

PARTICULARS OF INSTRUMENTS AND DETAILS OF ANALYTICAL METHODS AND PROTOCOLS FOR CHARACTERIZATION AND BIOLOGICAL STUDIES OF COMPLEXES

This chapter incorporates the details of methods employed for the quantitative analysis of various constituents and relevant particulars of the instruments/equipment used for the characterization and structural assessment of the compounds reported in the thesis.

Particulars of instruments/equipments

pH Measurement

The pH of reaction solutions were measured using a Systronics 362 pH meter and also by E Merck pH indicator paper.

Microanalysis

The microanalysis on carbon, hydrogen and nitrogen were obtained from Department of Inorganic Chemistry, IACS, Kolkata, SAIF, NEHU, Shillong , CSIR-NEIST, Jorhat, Assam, and Central Drug Research Institute(CDRI), Lucknow.

Conductance

Solution electrical conductances ($\sim 1 \times 10^{-3}$ M) were recorded in deionised water using a Systronics 30 direct reading conductivity bridge.

Electronic spectra

UV-visible absorption spectrum of compounds in aqueous medium was recorded on a Perkin Elmer Lambda 750 UV-visible spectrometer. Spectra of the complexes were recorded at room temperature in the wavelength range 200-800nm.

Infrared spectra

Infrared spectra were recorded on a Perkin Elmer Spectrum 100/System 2000 spectrometer on KBr disc in the $400\text{-}4000\text{ cm}^{-1}$ range at a spectral resolution of 4 cm^{-1} . For scanning below 400 cm^{-1} a Perkin-Elmer 983 spectrophotometer was used.

Thermal analysis

TGA, DTG and DTA analysis were carried out in a simultaneous TG-DTA analyser (SDT Q600, M/S TA Instruments, USA) using Al_2O_3 as reference at $20^\circ\text{C}/\text{min}$ heating rate in nitrogen.

XRD study

The XRD for the compound (**10**) was recorded on a Rigaku, Model : Micromax-007HF diffractometer.

Photoluminescence spectroscopy

Photoluminescence spectra were recorded on a Perkin Elmer LS 45 Fluorescence Spectrometer in aqueous solutions at ambient temperature.

Magnetic susceptibilities

Magnetic susceptibilities of the copper complexes were measured by a Gouy balance using $\text{Hg}[\text{Co}(\text{NCS})_4]$ as the calibrant.

Fluoride [1] (Volhard's method)

An accurately weighed amount of a fluoro-compound was dissolved in dilute nitric acid (0.1N, 25cm^3). The solution was then made alkaline by the addition of 0.1N NaOH (50cm^3). The mixture was then heated for ca. 20 min. to ensure complete decomposition. The hydrated metal oxide thus formed was separated by filtration and washed several times with water. The filtrate and washings were collected for fluoride estimation.

To the combined washing and filtrate, a pinch of bromophenol blue indicator and 3cm^3 of 10% sodium chloride solution were added and the whole was diluted to ca. 250cm^3 . Dilute nitric acid was added to it until the colour just changed to yellow followed by the addition of dilute sodium hydroxide solution until the colour just changed to blue. The mixture is then treated with 1cm^3 concentrated hydrochloric acid and 5.0g of lead nitrate and then heated on a steam bath. After all the lead nitrate had dissolved, 5.0g of crystallized sodium acetate was immediately added followed by vigorous stirring. The content was then digested on the steam bath for about half an hour with occasional stirring and allowed to stand overnight.

