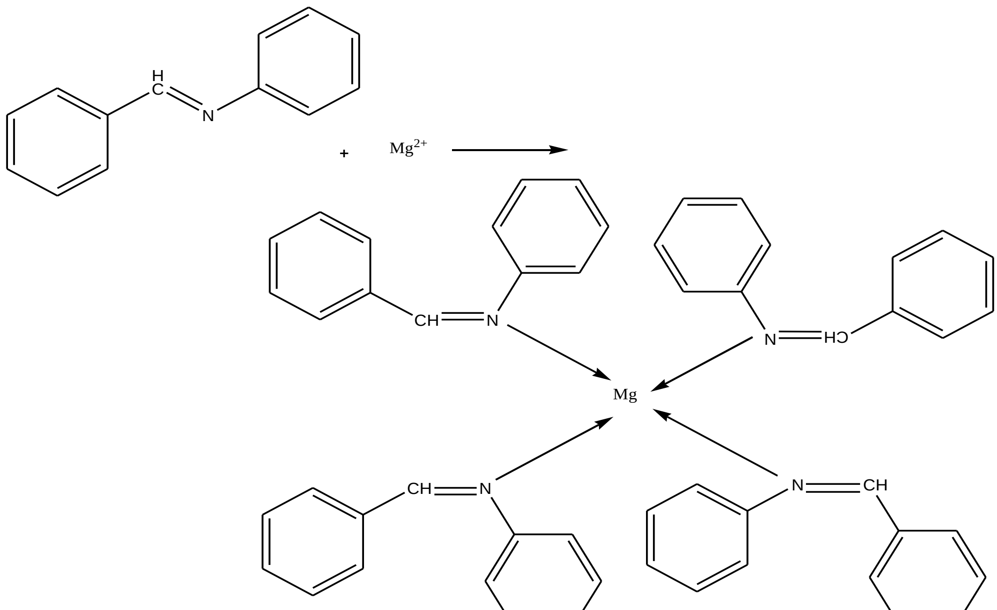



Fig 1.9:Expected structure of Schiff base-Magnesium complex

## 1.6 ABOUT THE METALS USED :

### 1.6.1 ZINC:

Zinc (Zn) is the first element in group 12 of the periodic table. In some respects zinc is chemically similar to magnesium: both elements exhibit only one normal oxidation state (+2), and the $Zn^{2+}$ and $Mg^{2+}$ ions are of similar size.The most common zinc ore is sphalerite (zinc blende), a zinc sulphide mineral.

Zn ion concentrations of $10^{-5}$-$10^{-7}$ M are required for optimal bacterial growth of most microorganisms in vitro. However , it is claimed that high zinc ion concentrations have some antibacterial properties .In dentistry, the antibacterial effects of Zinc oxide have been reported. Therefore in order to study the antimicrobial activity we complexed Zn with Schiff base.

Metal complexes of schiff bases have large numbers of application some of important application of Schiff base include its catalyst and biological activity. It is also used in dyes and in polymers.The biological activity of Schiff base include antimicrobial,antifungal antiviral activities and synergistic action on insecticides the Plants Growth Regulators and much more. Zinc is reported to show significant antimicrobial activitiy even at lower concentrations .The salts of zinc are common catalysts when used for their activity in a variety of organic reactions.

In this project we used Zn and Mg as the metals ,which thereby gets coordinated with the Schiff base to form Schiff base- metal complex.

### 1.6.2 MAGNESIUM :

Magnesium (Mg) is the ninth most abundant element in the universe. It occurs naturally only in combination with other elements, where it invariably has a +2 oxidation state. The free element (metal) can be produced artificially, and is highly reactive, it burns with a characteristic brilliant-white light.Magnesium is the eleventh most abundant element by mass in the human body and is essential to all cells and some 300 enzymes.Magnesium compounds are used medicinally as common laxatives,antacids and to stabilize abnormal nerve excitation or blood vessel spasm in such conditions as eclampsia.

## 1.7 CHARACTERIZATION TECHNIQUES

### 1.7.1 FT-IR SPECTROSCOPY

_Fourier transform infrared spectroscopy (FTIR) is a technique which is used to obtain an infrared spectrum of absorption or emission of a solid, liquid or gas. An FTIR spectrometer simultaneously collects high spectral resolution data over a wide spectral range. This confers a significant advantage over a dispersive spectrometer which measures intensity over a narrow range of wavelengths at a time. The term Fourier transform (a mathematical process) is required to convert the raw data into the actual spectrum.

Fourier transform spectroscopy is a less intuitive way to obtain the same information. Rather than shining a monochromatic beam of light at the sample, this technique shines a beam containing many frequencies of light at the sample and measures how much of that beam is absorbed by the sample. Next, the beam is modified to contain a different combination of frequencies, giving a second data point. This process is repeated many times. Afterward, a computer takes all this data and works backward to infer what the absorption is at each wavelength.

The beam described above in generated by starting with a broadband light source-one containing the full spectrum of wavelengths to be measured. The light shines into a Michelson interferometer – a certain configuration of mirrors, one of which is moved by a motor. As this mirror moves, each wavelength of light in the beam is periodically blocked, transmitted, by the interferometer, due to wave interference. Different wavelength are modulated at different rates so that at each processing is required to turn the raw data (light absorption for each mirror position) into the desired result (light absorption for each wavelength).the processing required turns out to be a common algorithm called the Fourier transform (hence the name “Fourier transform spectroscopy”). The raw data is sometimes called an “interferogram”.

