#### REMOVAL OF HEAVY METALS BY POLYMERIC MATERIALS

Submitted in partial fulfillment of the requirements for the award of Bachelor of Science in Chemistry

Ву

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# DEPARTMENT OF CHEMISTRY SCHOOL OF SCIENCE AND HUMANITIES

SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY

Accredited with Grade "A" by NAACI 12BStatus by UGC I Approved by AICTE

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#### **DEPARTMENT OF CHEMISTRY**

This is to certify that this Project Report is the bonafide work of NANSI POSHIYA A (Reg.No:39030018), SANTHOSH KUMAR N (Reg.No:39030030) and RAJALAKSHMI I (39030023) who this carried Out the project entitled Removal of heavy metals by polymeric materials under our supervision from November 2021 to May 2022.

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#### **DECLARATION**

We, NANSI POSHIYA A (Reg.No:39030018), SANTHOSH KUMAR N (Reg.No:39030030) and RAJALAKSHMI I (39030023). Hereby declare that the Project Report entitled Removal of heavy metals by polymeric materials done by us under the guidance of Dr. S. SUPRIYA is submitted in partial fulfillment of the requirements for the award of Bachelor of SCIENCE degree in CHEMISTRY.

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#### **ABSTRACT**

The present investigation reports, the complex formation of NiSO4 with polyvinyl alcohol (PVA) and the synthesis of PVA-stabilized NiSO4 particles. This PVA-NiSo4 composite has been prepared via chemical reduction method using PVA-NiSO4 complex as precursor. At first, Co (II) ions were stabilized in PVA matrix via complex formation with OH groups; subsequently, this PVA-NiSO4 macromolecular complex as precursor reacted with ascorbic acid as reducing agent at pH=12 to prepare PVA-Cuso4 composite. The products were characterized by FTIR, XRD, FE-SEM, HRTEM, Visible Spectroscopy and atomic absorption. In the following, the antibacterial properties of as-prepared composites were examined against Grampositive (Bacillus thuringiensis) and Gram-negative bacteria (Escherichia coli), and the results showed excellent antibacterial activity of these materials. Treated with polymers, this effluent will be safe to send to drain after being Run through a filter press to separate polymerized solids and water. The conventional processes for removing heavy metals from wastewater include many processes such as chemical precipitation, Adsorption, ion exchange, and electrochemical deposition. Chemical precipitation is the most widely used for heavy metal removal from inorganic effluent.

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# CHAPTER – 1 INTRODUCTION

National economic development is a process of improving the physical and spiritual living conditions of the country through the production of property, improvement of social relations, improvement of cultural quality. However, due to the reason changes in the negative trend of the environment. It has raised the issue that timely solutions should be taken to move towards a sustainable economic development, in association with naturally environmental production. Water is a valuable human resource but not permanent. Nowa-days source of water for living and natural water in developing countries. Such a Vietnam, Laos and Cambodia are now increasingly polluted, especially heavy metals contaminations in particular water environment in many urban areas, industrial parks and craft villages in these big cities. A novel conducting polymer-based absorbent, polyp role (PPy) fine powder has successfully been prepared as a new adsorbent and utilized in the adsorption of heavy metals ions like arsenic, Zinc and cadmium ions from aqueous solution. PPy was chemically synthesized by using FeCl<sub>3</sub>.6H<sub>2</sub>O as a oxidant. The prepared PPy adsorbent was characterized by Brunauer. Methods available for removal of heavy metals Factors to be considered in the choice of a method to be adopted for the treatment of heavy metal-bearing wastewater should include: high removal efficiency, economic feasibility in terms of materials, equipment and energy, applicability to small, intermediate and large scales, low productivity of highly enriched spent materials and capability of reducing heavy metal ion concentration to levels below established regulatory standards.