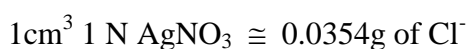
For gravimetric estimation, the precipitated lead chloride fluoride $PbClF$, was filtered through a sintered glass crucible (grade 4) and weighted as $PbClF$ after drying at 140° – $150^{\circ}C$ to constant weight. In the volumetric estimation, the precipitated $PbClF$ was quantitatively collected by filtration through a Whatman No. 42 filter paper and washed once with cold water, then 3 to 4 times with a saturated solution of $PbClF$ and finally once more with cold water. The precipitate was now dissolved in $100cm^3$ of 5% (v/v) nitric acid by heating over a steam bath for 4-5 min. A known excess of standard 0.1 N silver nitrate solution was then added to it followed by digestion on a steam bath for 30 min. and then cooled to room temperature in the dark. The precipitated silver chloride was filtered through a sintered glass crucible and washed with cold water. The residual silver nitrate in the filtrate and washings was then titrated with a standard 0.1 N potassium thiocyanate solution using ferric ion indicator. The amount of silver nitrate thus found in the filtrate was subtracted from that originally added and the content of fluoride calculated from the amount of silver nitrate consumed.

$$1cm^3 \text{ 1 N AgNO}_3 \cong 0.0190g \text{ of F}^-.$$

Chloride [2,3]

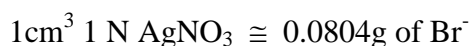
Chloride was determined by Volhard² method similar to that of fluoride and also by gravimetric³ method as silver chloride. An accurately weighed amount of the compound was dissolved in dilute nitric acid and metal (Cu, Zn or Cd) was precipitated as their corresponding hydrated oxide by filtration, the filtrate was adjusted to ca. $25cm^3$ and $5cm^3$ of 6 N nitric acid and a known excess of standard 0.1N silver nitrate solution were added to it. The resulting suspension was heated nearly to boiling for ca. 3 min. with constant stirring to coagulate the silver chloride and completion of precipitation was tested at this stage and then kept in the dark to cool to room temperature. For gravimetric

estimation, the precipitate was filtered through a previously weighed sintered glass crucible and washed thoroughly with very dilute nitric acid (1: 200) until the washings gave no turbidity with 0.1M hydrochloric acid. Chloride was estimated after drying the crucible at 130°-150°C to constant weight and weighing the precipitate as AgCl. In volumetric determination, the precipitate was filtered through a Whatman No. 41 filter paper, washed thoroughly with very dilute nitric acid and the amount of residual silver nitrate in the filtrate and washings found by filtration with a standard 0.1N potassium thiocyanate solution using ferric ion indicator. Chloride was estimated from the amount of silver nitrate consumed.



Bromide [4]

Bromide was estimated by Volhard's method similar to those for fluoride and chloride. Metal (Cu, Zn or Cd) was separated from the compound as hydrated oxide and the filtrate and washings, collected quantitatively, was acidified with dilute nitric acid. This solution containing bromide was treated with a known excess of standard silver nitrate solution and the residual silver nitrate determined by titration with standard potassium thiocyanate solution using ferric ion as indicator. As silver bromide is less soluble than silver thiocyanate, it was not necessary to filter off the silver bromide.



Iodide [5]

Iodide was estimated by Volhard's method. Metal (Cu, Zn or Cd) was separated from the compound as hydrated oxide and the filtrate and washings, collected quantitatively, acidified with dilute nitric acid. The solution was diluted in order to reduce adsorption

effects. The acidified dilute iodide solution was treated with a known excess of standard silver nitrate solution by adding slowly with vigorous stirring until the yellow precipitate coagulates and the supernatant liquid appears colourless. About 1cm^3 of iron(III) alum solution is added, and the residual silver nitrate is titrated with standard potassium thiocyanate. Silver iodide is comparatively much less soluble than silver thiocyanate. Iodide was estimated from the amount of silver nitrate consumed.

$$1\text{cm}^3 \text{ 1 N AgNO}_3 \cong 0.1269\text{g of I}^-$$

Copper [6]

Copper was determined iodometrically. An accurately weighed amount of the compound was dissolved in dilute nitric acid and the resulting solution was made alkaline by the addition of dilute sodium hydroxide solution. The mixture was then heated for ca. 20 min. to ensure complete decomposition. The hydrated copper oxide formed was collected by filtration through a Whatman No. 41 filter paper and washed several times with water and then dissolved in a minimum volume of dilute sulphuric acid (1:8). A few drops of dilute sodium carbonate solution was added to the copper solution until a faint precipitate persists which was then cleared by a few drops of dilute acetic acid and the volume made up to ca. 50 cm^3 . About 10cm^3 of a 10% potassium iodide solution was now added and the liberated iodine was titrated with standardized 0.1N sodium thiosulphate solution using starch as indicator. The amount of iodine and, hence, the amount of thiosulphate was used to calculate the amount of copper.