FTIR analysis is used to characterize and identify unknown materials like films, solids, powders, or liquids. it also help to identify oxidation, decomposition, or uncured monomers in failure analysis investigations. Using this technique easily identify additives after extraction from a polymer matrix

**1.7.2 SEM**

SEM uses a focused beam of high energy electrons to generate a variety of a signals at the surface of solid specimens. The signals that derive from electron-sample interactions reveal information about the sample including morphology(texture), chemical composition, crystalline structure, orientation of materials making up the sample.In a typical SEM an electron beam is thermionically emitted from an electron gun.These electrons are then accelerated to a voltage between 1-40 kV.A series of condenser lenses focus the electron beam as it moves from source down the column.This narrow beam is used to scan in a raster fashion over the sample.Samples are mounted and placed into a chamber that is evacuated. When electron beam comes and hits the atoms of specimen those atoms absorb their energy and give off their own electron-SE.There is a detector to pick up SE which has a positive charge on it about 300 V.Secondary Electrons(SE) emitted from very close to specimen surface and can produce very high resolution image of sample surface revealing details less than 1nm.Back scattered electrons(BSE) emerge from deeper locations within specimen and consequently BSE images are poorer than SE images.We mainly use SE and BSE in SEM study.It is called SEM because the primary beam that comes down makes pixel by pixel image of the surface hit from left to right,top to bottom and every time it hits we get electrons from that particular part of specimen.Thus SEM uses beam of electrons to form image of the surface.

The detectors of the SEM collect the electrons coming off the sample and image is obtained on screen.Electronic amplifiers of various types are used to amplify the signals which are displayed as variations in brightness on a computer monitor. The electron beam is generally scanned in a raster pattern and the beam's position is combined with the detected signal to produce an image of the surface.The composition of surface is not necessarily same as the bulk.Frequently,in otherwise homogenous solid systems,inhomogeneties occur as one approaches an interface.The distribution of surface species become an important parameter when considering chemical analysis of a surface.

Each pixel of computer video memory is synchronized with the position of the beam on the specimen in the microscope.The resulting image is therefore a distribution map of intensity of signal being emitted from the scanned area of specimen.Due to very narrow electron beams,SEM micrographs have a large depth of field yielding a characteristic three dimensional appearance useful for understanding the surface structure of the sample.

### 1.7.3 EDX Analysis

Energy-dispersive X-ray spectroscopy (EDS, EDX, EDXS or XEDS), sometimes called energy dispersive X-ray analysis (EDXA) or energy dispersive X-ray microanalysis (EDXMA), is an analytical technique used for the elemental analysis or chemical characterization of a sample

Every atom has a unique number of electrons that residue under normal conditions in specific positions. These positions belong to certain shells, which have different, discrete energies.

The generation of the X- rays is a two step process ‘in the first step, the electron beam hits the sample and transfers part of its energy to the atoms of the sample ‘this energy can be used by the electrons of the “ jump” to an energy shell with higher energy or be knocked off from the atom. If such a transition occurs the electron leaves behind a hole. Holes have a positive charge and in second step of the process, attract the negatively charged electrons from higher energy shell fills the hole of the lower energy shell, the energy difference of this transition can be released in the form of an X-ray

This X – ray has energy which is characteristic of the energy difference between these two shells. It depends on the atomic number, which is unique property f every element .in this way ,X-rays are a “fingerprint” of each element can be used to identify the type of elements that exist in a sample.

Unlike BSE and TE, X rays are electromagnetic radiation just like light, and consist of photons. To detect them, the latest systems use the so called silicon drift detectors (SDDSs).these are superior to the conventional Si (Li) detectors due to higher count rays, better resolution, and faster analytical capabilities .These detectors are placed under an angle,very close to the sample , and have the ability to measure the energy of the incoming photons that belong to the X-rays .The higher the solid angle between the detector and the sample , the higher the X-rays detection probability ,and therefore the likelihood of acquiring the best results . The data that is generated by EDX analysis consists of spectra with peaks corresponding to all the different elements that are present in the sample. Every element has characteristic peaks of unique energy, all extensively documented. Furthermore EDX can be used for qualitative (the type of elements)as well as quantitative (the percentage of the concentration of each element of the sample )analysis. In most SEMs dedicated software enables auto identification of the peaks and calculation of the atomic percentage of each element that is detected. One more advantage of this technique is that it is a non destructive characterization technique, which requires little or no sample preparation.Benefits from EDX analysis are improved quality control, rapid identification of contaminant and source, full control of environmental factors, emissions, higher production yield, easy to identifying the source of the problem in process chain.