# 1:1 Poly Vinyl Alcohol

Poly is a water-soluble synthetic polymer. It has the idealized formula [CH₂CH]□. It is used in papermaking, textile warp sizing, as a thickener and emulsion stabilizer in PVAc adhesive formulations, in a variety of coatings, and 3D printing. It is colourless and odourless: Formula: (C₂H₄O) xPVA is used in sizing agents that give greater strength to textile yarns and make paper more resistant to oils and greases. It is also employed as a component of adhesives and emulsifiers, as a water-soluble protective film, and as a starting material for the preparation of other resins

#### 1:2 Nickel and Uses

The most crucial use of this element is that it is used to make coins. It is used in making wires. It is used in gas turbines and rocket engines as it has the capability to resist corrosion even at high temperature. It is used to make a variety of alloys which are further used to make armour plating, nails, or pipes. Nickel-containing products play an important role in our daily lives. Compared with other materials, nickel-containing products possess better corrosion resistance, greater toughness, more strength at high and low temperatures, and a range of special magnetic and electronic properties. Therefore, most nickel production is used for alloying elements coatings, batteries, and some other uses, such as kitchen wares, mobile phones, medical equipment, transport, buildings, power generation and jewellery.

# 1:3 Heavy Metal

Heavy metal, genre of rock music that includes a group of related styles that are intense, virtuosic, and powerful. Various treatment technologies employed for the removal of heavy metals include chemical precipitation, ion exchange, chemical oxidation, reduction, reverse osmosis, ultrafiltration, electrodialysis and adsorption.

# **Heavy Metal Pollution**

Metal pollution has emerged due to anthropogenic activity which is the prime cause of pollution, primarily due to mining the metal, smelting, foundries, and other industries that Heavy are metal-based, leaching of metals from different sources such as landfills, waste dumps, excretion, livestock and chicken manure. Heavy metals normally occur in nature

and are essential to life but can become toxic through accumulation in organisms. Arsenic, cadmium, chromium, copper, nickel, lead and mercury are the most common heavy metals which can pollute the environment. The term heavy metal refers to any metallic chemical element that has a relatively high density and is toxic or poisonous at low concentrations. Examples of heavy metals include mercury (Hg), cadmium (Cd), arsenic (As), chromium (Cr), thallium (Tl), and lead (Pb). For example, "extreme" music genres, like heavy metal, appears to be great for reducing negative emotions and regulating anger and depression. Staving off these negative emotions will pay dividends for your brain's ability to function at peak performance. The ecosystem is being ruined to the fact that the heavy metals are entering the food chain. Heavy metals also affect the biodegradability of organic pollutants, making them less degradable and thus causing double the effect of polluting the environment. Heavy metals become toxic when they are not metabolised by the body and accumulate in the soft tissues. They may enter the human body through food, water, air or absorption through the skin when they come in contact with humans in agriculture, manufacturing, pharmaceutical, industrial or residential settings.

# 1:4 Nickel Sulphate

Nickel (II) sulfate, or just nickel sulfate, usually refers to the inorganic compound with the formula NiSO<sub>4</sub>(H<sub>2</sub>O)<sub>6</sub>. This highly soluble blue green colored salt is a common source of the Ni<sup>2+</sup> ion for electroplating. For blackening Brass and Zinc. As a mordant in dyeing and printing metals, textiles and ceramics. As a coating for many metals. In the production of driers that are used in protective shields.

# **Properties of Nickel Sulphate**

Its melting point is more than 100° C and the boiling point is 840° C. A metal Sulfate will have nickel ion with charge +2 as the metal ion. This chemical is yellow-green crystals which are soluble in water and insoluble in alcohol.

Also, it is blue, emerald-green, or green crystals that are soluble in water and alcohol. Nickel Sulfate is useful in plating baths. And it is useful as an intermediate in the production of nickel ammonium Sulfate. We may use it as a mordant in dyeing, and printing textiles;

coatings, and ceramics. It is a strong reducing agent. It is incompatible with oxidizers like chlorates, nitrates, peroxides, permanganates, perchlorates bromine, fluorine, etc.

