Chapter III

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EXPERIMENTAL TECHNIQUES ON SYNTHESIS AND CHARACTERIZATION

The reagents used and various analytical and physicochemical techniques employed in this chapter provide a quick overview of the current investigations, such as synthesis, characterization, and their applications.

3.1 REAGENT USED

Without additional purification, all chemical reagents and anhydrous solvents obtained from were used.

- 1-naphthoic acid
- 2-naphthoic acid
- Aminoguanidine
- Guanidine

These were purchased from Sigma Aldrich Chemical Private Limited. A solvent like ethanol, methanol, and additional chemicals were used for the experimental studies.

3.2 METHODS FOR ANALYTICAL TECHNIQUES

The analytical techniques are used to determine the quantitative estimation of hydrazine, and metals like Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Li^{+}, Na^{+}, and K^{+} ions.

3.3 HYDRAZINE ESTIMATION

Under Andrew's conditions, complexes were analyzed by volumetrically consuming a standard KIO3 (0.025 M) solution to determine the content of hydrazine present [116].

$$IO_3^- + N_2H_4 + 2H^+ + Cl^- \longrightarrow ICl + N_2 + 3H_2O$$

0.0008013g hydrazine is contained in 1mL of 0.025M KIO_3.

To analyze the presence of base volumetrically, 60 mg of the sample was added in the iodometric flask and dissolve it in 5 mL of concentrated HCl, 5 mL of Milli-Q water, and 5 mL of CCl_4. The above-mentioned solution was titrated against std. KIO_3 which was taken in a burette with shaking thoroughly after every drop. The disappearance of the pink color is the endpoint.

3.4 DETERMINATION OF NITROGEN

The nitrogen content in the metal complexes was determined either by performing sodium fusion test or lassaigne's test .

In a test tube, 2 ml of sodium fusion extract was taken, and 1 ml of freshly prepared ferrous sulfate solution was added. The solution was uniformly boiled and cooled and acidified using dilute HCl. If the color of the solution changes into deep blue which indicates the presence of nitrogen.

3.5 METAL ION ESTIMATION

A standard amount of the compound was dissolved in few drops of concentrated nitric acid in a silica crucible and then heated in a heating mantle three times for the complete decomposition of organic moiety. EDTA complexometric titrations was performed to the decomposed sample to calculate the metal ion concentration. 1.8614 g of EDTA disodium salt were dissolved in 500 mL of Milli-Q water to create a 0.01 M EDTA solution. The known quantity of the synthesized complex was titrated against EDTA till the end point was reached and from the titre value the amount of metal ion was determined.

3.5.1 Manganese

Hydroxyl ammonium chloride was added and warmed to a known volume of the sample solution. 3 mL of triethanolamine and 2 mL of ammonia buffer ($NH_4Cl + NH_4OH$) were added to this hot solution to maintain the pH at 10, the addition of Eriochrome Black T indicator were added. They were titrated against the standard EDTA until color change from red to blue.

3.5.2 Cobalt and cadmium

The known quantity of cadmium complex was transferred to a conical flask and diluted with100 ml water. A pinch of hexamine was added to maintain pH 6 and xylenol orange indicator was added. The resultant mixture was titrated with std. EDTA untill the solution was changed into yellowish orange color.

The cobalt solution was heated to 40 ^{o}C before titration against standard EDTA.

3.5.3 Nickel

50mg of an indicator mixture (0.1g Murexide with 10mg Ar KNO3) to the sample compound. The pH of the solution to 10 by adding NH_4Cl and buffer NH_3. The resulting yellow solution was titrated against standard EDTA. The final result is a color shift from yellow to wine red.

3.5.4 Copper

Ammonium hydroxide was added to the copper solution until a permanent precipitate formed, and a small amount of acetic acid was added to dissolve the precipitate. After adding 2 grams of solid KI, the compound was titrated against an std. sodium thiosulfate solution with starch as an indicator, and the solution turned yellow. The titration was carried out till the color change from blue to dirty white.

3.5.5 Zinc

The zinc sample was transferred to a conical flask and diluted with 100 ml water. The pH was maintained at 10 by adding NH_4Cl and NH_3 buffer followed by the addition of Erichrome black-T indicator. The resulting mixture was then titrated against standard EDTA until the endpoint change red to blue.

3.5.6 Calcium

To the known amount of calcium complex NH_4Cl and NH_3 buffer was added to maintain basic pH and the indicator Eriochrome black -T was added and then titrated with standard EDTA solution, until the endpoint was reached.

3.5.7 Strontium

The decomposed sample was transferred from the crucible to the conical flask and diluted to 100 ml. By adding NaOH and methylthymol blue indicator, the pH of the solution was maintained at 12. The resulting solution was titrated with standard EDTA until the end color changed from blue to grey.

3.6 PHYSICO-CHEMICAL METHODS

In order to determine the presence of functional group, nature of the bonding, particle size, isomorphism and thermal stability, characterization studies like elemental analysis, spectral, thermal, powder XRD, single crystal-XRD, SEM, TEM, electronic impedance spectra, photoluminescence, antimicrobial, and cytotoxic studies was carried out for all the complexes.