## 1.8 ANTIMICROBIAL ACTIVITY

An antimicrobial is an agent that kill the microorganisms or inhibit their growth. Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. Antibiotics are used against bacteria and antifungal used against fungi are the best example for this. Another group of classification is based according to their function.Microbicidal are the agents that kill microbes, while those that merely inhibit their growth are called biostatic. Antimicrobial chemotherapy is the therapy which use antimicrobial medicines to prevent infection. Antimicrobial prophylaxis is the use of antimicrobial medicines to prevent infection.Disnifecants are the main class of antimicrobial agents ("non selective antimicrobials" such as bleach), which kill a wide range of microbes on non-living surfaces to prevent the spread of illness, antiseptics(which are applied to living tissues and help to reduce the infection during surgery),and antibiotics(which destroy microorganisms within the body).the term "antibiotic" originally described only those formulations derived from living organisms but is now also applied to only synthetic antimicrobials.

Metals have a wide range of chemical properties that govern their reactivity in living cells. Metals have been used as a antimicrobial agents since antiquity. From the recent studies indicates that, different metal cause discrete and distinct types of injuries to microbial cells as a result of oxidative stress, protein dysfunction or membrane damage. Some have metals have been used as antimicrobial agents because of their potent toxicity to bacteria and yeast. Now antimicrobial metal compounds including metallic surfaces and coatings, chelates and nanomaterials have a various application in the fields like industry, agriculture and health care. These innovations were made possible following the discovery that certain metal disrupt antibiotic –biofilms, exert synergistic bactericidal activities with other biocides, inhibit metabolic pathways in a selective manner and kill multidrug resistant bacteria.

With the global rise of antibiotic resistance and the lack of news antibiotics reaching the market, research metal based antimicrobial therapy has now moved towards clinical studies. Because of the outbreak of infectious diseases caused by different pathogenic bacteria and the development of antibiotic resistance researchers are searching for new antibacterial agents.Therfore new antimicrobial agents materials have to synthesize for the treatment of resistant bacterial diseases.

Different biologically active compounds used as drugs possess modified pharmacological and toxicological potentials when administered in the form of metal based compounds. Mainly ions like cobalt, copper, nickel and zinc because of their low molecular weight complexes and therefore, prove to be more beneficial against several diseases. The antimicrobial activities of metal complexes depended more on the metal center itself than on the geometry around metal ion.chelation of bulky ligands to metal cations reduces the polarity of the ion.Due to the glycolipophilic nature of cell wall, an increase in the lipophicity of a coordination compound enhances its ability to penetrate bacterial cell memberane.

The antimicrobial activities of the synthesized Zn (II) complexes was done by using gram positive organisms–Escherichia coli. This bacterial strain was choosen as it is a known human pathogen. Escherichia coli, also known as E.coli is a facultative anaerobic, rod shaped non –spore-forming rod, 1-2micrometer wide3-30micro meter long bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms (endotherms). Some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination.Virulant strains of E.coli can cause gastroenteritis,urinary tract infections,neonatal meningitis,hemorrhagic colitis,Crohn's disease.E.coli is expelled into the environment within fecal matter.The bacterium grows massively in fresh fecal matter under aerobic conditions.

# Chapter 2

## MATERIALS AND METHODS

All reagent used were of A.R grade .The Scanning electron micrographs were taken using a Hitachi S-2400 instrument.EDX image was obtained from EDX-800,Japan instrument.The FTIR spectra was recorded on a broker IFS – 55 spectrometer using KBr pellets :

REQUIREMENRTS:

- Benzaldehyde
- Aniline
- Ethanol
- Zinc chloride
- Magnesium chloride
- Ammonia solution
- Measuring cylinder
- Glass rod
- Filter paper

### 2.1 SYNTHESIS OF BENZALDEHYDE-ANILINE SCHIFF BASE

50 ml of Benzaldehyde and 50 ml of Aniline is measured using a measuring cylinder and poured into a 250 ml beaker containing crushed ice.This beaker is placed over an ice bath and stirred well using a glass rod . A solid yellow precipitate was obtained. The mixture formed was filtered using filter paper and dried. The solidified substance obtained is the Benzaldehyde-Aniline Schiff base .

## 2.2 SYNTHESIS OF SCHIFF BASE-METAL COMPLEXES

1.81 gram of Schiff base(0.01 mol) was dissolved in a minimum quantity of (10 ml) ethanol. 1.36 gram(0.01 mol) of Zinc Chloride was dissolved in minimum quantity of distilled water. 5 drops of ammonia solution was added and the mixture was shaken well. It was refluxed for 1 hour using water condenser. After refluxing,crushed ice was added into it and metal complexe was precipitated, The obtained precipitate was filtered using filer paper and dried at room temperature. The precipitate hence obtained is the Schiff base-Zinc complex.

Schiff base – magnesium complex was synthesized using Magnesium acetate as one of the reagent.1.8 gram of Schiff base was dissolved in a minimum quantity of (10 ml) ethanol.1.423gram of magnesium acetate(0.01 mol) was dissolved in minimum quantity of distilled water.5 drops of ammonia solution was added.it was refluxed for 1 hour using water condenser. Then crushed ice was added into it and metal complex was precipitated,.The obtained precipitate was filtered using filter paper and dried at room temperature. The precipitate hence obtained is Schiff base – Magnesium complex.

## 2.3 PROCEDURE FOR ANTI MICROBIAL STUDIES (BROTH DILUTION)

### 2.3.1 Preparation of microbial culture

Using aseptic techniques a single pure colony was transferred into a 10 ml of nutrient broth, capped and placed in incubator overnight at 37°C.