## 1:5 Preparation of Nickel Sulphate

We may get this Nickel Sulfate by several methods. Scientists prepare it by dissolving nickel metal, its oxide, or its carbonate in sulfuric acid. In these methods, powdered metal or black nickel oxide is added to hot dilute sulfuric acid, or nickel carbonate is added to dilute sulfuric acid

#### **Uses of Nickel Sulfate**

#### Some main uses of it are as given below:

- It is useful in laboratories.
- It is useful as a calibrate for magnetic susceptibility measurements.
- Also, it is useful to make other nickel compounds
- It is useful in the electroplating of nickel on other metals.

# 1:6 Chemical Properties of NiSO<sub>4</sub>

- 1. It is non-flammable.
- 2. It is insoluble in alcohol and ether.
- 3. It is not compatible with strong acids.
- 4. After dissolving in water, it forms an acidic corrosive solution. On corrosion, it emits fumes of oxides of sulfur.
- 5. When it is dissolved in water, and the solution is heated to decompose, it liberates fumes of nitrous oxide. Fumes of Nickel Sulphate are highly toxic.

- 6. As its aqueous solution is acidic, the pH value is 4.5.
- 7. On heating at 103°C, aqueous Nickel Sulfate loses the water molecules. And heating up to 848°C, the anhydrous form decomposes to form sulfur trioxide and nickel oxide. The chemical expression is
- 8. NiSO<sub>4</sub>→NiO+SO<sub>3</sub>NiSO<sub>4</sub>→NiO+SO<sub>3</sub>
- Nickel Sulfate gives double by-products of salts on reacting with alkali metals or ammonium sulfates. These sulfates are isomorphous.
- 10.Reacting with hydrochloric acid gives Sulphur acid and Nickel Chloride. The chemical reaction is as shown,

#### Effects of NiSO<sub>4</sub> on Human Health

Nickel Sulfate can be assimilated into the human body by inhalation or ingestion. Once it is absorbed in the body, it concentrates in the lungs, gut, kidneys and liver. Exposure to NiSO<sub>4</sub> causes:

- Skin irritations
- 2. Giddiness
- 3. Lassitude
- 4. Headache
- 5. Myalgia
- 6. Gastrointestinal effects such as abdominal pain, nausea, vomiting, diarrhea
- 7. Hemorrhagic gastritis
- 8. Transient hyperbilirubinemia and albuminuria
- Bronchial asthma
- 10. Dyspnoea

- 11. Mild cyanosis
- 12. Chronic obstructive airways syndrome
- 13. Chronic urticaria
- 14. Dermatitis

#### Effects of Nickel Sulfate

#### **Effects of Nickel Sulfate Include**

Nickel sulfate can be incorporated into the human body by inhaling or ingesting it. Nickel sulfate concentrates in the lungs, intestines, kidneys, and liver once it is taken into the body. Nickel sulfate causes poisoning by being exposed to it. Nickel Sulfate causes Chronic obstructive pulmonary disease, Dyspnoea, Mild cyanosis, Chronic urticaria, Dermatitis. Nickel Sulfate also causes Irritation of the skin, giddiness, lassitude, headache, myalgia, abdominal pain, nausea, vomiting, diarrhea, hemorrhagic gastritis, transient hyperbilirubinemia, and albuminuria are some of the gastrointestinal side effects.

# 1:6 What are transferring and packaging specifications while handling NiSO<sub>4</sub>

While treating Nickel sulphate, toxic fumes and heat are generated.it has hazardous effects on health, it should be handled, packed and shaped cautiously. Follow are the safety measures while handling NiSO<sub>4</sub>.

- Proper training should be given before handling NiSO<sub>4</sub>
- The area where treatment, storage of NiSO<sub>4</sub> is going to take place should be marked and isolated from other activities.
- As it is not compatible with oxidizing agents, it should not be treated or kept near them.
- Use masks, gloves, goggles and aprons while handling NiSO<sub>4</sub>

# Why Removal of Heavy Metals is important

Due to the discharge of large amounts of metal-contaminated wastewater, industries bearing heavy metals, such as Cd, Cr, Cu, Ni, As, Pb, and Zn, are the most hazardous among the chemical-intensive industries. Because of their high solubility in the aquatic environments, heavy metals can be absorbed by living organisms.