3.6.1 Melting point and Elemental analysis (CHNS)

Using Sigma melting point equipment, melting points were calculated. The Vario EL III CHNS analyzer was used forelemental analysis.

3.7 SPECTROSCOPY TECHNIQUES

3.7.1 Infrared spectra

Infrared spectra of the solid samples were recorded by a Shimadzu FTIR-8000 spectrophotometer in an range of 4000-400 cm^{-1}.

3.7.2 Electronic spectra

UV-visible spectroscopy was performed using a Varian-Cary 5000 spectrophotometer with a wavelength ranging from 200 to 800 nm.

3.7.3 Thermal Study

A thermal examination involves subjecting a compound to a controlled temperature while determining physical or chemical properties of the substance related to temperature or time. It involves dynamic heating or cooling, maintaining a constant isothermal temperature, or any combination of these.

Thermal methods involve thermogravimetry, differential scanning calorimetry (DSC) techniques, and differential thermal analysis (DTA). These methods are extensively used in quality control and research on metals, alloys, etc,

3.7.4 Thermogravimetry (TG)

The mass loss of a sample is determined in a thermogravimetric (TG) analysis. Any reaction involving a gaseous phase, like oxidation or dehydration, can be easily observed in TG. The purity of the sample, its water, carbonate, and organic content, as well as the rate of decomposition reactions, can be determined using this method. The thermogram shows a weight vs. temperature or time graph and provides valuable information about sample's composition, reaction rates, and thermal stability.

3.7.5 Differential thermal analysis (DTA)

The differential thermal analysis was recorded in the DTA instrument. Through a programmed temperature variation, the temperature of a sample is linked to that of an inert reference compound in DTA. Until a thermal event occurs, like melting, decomposition, or a change in the structure, it will remain constant. When there is an endothermic occurrence in the sample, the temperature was noted, and a downward peak is noticed on the curve. The range beneath the endotherm or exotherm is compared to ΔH.

The TG-DTA studies were performed on an EXSTAR/6300 (Thermogravimetric analyzer) with 5-10 mg of the compound by heating range of 10° C/min up to 700 °C.

3.7.6 Powder X-Ray Diffraction

Powder -X-ray diffraction is a technique for determining a crystal’s phase and unit cell dimension. It obeys Bragg's law and relates d-spacings to standard reference patterns.

P-XRD was determined at using an X'PERT-PRO in the range of 0-500 at wavelength 1.54056.

3.7.7 Single Crystal X-Ray Diffraction

Single-crystal XRD requires a high degree of homogeneity in the selected crystal. The intensity of the crystal was measured using a Bruker Axs diffractometer at wavelength of 0.71073.

3.7.8 Scanning electron microscopy-Energy dispersive X-ray analysis (*SEM-EDAX*)

It is a kind of electron microscope that rapidly scans the sample surface with a high-energy electron beam to create images of the surface. The sample's atoms and electrons interact to produce signals that provide details about the sample's surface topography, composition, and other characteristics like electrical conductivity.EDAX is used to deliver elemental and quantitative evidence about the compositions. The SEM-EDAX images were recorded using a Jeol 6390LA/OXFORD XMX-N instrument.

3.8 PREPARATION OF MILD STEEL SPECIMENS

The specimen's (Wt %) arrangement is C- 0.078, Mn- 0.019, S- 0.020, and P- 0.026, the remaining portion consists of Fe^{2+}. The trial steel plate was cut pieces into 3.0 cm X 1.0 cm X 0.05 cm then grazed with emery sheets (200, 400, and 800). The coupons were carried away to remove impurities, then washed with acetone, dried, and kept desiccated.

3.8.1 Preparation of epoxy-resin

The epoxy and hardener were mixed in a 4:1 ratio. Finally, the combination was applied on the steel through a brush and dried out in a room environment.

3.8.2 Weight loss method

The mild steel was tested and hung on glass hooks for three hours in a room temperature, both with and without an inhibitor. The samples were later removed, cleaned through Milli-Q water, dry out, and re-weighted both before immersion and after immersion. According to ASTM standard, the mass loss was estimated. A thermostatic water bath was used to repeat the procedure at higher temperatures.