### 2.3.2 **Sample preparation**

Samples was prepared in a concentration of 0.0125g/ml,0.025g/ml,0.037g/ml, 0.05g/ml, 0.075g/ml ethanol was used as solvent.

### 2.3.3 **Preparation of the microtiter plate plates**

Microtitre plates were prepared under aseptic conditions. A sterile 96 well plate was labeled. 100µl of test material was added into each wells, 100µl of nutrient broth was added to each well, Finally 100µl of microbial suspension was added to each well (test). Control dilutions of test material were also kept (extract control). A column with all solutions except the test compound was prepared as organism controls (nutrient broth and microbial suspension). A well with 100µl of microbial suspension, 100µl nutrient broth and 100µl of ethanol was kept as solvent control. Plate was wrapped loosely with cling film to ensure that organism did not become dehydrated. The plates were incubated at $37^0$ C for 24 hours and OD reading was taken ($OD_{600}$) after sufficient incubation.

Optical density of final test was obtained from subtracting the extract control OD from the test OD. The % of inhibition was calculated from the following equation:

$$\% \text{ of inhibition} = (\text{Control- final Test})/\text{Control} \times 100$$

Where control = organism control, growth in this well is considering as 100% growth.

% of inhibition indicate the antimicrobial activity of the sample.

Solvent control was kept to knew the antimicrobial activity of solvent.