## **Methods of Removing heavy metals**

- Chemical precipitation, ion exchange, chemical oxidation, reduction, reverse osmosis.
- Ultrafiltration, electrodialysis and adsorption.
- Chemical precipitation is one of the efficient removal processes for the heavy metal- containing waste effluent treatment because of its ease to use and lower operational cost.
- In this technique the metals are precipitated as insoluble hydroxide, carbonates or sulfides and removed by sedimentation and filtration.
- Most acidic heavy metal waters are neutralized by adding alkaline solutions, whereby the heavy metals are precipitate as a hydroxide they are bound to hydroxide ions.
- Neutralizing agents such as caustic soda (sodium Hydroxide) or lime milk (calcium hydroxide) are also used as precipitants in this process.
- Typical techniques include sedimentation and decantation, filtration or centrifugation. A key difficulty is that most iron-bearing precipitates are notoriously difficult to filter because of their gelatinous nature so that n expensive filtration plant may be needed.
- The adsorption process is widely used for the removal of heavy metals from wastewater. Because of its low cost, availability and eco-friendly nature. Both commercial adsorbents and bio adsorbents are used for the removal of heavy metals from wastewater, with high removal capacity.

# CHAPTER 2 LITERATURE SURVEY

In recent years, the problem of heavy metal pollution has become increasingly prominent, so it is urgent to develop new heavy metal adsorption materials. Compared with many adsorbents, the polyamide-amine dendrimers (PAMAMs) have attracted extensive attention of researchers due to its advantages of macro-molecular cavity, abundant surface functional groups, non-toxicity, high efficiency and easy modification.

Femina Carolin, p.Senthil Kumar Efficient technique for the removal of toxic heavy metals from aquatic environmen:8 This paper contributes the outline of new literature with two objectives, first its provides the sketch about treatment technologies followed by their heavy metal capture capacity from industrial effluent. Conclusively, this review paper furnishes the information about the important methods incorporated in lab scale studies which are required to identify the feasible and convenient wastewater treatment. 2019

Ahmed M.Elbedwehy Super Effective Removal of Toxic Metals Water Pollutants using Multi Functionalized polyacrylonitrile .Super adsorbent polymers can be considered to be a very efficient solution for wastewater treatment. In general, their adsorption capacities depend on the type and amount of the functional groups present on the surface of the polymers, while their economic value is affected by their cot. Therefore, this study aims to understand the effect of multi-functionalization of cheap Arabic gum on the adsorption capability toward heavy metals. Graft copolymers of polyacrylonitrile onto Arabic gum were prepared in aqueous solution using as redox initiator. chemical modification of the graft copolymer was carried out by reaction with hydrazine hydrochloride followed by hydrolysis in the basic medium.

#### **CHAPTER-3**

# **AIM and Scope of the Present Investigation**

#### Aim

Removal of heavy metals by doping it with

## Scope

Heavy metals appear in the environment through various anthropogenic activities like copper and cadmium from electro plating industry, chromium from tanning industry, wood preservative, textile industry, mercury from caustic soda and chlorine industries, arsenic from fertilizers etc. Heavy metals are toxic and harmful even at low concentrations and limits have been placed on their concentration in potable water supplies and effluent discharges by various agencies throughout the world. Considering the merits and limitations of various techniques reported in literatures, adsorption is considered an efficient technique for the removal of metal ions.

With proper control of the process, it is possible to meet the discharge limits and no harmful by-products are generated like ion exchange and reverse osmosis. Further, when metal ion recovery is possible with reuse of sorbents, adsorption becomes highly cost effective. Literature review highlighted the effectiveness of various functionalized polymers for removals of heavy metal ions form wastewater. Functionalized polymers with amine (NH2) (derivative of ammonia) as functional group are known to have affinity for metal ions. Nitrogen atom in amine, can make bond with positive charge due to presence of electron in sp3 orbital of nitrogen. Polyacrylonitrile fibers, polyacrylamides and polyethyleneimine are variousfunctionalized polymers with amine groups, which were successful in removing several heavy metal ions from wastewater. However, the synthesis procedures of many functionalized polymers like polyethylene, polyvinyl pyridine are complex. Also, synthesis of many functionalized polymers involved high cost. Therefore, there exists a need for synthesis of low-cost functionalized polymer with affinity for metal ions.