$$1\text{cm}^3 \text{ 1 N Na}_2\text{S}_2\text{O}_3 \cong 0.06354\text{g of Cu}^{2+}.$$

Zinc [7]

An accurately weighed amount of the zinc compound is dissolved in 10ml 50% (v/v) nitric acid. The solution was heated to reduce the volume to about 1/3rd of its original volume to ensure complete decomposition. Neutralized the solution with dilute sodium hydroxide solution so that its pH value within 8. To it 20ml water and about 2ml ammonium buffer solution is added (pH = 10). About 30mg Erichrome Black-T indicator mixed with potassium nitrate is added to it when violate colour produces. It was titrated with 0.01M EDTA solution. Near the end point very slow addition of EDTA solution was done to get no violate colour at the end point.

$$1 \text{ cm}^3 \text{ of } 1 \text{ N of EDTA} \cong 0.06538 \text{ g of Zn}^{2+}.$$

Cadmium [8]

Cadmium was estimated by direct titration with EDTA. An accurately weighed amount of the cadmium compound was dissolved in dilute nitric acid and the resulting solution was made alkaline by addition of dilute sodium hydroxide solution. The mixture was then heated for ca. 20 min. to ensure complete decomposition. Hydrated cadmium oxide thus formed was collected by filtration through a Whatman No. 41 filter paper and washed several times with water then dissolved in a minimum volume of dil. H₂SO₄. The solution was put into a 500ml conical flask and diluted to 150ml. The pH of the solution was adjusted to ca. 5 by adding hexamine, xylon orange indicator (30mg, 1% mixture with KNO₃) was added to it and titrated with 0.01M EDTA solution until the red colour starts to fade then titrated slowly until the end point is reached from red to yellow.

$$1 \text{ cm}^3 \text{ of } 1 \text{ N of EDTA} \cong 0.11241 \text{ g of Cd}^{2+}.$$

Xylon orange indicator: The indicator solution is freshly prepared by dissolving 0.5g of xylon orange in 100ml water.

DFT study

Quantum chemical calculations were carried out using density functional theory (DFT) as implemented in DMol3 package at B3LYP level [9,10].

Preparation of Hydrated metal(II) oxide, $MO.nH_2O$ (M = Cu, Zn, Cd)

An aqueous solution of (0.004 mol) metal sulphate $MSO_4.nH_2O$ in a 500cm^3 beaker was treated with 10% AOH (A = K or Na) solution drop by drop until the precipitation of hydrated metal oxide completed. An excess of alkali was avoided. To this, 200cm^3 of water was added and the precipitated solid was allowed to settle and finally the supernatant liquid was decanted off. The process was repeated 4-5 times to ensure complete removal of excess alkali and anions. The pH recorded at this stage was ~ 7 . This suspension of hydrated oxide $MO.nH_2O$ (M = Cu, Zn, Cd) was used as precursors for the syntheses of the compounds.

Antimicrobial activity

Cadmium(II) being biologically non-essential and toxicologically relevant, it was considered worth to screen some selected water soluble complexes of fluorocadmium(II) for their antimicrobial activity against two gram positive strains, [A] *Staphylococcus aureus* and [B] *Bacillus subtilis*, and two gram negative strains, [C] *Klebsiella pneumoniae* and [D] *Escherichia coli* (*E.coli*). The study was carried out using Kirby-Bauer disc diffusion technique [11, 12] and the extent of antimicrobial activity was evaluated by measuring the diameter of zone of inhibition. To obtain bacteria in the mid-logarithmic phase 100 μl of nutrient of an overnight culture made in nutrient broth was added to 10 ml of nutrient broth and incubated for 5 hours at 37 °C with orbital shaking

for each strain. The strains were separately plated on nutrient agar media by pour plate method. The test samples were then dissolved in water at a concentration of 50µg/ml and adsorbed on the sterile paper discs. The discs were placed on the agar medium and the plates were incubated at 37 °C for 24 hours. The resulting inhibition zones on the plates were measured after 24 hours. The tests were carried out in triplicate and the results were expressed as mean. Two standards viz. ceftazidime and azithromycin for the gram-positive and gentamicin and imipenem for the gram-negative strains were chosen.