Using the following formula, the proportion of protection efficiency (%IE) was determined.

$$Weight\ loss = \frac{Blank - Inhibitor}{Blank} \text{ x } 100 \qquad \textbf{Eq. (3.1)}$$

$$Inhibition\ Efficiency\ (\%) = \frac{W_{\circ} - W_i}{W_{\circ}}\ X\ 100 \qquad \textbf{Eq.(3.2)}$$

where, $W_{\circ}$ = Mass loss absence inhibitor, W_i = Mass loss presence inhibitor.

$$Corrosion\ rate = \frac{87.6W}{Area\ (Cm)X\ Time(h)\ X\ Density(\frac{g}{Cm^3})}$$ **Eq.(3.3)**

3.8.3 Electrochemical Impedance Spectroscopy (EIS)

The Ivium base electrochemical work station with a three-electrode setup was used for impedance measureent. Metal with an unprotected part consist of 0.785 cm^2 was used as a working electrode. OCP reaches a steady state, allowing frequency measurements from 0.01 Hz to 10 kHz with an signal excitation of 5 mV/S. The charge transfer resistance equation was used to determine the percentage of inhibition in EIS.

$$Inhibition\ efficiency\ (\%) = \frac{R_{t(i)} - R_{t(b)}}{R_{t(i)}}\ X\ 100$$ **Eq.(3.4)**

where, $R_{t(i)} and\ R_{t(b)}$ stands the electrochemical resistances presence and absence of inhibitor, respectively, and C_{dl} values was measured using by the following equation:

$$C_{dl} = \frac{1}{2\pi f_{max}} * \frac{1}{R_{ct}}$$ **Eq.(3.5)**

3.8.4 Potentiodynamic Polarization

Potentiodynamic olarization was demonstrated by capturing anodic and cathodic signals that occur between -200 mV and +200 mV at a scan rate of 1 mV/sec. Ivium software was used to generate the corrosion potential and current (E_{corr}, I_{corr}), as well as the Tafel curves (b_c, b_a). The following equation was used to evaluate the protective effectiveness of polarization:

$$Inhibition\ efficiency\ (\%) = \frac{I_{corr\ (b)} - I_{corr\ (i)}}{I_{corr\ (b)}}$$ **Eq(3.6)**

which $I_{corr\ (b)} and\ I_{corr\ (i)}$ refers to the corrosion current presence and absence of the inhibitor.

3.9 HYDROGEN EVOLUTION REACTION

3.9.1 Basics of Electrocatalytic HER

i) Mechanism of HER

According to the HER mechanism, the electrochemical system includes two essential half-processes known as the cathodic and anodic reaction, which were shown by the equation below.

$$Volmer\ reaction = H_3O^- + e^- \rightarrow H^* + H_2O \quad \textbf{Eq.(3.7)}$$

$$Heyrovsky\ reaction = H_3O^- + e^- \rightarrow H_2 + H_2O \quad \textbf{Eq.(3.8)}$$

$$Tafel\ reaction = H^* + H^* \rightarrow H_2 \quad \textbf{Eq.(3.9)}$$

In the HER process, the first step is the Volmer reaction, in which the hydrogen atom is generated by the discharge of H_2O or H_3O^+ on the active position. Hydrogen formation can also, occurs in other two pathways namely: i) the Heyrovsky reaction, where the hydrogen is formed by electrochemical desorption, and ii) the Tafel reaction. HER can be assigned as Volmer-Heyrovsky and Volmer-Tafel mechanisms which can be activated by the acidic solution.

To evaluate the HER activity, the overpotential (η), exchange current density (j_0), Tafel slope (b), TOF, and ESCA data's are required.

iii) Overpotential

Overpotential corresponds to difference between applied potential and equilibrium potential and it overcomes the barrier of energy from the transmission of electrons, weight diffusion, and the interaction of the surface of the electrode. From the overpotential, the linear sweep polarization (LSV) curve was determined, which is used to correlate the intrinsic properties and estimate the electrocatalytic performance.

iv) Tafel slope

$$\eta = b.logj + a \quad \textbf{Eq.(3.10)}$$

The Tafel equation is used to calculate the Tafel slope, current density, and overpotential. Depending on the 'b' values, the Volmer Heyrovsky or Tafel plot mechanism determines the HER process. The faster the kinetics of the reaction, the smaller

the 'b' value. The Tafel equation was used to compute the current density of the (j) value; the j value is a key indicator for determining the catalytic efficiency and is also correlated with the electron surface and catalytic surface area. Fast electron transmission and a good catalytic surface area are indicated by high values of j.

3.10 ANTIBACTERIAL STUDIES

Antibacterial growth progress against *Staphylococcus aureus* and *Escherichia coli* were examined. The Petri plate was separated into four parts, where one portion possess standard disc and the remaining three contains samples. The petri plates were then kept at 4ºC for 1 hour and incubated at 37ºC for 24 hours for antibacterial study. For antifungal investigation, the petri plate was incubated for 48hours at 28ºC. The zone of inhibition was measured by scale.

3.11 ANTICANCER STUDIES

The anticancer study was performed against Human breast cancer cells (MCF7) and lung cancer cells (A-549). The cancer cells were grown for about 48 hours in 96-well plate in to 75% convergence and the sequence of the cells was incubated. After that, they were treated for 4 hours at 27^0Cin 50µL of DMSO. The optical Density (OD) value, or percentage was calculated using the below equation.

$$Viability\ (\%) = \frac{OD\ value\ of\ the\ sample}{OD\ value\ of\ control\ (Doxorubicin)} \text{ X } 100 \qquad \textbf{Eq(3.13)}$$