#### CHAPTER - 4

#### **EXPERIMENTAL OR MATERIALS AND METHODS**

#### **MATERIALS**

Beaker, Test tube, Watch glass, Filter paper, PH-Papers, shaker, Magnetic stirrer, Spatular Glass rod, Poly Vinyl Alcohol and Nickel Sulphate, Hot air oven, Acetone

#### 4:1 METHODS

#### **Acidic Condition**

- ➤ Then this solution is made up to 25mL by adding HCl drop by drop

and maintaining the acidic condition of pH=3

$$HCI + NiSO_4 \rightarrow H2SO_4 + NiCl_2$$
.

- ➤ Add 2gram of PVA (Poly vinyl alcohol) with constant stirring.
- Then the metal is separated out in the form of polymer by removing it with acetone

#### **Base Condition**

1gram of copper sulphate is dissolved in 20mL of distilled water.

Then this solution is made up to 25mL by adding NaOH drop by drop and maintaining the basic condition of pH=8

$$NiSO_4 + NaOH \rightarrow Ni (OH)_2 + Na2SO_4$$

Add 2gram of PVA (Poly vinyl alcohol) with constant stirring.

Then the metal is separated out in the form of polymer by removing it with acetone

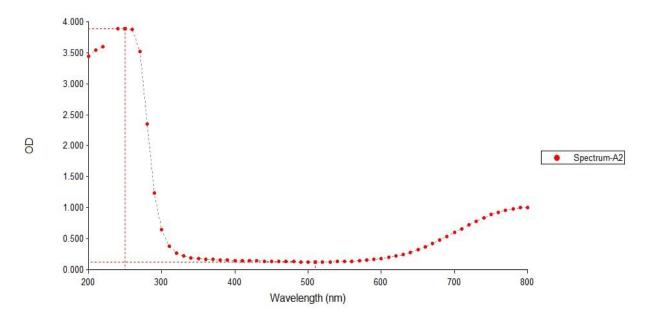
#### **Neutral condition**

1gram of copper sulphate is dissolved in 20mL of distilled water.

- Then this solution is made up to 25mL by distilled water.
- Then to this 2gram of PVA is added and stirred continuously.
- Then the metal is separated out in the form of polymer by removing it with acetone

## Applications of UV-Spectroscopy

The Principle of UV-Visible Spectroscopy is based on the absorption of ultraviolet light or visible light by chemical compounds, which results in the production of distinct spectra. Spectroscopy is based on the interaction between light and matter. UV-Visible spectroscopy is widely used in the field of analytical chemistry, especially during the quantitative analysis of a specific analyte. For example, the quantitative analysis of transition metal ions can be achieved with the help of UV-Visible spectroscopy. Furthermore, the quantitative analysis of conjugated organic compounds can also be done with the help of UV-Visible spectroscopy. It can also be noted that this type of spectroscopy can also be carried out on solid and gaseous analytes in some conditions. Spectroscopy is the measurement and interpretation of electromagnetic radiation absorbed or emitted when the molecules or atoms or ions of a sample move from one energy state to another energy state. UV spectroscopy is a type of absorption spectroscopy in which light of the ultra-violet region (200-400 nm) is absorbed by the molecule which results in the excitation of the electrons from the ground state to a higher energy state. It is used in characterization of aromatic compound and in detection of conjugation. The unknown concentration of a solution can be determined using this spectroscopy. It is used in determination of impurities present in the sample. Ultraviolet-Visible Spectrometry is one of the most frequently employed technique in pharmaceutical analysis. It involves measuring the amount of ultraviolet or visible radiation absorbed by a substance in solution.



## Principle difference between IR and UV spectroscopy

UV (Ultra-violet) spectrophotometers use visible light to determine the concentration of chemicals in a mixture. FTIR (Fourier-Transform Infrared (Spectroscopy) uses infrared light to the same purpose. Atoms and molecules absorb the energy from the light and under-go electronic transitions.