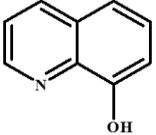
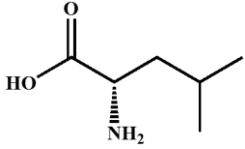
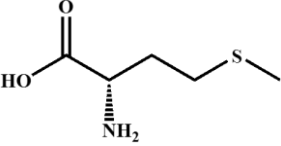
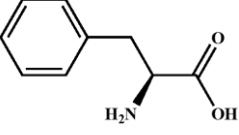
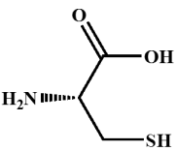
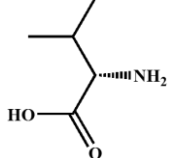
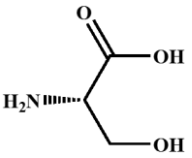
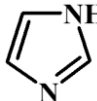
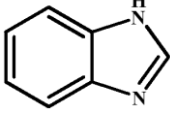
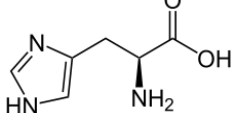
Protocol for study of silk productivity in eri silkworm (*Samiya cynthia ricini*)

Based on nutritional role of copper and zinc and the solubility of their complexes in water, the compounds $[\text{Cu}(\text{val})_2(\text{H}_2\text{O})]$ (**10**), $[\text{ZnF}_2(\text{Hser})(\text{H}_2\text{O})].4\text{H}_2\text{O}$ (**13**) and $\text{NH}_4[\text{ZnF}(\text{ser})_2(\text{H}_2\text{O})].5\text{H}_2\text{O}$ (**14**) were selected to assess the effect of their supplementation on silk production by the *Samiya cynthia ricini* (common name : eri silkworm) variety. The disease free lay (DFL) were initially collected from Central Silk Board, Diphu, Assam and applied on castor leaves as 1% aqueous solution (in distilled water) to 5th instars larva at a dose of 5µL per larva. Subsequent cycle of experiments were conducted using the lay (egg) obtained from the adult moth of the first, second and third run separately. The growth was monitored by measuring weight and length of the larva every day since the beginning of the 5th instar. Silk production was monitored by recording the final weight of cocoon and pupa after 25 days of the 5th instars. A total of 12 species were considered in each of the three replicates. Further details are given in the experimental section of **Chapter 6**.

Ligands

The source of halide ligands were their corresponding mineral acids or salts. The organic ligands were procured as analytical grade chemicals (**Table2.1**) directly from reputed chemical suppliers.

Table 2.1: List of organic ligands, their abbreviation, formula, structure and molecular mass

Sl. No.	Ligand	Abbreviation	Formula (Isoelectric point)	Structure	Molecular mass
1	8-hydroxyquinoline (oxine)	oxin	C_9H_7NO		145
2	l-leucine	Hleu	$C_6H_{13}NO_2$ (6.0)		131.18
3	l-methionine	Hmet	$C_5H_{11}SNO_2$ (5.7)		149.21
4	phenylalanine	Hphe	$C_9H_{11}NO_2$ (5.9)		165.19
5	l-cysteine	Hcys	$C_3H_7NO_2S$ (5.1)		121.07
6	Valine	Hval	$C_5H_{11}NO_2$ (6.0)		117.03
7	l-serine	Hser	$C_3H_7NO_3$ (5.7)		105.00
8	Imidazole	im	$C_3H_4N_2$		68.08
9	Benzimidazole	bim	$C_7H_6N_2$		118.07
10	Histidine	Hhis	$C_6H_9N_3O_2$		115.16

References

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