# Chapter 3

## Results and discussion

3.1 <u>IR SPECTRAL STUDIES OF SCHIFF BASE AND ITS METAL COMPLEXES</u>

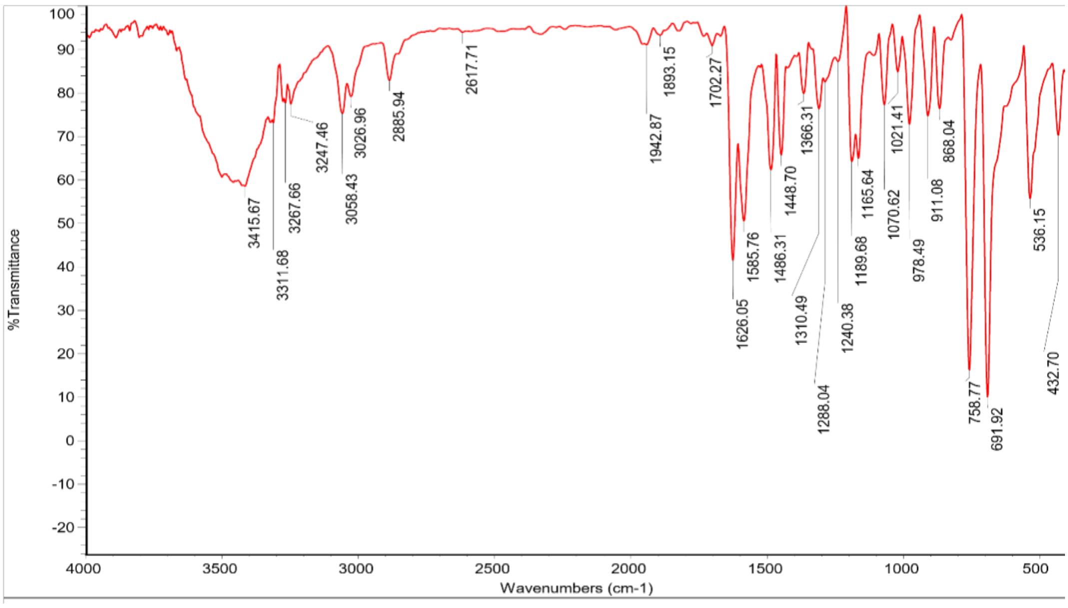



Fig 3.1.1 IR Spectra of Schiff base

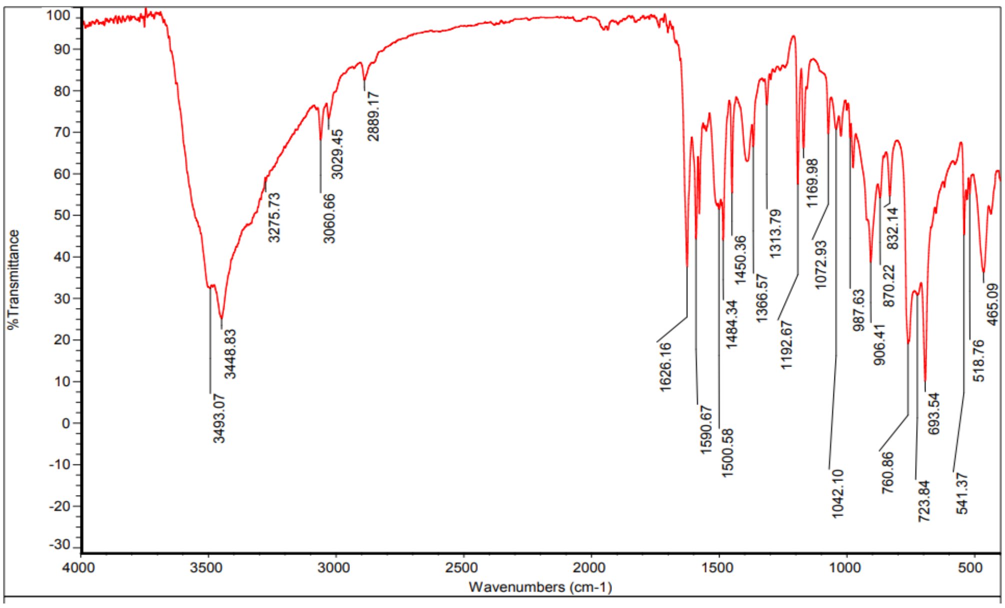



Fig 3.1.2 IR Spectra of Zinc Schiff base metal complex

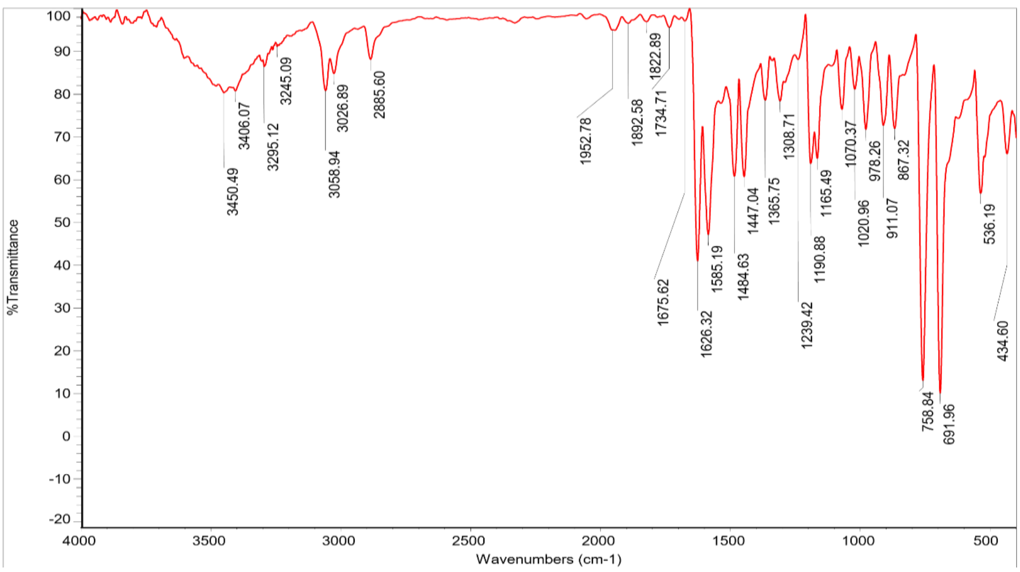



Fig 3.1.3 IR Spectra of magnesium-Schiff base complex

Table 1.1: IR-analysis of Schiff base and its metal complexes

| SL.NO | COMPOUND | ʋ (C=N)$cm^{-1}$ | ʋ (O-H)$cm^{-1}$ | ʋ (M-N)$cm^{-1}$ |
|---|---|---|---|---|
| 1 | Schiff base | 1626.05 | 3415.67 | - |
| 2 | Zinc Schiff base | 1626.16 | 3493.07 | 465.09 |
| 3 | Magnesium Schiff base | 1626.32 | 3450.49 | 434.60 |

The IR spectral data of ligand shows a band at a region of 1580–1680 which is assigned to C=N stretching frequency, a feature found in sciff base. This band is also observable in complexes, suggesting that ligand has coordinated to the metal.In case of complexes, the band in the region 440-495$cm^{-1}$ is attributed to ν(M-N) stretching frequencies respectively , conforming coordination of Schiff base to metal ions.