# 4:3 Principle of UV

- 1. Basically, spectroscopy is related to the interaction of light with matter.
- 2. As light is absorbed by matter, the result is an increase in the energy content of the atoms or molecules.
- 3. When ultraviolet radiations are absorbed, this results in the excitation of the electrons from the ground state towards a higher energy state.
- 4. Molecules containing  $\pi$ -electrons or nonbonding electrons (n-electrons) can absorb energy in the form of ultraviolet light to excite these electrons to higher anti-bonding molecular orbitals.
- 5. The more easily excited the electrons, the longer the wavelength of light they can absorb. There are four possible types of transitions  $(\pi-\pi^*, n-\pi^*, \sigma-\sigma^*, \text{ and } n-\sigma^*)$ , and they can be ordered as follows:  $\sigma-\sigma^* > n-\sigma^* > \pi-\pi^* > n-\pi^*$

**6.** The absorption of ultraviolet light by a chemical compound will produce a distinct spectrum that aids in the identification of the compound.

## **Applications of UV-Spectroscopy**

It is one of the best methods for the determination of impurities in organic molecules.

Additional peaks can be observed due to impurities in the sample and it can be compared with that of standard raw material.

By also measuring the absorbance at a specific wavelength, the impurities can be detected.

# 4:4 Principle of Infrared Spectroscopy

IR spectroscopy works on the principle that molecules absorb specific frequencies that are characteristic of their structure. At temperatures above absolute zero, all the atoms in molecules are in continuous vibration with respect to each other. The typical IR absorption range for covalent bonds is **600 - 4000 cm<sup>-1</sup>**. The graph shows the regions of the spectrum where the following types of bonds normally absorb.

The method or technique of infrared spectroscopy is conducted with an instrument called an infrared spectrometer to produce an infrared spectrum. The energy required to excite the bonds belonging to a molecule, and to make them vibrate with more amplitude, occurs in the Infrared region. A bond will only interact with the electromagnetic infrared radiation, however, if it is polar.

The presence of separate areas of partial positive and negative charge in a molecule allows the electric field component of the electromagnetic wave to excite the vibrational energy of the molecule.

The change in the vibrational energy leads to another corresponding change in the dipole moment of the given molecule. The intensity of the absorption depends on the polarity of the bond. Symmetrical non-polar bonds in N≡N and O=O do not absorb radiation, as they cannot interact with an electric field.

## **Principle of IR-Spectroscopy**

- 1. Infrared Spectroscopy is the analysis of infrared light interacting with a molecule.
- 2. The portion of the infrared region most useful for analysis of organic compounds have a wavelength range from 2,500 to 16,000 nm, with a corresponding frequency range from 1.9\*1013 to 1.2\*1014 Hz.
- 3. Photon energies associated with this part of the infrared (from 1 to 15 kcal/mole) are not large enough to excite electrons, but may induce vibrational excitation of covalently bonded atoms and groups.
- 4. It is known that in addition to the facile rotation of groups about single bonds, molecules experience a wide variety of vibrational motions, characteristic of their component atoms.
- 5. Consequently, virtually all organic compounds will absorb infrared radiation that corresponds in energy to these vibrations.
- 6. Infrared spectrometers, similar in principle to other spectrometer, permit chemists to obtain absorption spectra of compounds that are a unique reflection of their molecular structure.
- 7. The fundamental measurement obtained in infrared spectroscopy is an infrared spectrum, which is a plot of measured infrared intensity versus wavelength (or frequency) of light.

The main parts of the IR spectrometer are as follows:

Radiation source

Sample cells and sampling of substances

Monochromators

**Detectors** 

Recorder

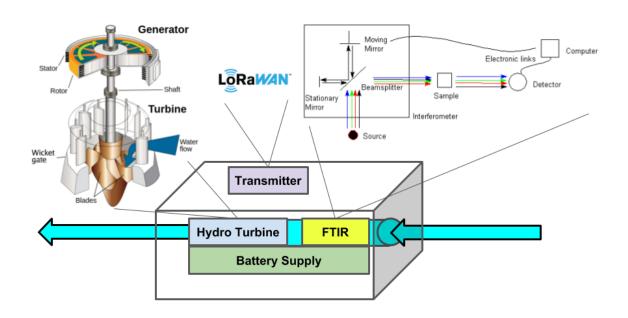
#### **4:5 FTIR**

Fourier transform infrared spectroscopy (FTIR) is a technique which is used to obtain infrared spectrum of absorption, emission, and photoconductivity of solid, liquid, and gas. It is used to detect different functional groups in PHB. FTIR spectrum is recorded between

