Chapter II
EXPERIMENTAL METHODS

This chapter gives the details of the reagent employed for the present study, procedure for the preparation of the complexes and general method for the analysis of the complexes. A brief description of the various instruments employed for the physicochemical studies of the complexes are also given in this chapter.

2.1 Materials and Methods

2.1.1 Metal salts

AR grade sample of nitrates of Co(II), Ni(II), Zn(II) and Cd(II) were used for the synthesis of complexes.

2.1.2 Solvents

Acetone, methanol, ethanol, DMSO, acetonitrile, nitrobenzene, petroleum ether etc., are the solvents used.

Commercial grade acetone was purified by standard procedure. BDH spectroscopic grade solvents were used for the conductivity measurements. Acetonitrile and nitrobenzene used for conductance measurements were purified by repeated distillation of BDH samples over P₂O₅.
2.1.3 Other reagents

All other reagents like Con.HNO$_3$, NH$_4$OH, glacial acetic acid etc., used in the present investigation were of high quality, BDH, AR or other analytical reagent grade.

2.1.4 Ligands

The ligands used for the preparation of the complexes were ethylenediamine, triethylenetetramine and o-toluidine. Commercial ethylenediamine, triethylenetetramine and o-toluidine were used without further purification.

2.1.5 Preparation of the complexes

The complexes [Co(en)$_3$(NO$_3$)$_2$], [Ni(en)$_3$NO$_3$)$_2$, [Zn(en)$_3$(NO$_3$)$_2$] and [Cd(en)$_3$(NO$_3$)$_2$] were prepared by known methods.$^{266}$ The ligand was added drop wise to the corresponding metal nitrate solution in ethanol with continuous stirring in the ice cold condition. The precipitated complexes were washed with ethanol and dried.

The complexes [Co(trien)(NO$_3$)$_2$], [Ni(trien)(NO$_3$)$_2$], [Zn(trien)(NO$_3$)$_2$] and [Cd(trien)(NO$_3$)$_2$], were prepared by known methods.$^{266}$ [trien = triethylenetetramine]. Metal nitrate was dissolved in ethanol and triethylenetetramine was added dropwise to the metal salt solution with constant stirring in the ice cold condition. The precipitated complexes were washed with ethanol and dried.

The complexes [Co(o-tol)$_2$(NO$_3$)$_2$], [Ni(o-tol)$_2$(NO$_3$)$_2$], [Zn(o-tol)$_2$(NO$_3$)$_2$] and [Cd(o-tol)$_2$(NO$_3$)$_2$] were prepared by known$^{267}$ methods.
Ortho toluidine was added drop wise to an ethanolic solution of metal nitrate. The mixture was stirred and refluxed on a water bath for 5-6 hrs and left for a day. The precipitated metal complexes were washed with ethanol and ether.

The prepared complexes were purified by repeated recrystallisation, dried over P₂O₅ in vacuum and stored in a desiccator.

2.2 Characterization of the complexes

The complexes were characterized by chemical analysis, spectral and magnetic studies. Cobalt was estimated by pyridine method, nickel by dimethyl glyoxime, zinc and cadmium by AAS. Nitrate ions in all complexes were determined using nitron reagent. The structure and geometry of complexes were confirmed by spectral and magnetic studies. The Infra-red spectra of the complexes were recorded in a Perkin-Elmer FT-IR spectrophotometer for the range 4000-200cm⁻¹. Magnetic susceptibility was measured by Gouy method.

2.2.1 Estimation of metal

Estimation of the metals in the complexes was carried out by volumetric and gravimetric methods. For this, the complexes were decomposed with concentrated nitric acid and a few drops of perchloric acid. The resulting solutions were evaporated to dryness and the residue extracted with water. The aqueous solutions, thus obtained were used for the estimation. Nickel was estimated gravimetrically as nickel
dimethylglyoximate and cobalt by using pyridine thiocyanate. Zinc and cadmium were estimated by AAS.

### 2.2.2 Estimation of nitrate

The nitrate was determined as nitron-nitrate using nitron reagent.

### 2.3 Irradiation

The dried samples were sieved to uniform mesh size of 100-120 and were encapsulated under vaccum in glass ampoules. The samples were exposed to radiation using $^{60}$Co-$\gamma$ rays upto 800 kGy at constant intensity under room temperature at a dose rate of 2.8 kGy/hr. The irradiated samples were taken out and each sample was mixed uniformly and was stored over P$_2$O$_5$.

### 2.4 Detection of radiolytic fragments

Ammonia in the irradiated samples was detected using nessler's reagent and the damage nitrite by the azo dye test.

### 2.5 Thermal Decomposition

Thermograms providing TG, DTG and DTA were recorded in nitrogen atmosphere in an automatically recording thermal analyzer, ‘METLER TOLEDOSTAR SYSTEM’ for both irradiated and unirradiated samples. The heating rate was 10°C /min. In all experiments about 3 mg of the substance was taken.
2.6  X-ray Diffraction-Powder Method

The X-ray powder diffraction pattern of some of the complexes, before and after irradiation was recorded on Philips diffractometer using nickel filter (Cu-K\textsubscript{α} radiation \(\lambda = 1.5406\) Å). They were indexed by Hesse and Lipson method\(^\text{272}\). Lattice constants and lattice parameters were calculated for the unirradiated and irradiated samples.

2.7  Electronic Spectra

Electronic spectra were recorded on a Schimadzu UV-1601 UV-visible using methanol as solvent in the range 1000-200nm before and after \(\gamma\) irradiation. Reflectance spectra of insoluble or very soluble ones were also recorded on a Schimadzu UV-2450 UV-visible spectrophotometer.

2.8  Antimicrobial Screening

Complexes \([\text{Zn}(\text{en})\text{\textsubscript{3}}](\text{NO}_\text{3})\text{\textsubscript{2}}]\), \([\text{Cd}(\text{en})\text{\textsubscript{3}}](\text{NO}_\text{3})\text{\textsubscript{2}}]\), \([\text{Zn}(\text{trien})(\text{NO}_\text{3})\text{\textsubscript{2}}]\), \([\text{Cd}(\text{trien})(\text{NO}_\text{3})\text{\textsubscript{2}}]\), \([\text{Zn}(\text{o-tol})\text{\textsubscript{2}}](\text{NO}_\text{3})\text{\textsubscript{2}}]\) and \([\text{Cd}(\text{o-tol})\text{\textsubscript{2}}](\text{NO}_\text{3})\text{\textsubscript{2}}]\), were screened for their antimicrobial activity against E.Coli, staphylococcus, mycobacterium smegmatis, fast growing non pathogenic myco bacteria and sacharomyce cerevisiae (yeast-fungus) by disc diffusion method using agar nutrient as medium. Activity was tested both for the irradiated and unirradiated samples. Change in zone of inhibition due to irradiation was noted.