3.2.<u>SEM OF SCHIFF BASE AND ITS METAL COMPLEXES</u>

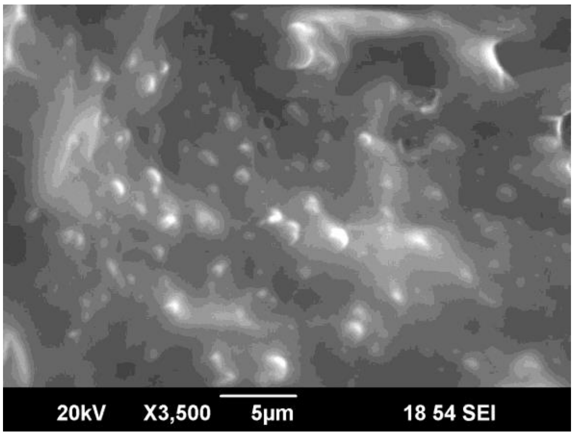



Fig 3.2.1 :SEM of Schiff base

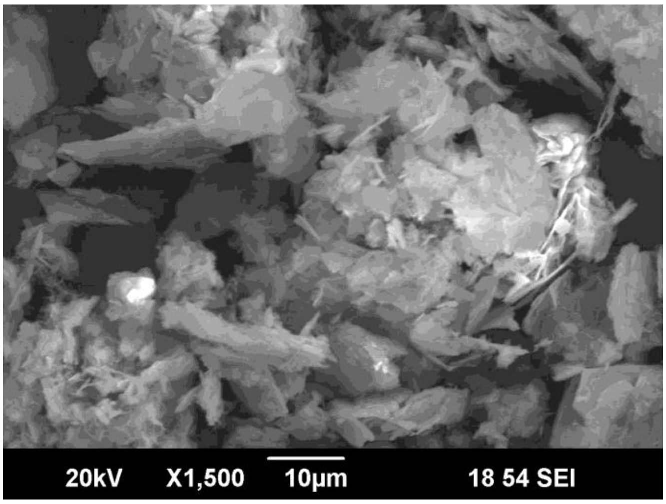



Fig 3.2.2 :SEM of Schiff base-Zn metal complexes

The surface morphology of Schiff base and the metal complexes have been examined using scanning electron microscope and the respective images are shown in the figure above. From the figure, Schiff base ligand shows a microporous appearance. Metal complexes contribute to greater segments or crystalline region compared to the parent ligand which may arise from the contraction of the voids by the cooperative contribution of ligand for complexion with metal ions or the disappearance of the voids due to complexation with metal ions. This is a further evidence for complexation.

## 3.3 EDX OF SHIFF BASE AND ITS COMPLEXES

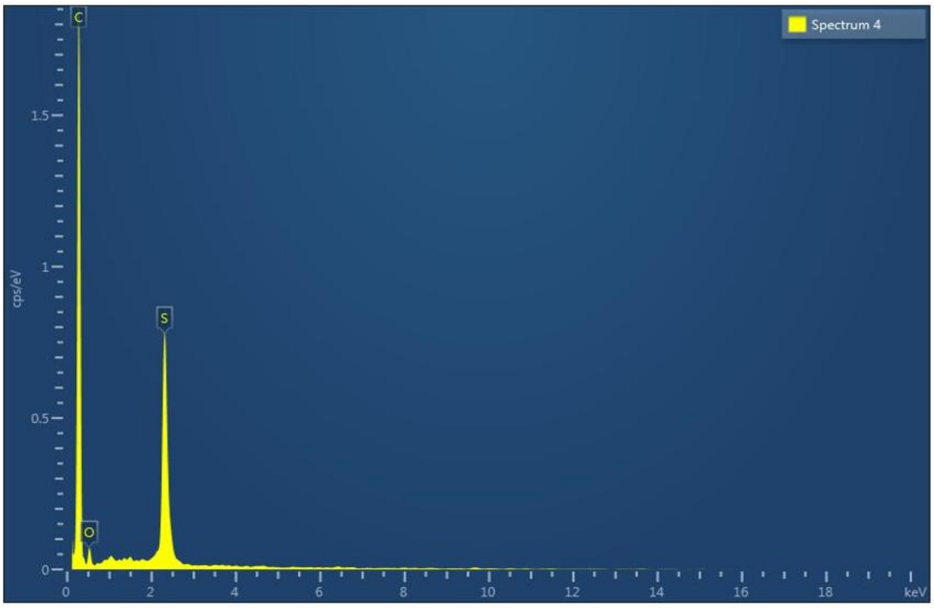



Fig 3.3.1 :EDX spectrum of Schiff base

Table 3.3.1: EDX analysis of Schiff base

| ELEMENT | Weight% | Atomic% |
|---|---|---|
| C | 87.01 | 92.51 |
| O | 5.8 | 4.63 |

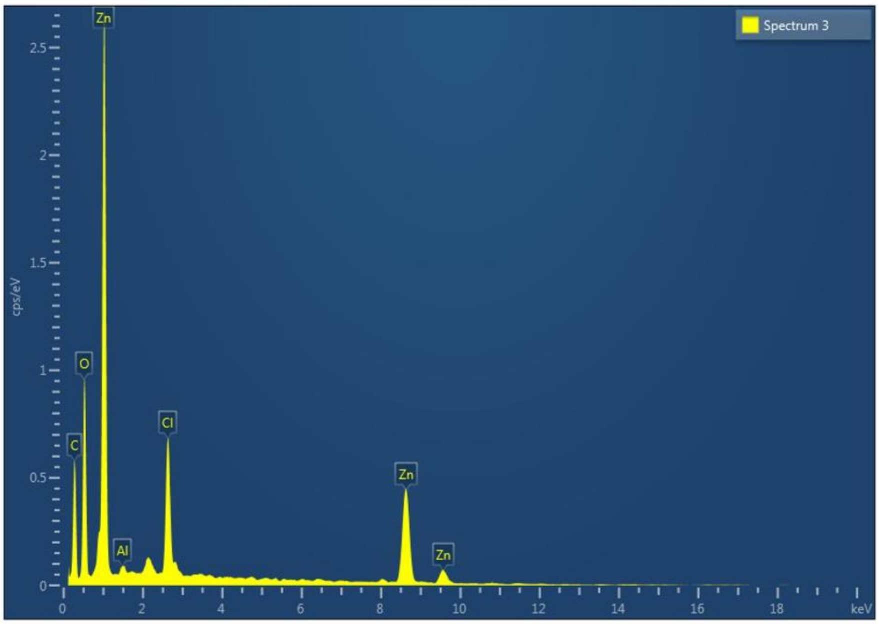



Fig 3.3.2 :EDX spectrum of Schiff base-Zn metal complex

Table 3.3.2: EDX-analysis of Schiff base-Zn metal complexes

| ELEMENT | Weight% | Atomic% |
|---|---|---|
| C | 36.22 | 57.62 |
| O | 24.69 | 29.5 |
| Zn | 33.41 | 9.77 |

EDX spectra is used to calculate the percentage level of the elements present in the metal complex like C, O,Zn in Zn-Schiff complex.