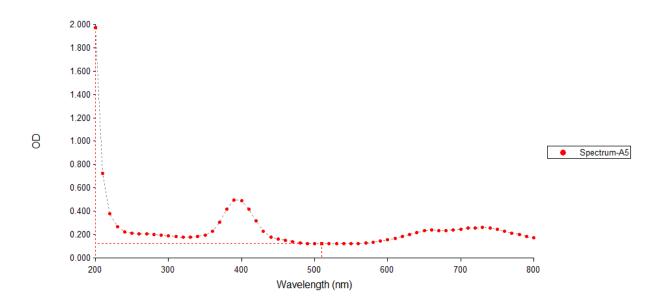
4000 and 400 cm<sup>-1</sup>. Fourier-transform infrared spectroscopy (FTIR) works by analyzing the infrared spectral samples of the scan absorbed infrared light samples. If the scan material does not absorb infrared light spectral, they are categorized as metals, which do not absorb infrared light. The major difference between an FTIR spectrometer and a dispersive IR spectrometer is the Michelson interferometer.

#### **FTIR** instrument

A Fourier Transform InfraRed (FT-IR) Spectrometer is an instrument which acquires broadband Near InfraRed (NIR) to Far InfraRed (FIR) spectra. Unlike a dispersive instrument, i.e. a grating monochromator or spectrograph, FTIR spectrometers collect all wavelengths simultaneously



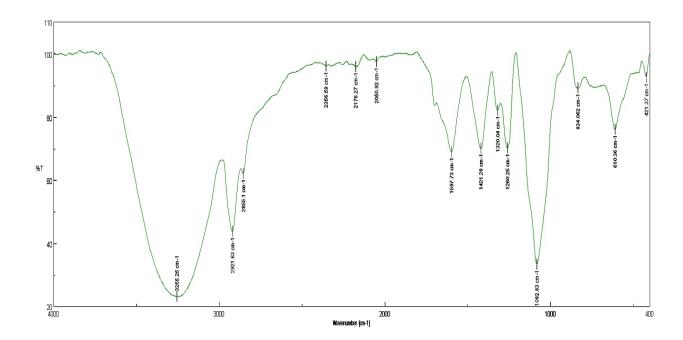
# CHAPTER-5 RESULT AND DISCUSSION 5.1 UV SPECTROSCOPY



This graph shows the absorption of the given compound can be confirmed by the sharp absorption at 395 wavelengths with 0.56 absorbance.

#### **5.2 FTIR SPECTROSCOPY**

FTIR spectra reveal the composition of solids, liquids, and gases. The most common use is in the identification of unknown materials and confirmation of production materials (incoming or outgoing). The information content is very specific in most cases, permitting fine discrimination between like materials.



This spectroscopic technique is used to determine the functional group present in the compound at different frequency levels.

Frequency range	Group
3255.25 cm <sup>-1</sup>	O-H Stretching
2921.63 cm <sup>-1</sup>	C-H stretching
2855.1cm <sup>-1</sup>	C-H Stretching
2366.69cm <sup>-1</sup>	O=C=O Stretching
2176.27cm <sup>-1</sup>	S-C≡N stretching
2050.92cm <sup>-1</sup>	N=C=S Stretching
1421.28cm <sup>-1</sup>	O-H Bending
1260.25cm <sup>-1</sup>	C-O Stretching
1082.83cm <sup>-1</sup>	C-O Stretching
834.062cm <sup>-1</sup>	C=C Bending

# **CHAPTER-7**

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# CHAPTER-4 EXPERIMENTAL OR MATERIALS AND METHODS