The results by energy dispersive X-ray analysis having indicated that there were Zn peaks, which meant there were metal attachment to the Schiff base and the % of metal attached to the Schiff is obtained from EDX.

## 3.4 ANTIMICROBIAL ACTIVITY

Anti-microbial activity of samples were studied against against E.coli.

### ANTIMICROBIAL ASSAY BY BROTH DILUTION METHOD

Table 3.4.1 :Antimicrobial study for Schiff base

| Sample | **Concentration (g/ml)** | **0.0125 g/ml** | **0.025 g/ml** | **0.037 g/ml** | **0.05 g/ml** | **0.075 g/ml** |
|---|---|---|---|---|---|---|
| Benzaldehyde-Aniline Schiff Base | **Control OD** | 1.169 | 1.169 | 1.169 | 1.169 | 1.169 |
| | **Test OD** | 0.903 | 1.078 | 1.346 | 1.637 | 1.870 |
| | **OD of extract** | 0.311 | 0.497 | 0.798 | 1.128 | 1.392 |
| | **Final Test OD** | 0.592 | 0.581 | 0.548 | 0.509 | 0.478 |
| | **% of inhibition** | 49.35 | 50.29 | 53.12 | 56.45 | 59.11 |

Table 3.4.2 :Antimicrobial study for Schiff base-Zn metal complex

| Sample | Concentration (g/ml) | 0.0125 g/ml | 0.025 g/ml | 0.037 g/ml | 0.05 g/ml | 0.075 g/ml |
|---|---|---|---|---|---|---|
| Schiff Base-Zn complex | **Control OD** | 1.169 | 1.169 | 1.169 | 1.169 | 1.169 |
| | **Test OD** | 1.484 | 2.118 | 2.434 | 2.509 | 2.731 |
| | **OD of extract** | 1.116 | 1.900 | 2.257 | 2.419 | 2.646 |
| | **Final Test OD** | 0.368 | 0.218 | 0.177 | 0.09 | 0.085 |
| | **% of inhibition** | 68.52 | 81.35 | 84.85 | 92.30 | 92.72 |

Table 3.4.3:Ethanol control

| | | |
|---|---|---|
| **Ethanol control** | **Control OD** | 1.169 |
| | **Test OD** | 0.609 |
| | **OD of extract** | 0.010 |
| | **Final Test OD** | 0.599 |
| | **% of inhibition** | 48.75 |

The sensitivity of gram negative bacteria E.coli, towards the Schiff base and their metal complexes were analyzed by broth dilution method. The analysis of antimicrobial activity of 0.0125g/ml, 0.025g/ml, 0.037g/ml, 0.05g/ml,0.075g/ml of Schiff base and its metal complexes were done.It was found that 0.075g/ml sample of metal complex showed greater antibacterial activity than 0.0125g/ml of sample..This indicates that as the amount of metal complex is increased the percentage of inhibition also increases.From the result it is evident that regardless of the concentration of the sample,the percentage inhibition shown by Schiff base-Metal complex is higher than that of Schiff base at any instant. At the highest concentration of samples taken Benzaldehyde-Aniline Schiff Base showed a percentage inhibition of 59.11 while complex showed a percentage inhibition of 92.72 against E.coli bacteria.

The coordination of metal ions with bulky Schiff base through chelation decreases the polarity of metal ions .This due to the overlap of the ligand orbital and partial sharing of positive charge of the metal ion with donor groups.it also increases the delocalization of pi electrons over whole chelate ring. This enhances the lipophilic nature of complexes. The lipophilicity of the lipid membrane is an important factor in determining antibacterial activity. Lipid membrane of cell favors the passage of lipid soluble materials. This increased the lipophilicity enhance the penetration of complexes into the lipid membrane and thus block the metal binding site on enzymes of microorganisms .The metal complexes also disturb the respiration of cell and inhibit the cell growth by interfering bacterial protein production, DNA replication, or other aspects of bacterial cell metabolism .The antibacterial potency of metal complexes depends on the impermeability of the cells of the microbes.

# Chapter 4

## CONCLUSIONS

Synthesis of schiff base from benzaldehyde and aniline was carried out. The synthesized schiff base ligands have been successfully complexed with the metal ions Zn and Mg.

Characterization of the above schiff base and its metal complexes were carried out. The FTIR spectral data of ligands shows a band at a region of 1580-1680 $cm^{-1}$ which is assigned to C=N stretching frequency, a feature of schiff base. This band is also observable in complexes, suggesting the ligand has coordinated to the metal. In the case of complexes, the band in the region 440-495$cm^{-1}$ are attributed to $\upsilon$ (M-N) stretching vibrations, conforming coordination of schiff base to metal ions.

Surface morphology of the Schiff base and the metal complexes have been examined using scanning electron microscopy. Metal complexes showed greater segments or crystalline region compared to the parent ligand which may arise from the contraction of the voids or the disappearance of the voids by the cooperative contribution of ligand for complexation with metal ions or the disappearance of the voids due to the complexation with metal ions. This gave a further evidence for complexation.

Another evidence obtained for complexation is from EDX spectra. It is used to calculate the percentage of elements present in the metal complex. It showed the percentage of Zn metal present in the metal complex . The results from this analysis having indicated that there were Zn peaks, which meant attachment to the Schiff base and the % of metal attached to the Schiff base is obtained.

The inhibitory action of schiff base and their complexes against E.coli bacteria were studied using broth dilution method. Based on antibacterial study, it was concluded that metal complexes of schiff base are more efficient antimicrobial agents than its native form. The increased inhibitory action of metal complexes than the parent ligand under experimental conditions could be explained on the basis of chelation. However the antimicrobial potentiality of metal complex is highly dependent of the metal ion used for formation of metal complexes. It has the ability to kill the bacteria which is indicated by the percentage of inhibition. Result of antimicrobial activity reflect that the schiff base metal complex has an antimicrobial activity The analysis of antimicrobial activity of 0.0125g/ml,0.025g/ml,0.037g/ml,0.05g/ml,0.075g/ml of Schiff base and its metal complexes were done and was found that 0.075g/ml sample showed greater antibacterial activity than 0.0125g/ml of sample. This indicates that as the amount of metal complex is increased the percentage of inhibition also increases. It is found that regardless of the concentration of the sample,the percentage inhibition shown by Schiff base-Metal complex is higher than that of Schiff base at any instant.Thus the metal complexes of benzadehyde-aniline schiff base could find potential application as an antibacterial agent.

1. Dr.S.Arockia Edwin Xavier,N B Srividya “Synthesis and study of Schiff base ligands”-published 2014.
2. https://en.m.wikipedia.org/wiki/Schiff_base
3. NahidNishant,FahminaZafar,Synthesis,characterization andbiological activity of transition metal ions based on Schiff base ligand
4. ShalinKumar,DurgaNathDhar.p.NSaxena,Applications of metal complexes of Schiff base/A review,Journal of scientific and industrial research Volume23,2011.
5. A.S.P.Azzouz,R.T.Ali,Synthesis of Schiff bases derived from Benzaldehyde and salicylaldehyde with some amino acids by a new developed method-National journal of chemistry volume37,2010.
6. M.S.MohyEldin,A.I.Hashem.A.M.Omer-Preparation,characterization and antimicrobial evaluation of novel cinnamyl chitosan Schiff base international journal of advanced research 2015.
7. S.Sreekala.S.Syed Shafi Synthesis and characterization of Schiff base derivative,schiolars research library.
8. N.K.Gondia,S.K.Sharma Spectroscopic characterization and photophysical properties of Schiff base metal complex,Journal

of molecular structure Volume1171,5 November 2018,pages 619-625

9. Saudi Chemical society and the Arab union of chemist,Arabian journal of chemistry ,volume12,issue 1,ISSN1878/5352/Jan 2k19
10. Kostava Irena,Advances in Schiff base metal complexes as potent antioxidants,Article in current medicinal chemistry 20(36).June 2013
11. Amer Said,El-Ghamry Hoda;Synthesis ,spectal,anti tumour and anti microbial studies on Cu(II) complexes of purine and triazole schif base derivatives,Journal of molecular structure volume 1049,8 october 2013,pages 325-326.
12. https://en.m.wikipedia.org/wiki/benzaldehyde
13. https://en.m.wikipedia.org/wiki/aniline
14. Etroki Mohamed A,Modificationnon Synthesis of mixed ligand chelates by using di and tri valent transition metal ions with Schiff base as primary ligand,Volume 4 No.3,August 2014
15. Al Zoubi,W.(2013) Biological activities of Schiff base and their complexes;A review of recent works,International journal of organic chemistry,3,73-95.http://dx.doi.org/10.4236/ijoc.2013.33A008
16. https://en.m.wikipedia.org/wiki/zinc
17. https://en.m.wikipedia.org/wiki/magnesium
18. Manpreet,Divya ,Singh Schiff bases and their metal complexes as anti cancer agents:a review,home/current bioactive compounds,volume 11,number 4.
19. Suman Malif,Bharathi jain,Amar Sohali Mizra,Archana Singh ,Pharmachutical and Chemical journal,2016,3(4);119-124

20. https://en.m.wikipedia.org/wiki/Schiffbasemetalcomplex
21. Reda F M.Elshaarawy,Journal of molecular liquids,volume223,November2016,pages1123-1132
22. Wail Al Zoubi,A new azoSchiff base: Synthesis,Characterization,biological activities and theoretical studies of its complexes
23. Safa S Hussan ,Synthesis , Characterization,biological and molecular docking studies of some amino acid Schiff base with their cobalt (II)complexes.Vol.7 no. 5,October 2017
24. Wali al Zoubi ,biological activities of Schiff bases and their metal complexes:a review of recent works.Volume 3,(November 2013).
25. Ashu Chaundhary and Anshul Singh,Schiff bases:an emerging potent class of chemicals , Volume 3,issue 6-2017