CHAPTER 3

GENERAL EXPERIMENTAL PROCEDURES
Section 1: Zero order spectrophotometry

a) Absorption spectra of reagent blank and metal-reagent coloured solutions

5ml of buffer solution of suitable pH, appropriate volume of the reagent were mixed in each of two 10ml volumetric flasks. A known volume of metal solution in required concentration was added to one of the flasks and the contents of the flasks were made up to the mark with distilled water. The absorbances of the solutions were measured in appropriate wavelength region against appropriate blank. Plots were drawn between the absorbance values and the wavelength.

For the coloured solutions of different metals with HMBATSC obtained as above, the derivative spectra of different orders were recorded in the wavelength region 350-600nm with a scan speed of 2400 nm/min and at 9 degrees of freedom. The derivative plots were obtained by plotting the measured derivative amplitudes using peak-zero method or trough-zero method against the wavelength from which the analytical wavelengths were ascertained.

b) Effect of pH on the absorbance of the experimental solution containing complex species.

For investigating the effect of pH on the absorbance of the coloured species, appropriate volume of metal ion, and suitable volume of reagent solution were taken in each of a set of different 10 ml flasks. To these, 5 ml of buffer solutions of different pH values were added and the contents were made up to the mark with distilled water. Absorbance of these solutions was measured against the corresponding reagent blank at suitable wavelengths. Plots were drawn between absorbance and pH, from which the working pH was chosen.
c) **Effect of reagent concentration**

In a series of 10ml volumetric flasks, a known aliquot of metal ion solution and 5ml of buffer solution of the appropriate pH were taken. For obtaining coloured species of optimum stability and sensitivity, different known aliquots of the reagent solution of suitable concentration were added to these flasks and the contents were diluted to the volume with distilled water. The absorbance of the solutions was measured against the reagent blank at the analytical wavelength from which the required molar excess of the reagent for complete colour formation was ascertained.

d) **Adherence of the system to Beer’s law**

To ascertain the range of determination of the metal ion in microgram amounts by the proposed methods, the following general procedure was adopted. To a set of 10ml volumetric flasks, each containing 5ml of buffer solution of suitable pH, appropriate volume of reagent solution, various known amounts of metal ion were added and the contents in each flaks were made the analytical wavelength against the reagent blank. A plot between absorbance and the amount of metal ion was drawn and the validity of Beer’s law for the given system was verified.

For the experimental solutions containing different amounts of metal ion as prepared above, derivative spectra were drawn in the appropriate wavelength region. The derivative amplitudes were measured at analytical wavelengths and plotted against the amount of metal ion taken. Linear plots were obtained in some concentration regions for variable metal ions indicating the validity of Beer’s Law.
e) Effect of foreign ions

In order to assess the applicability of the proposed methods for analysis of real samples containing the metal ions, the effect of the presence of various foreign ions which are generally associated with the test metal ions in various real samples, on the absorbance of the reaction mixtures was studied by adopting the following procedure.

In different 10ml volumetric flasks, each containing an interfering ion of known amount, 5ml of buffer solution of desired pH, known aliquots of metal ion, and appropriate amount of reagent solution were added. The contents were diluted to the mark with distilled water. The absorbance was measured at the analytical wavelength against reagent blank and compared with the absorbance of solution containing no interfering ion. From this, the tolerance limits of the foreign ions in the determination of the target metal ion were evaluated. Amount of foreign ion, which brings about a change in absorbance by \( \pm 2\% \), was taken as its tolerance limit.

Composition and stability constant of the complex species

The composition of the complex species was determined and confirmed by different spectrophotometric methods described below.

f) Job's Method

Equimolar solutions of the metal ion and the reagent solutions were mixed in different volume proportions, keeping the total volume of the mixture constant in a series of 10ml standard flasks. 5ml of buffer solution of desired pH were added to each flask and made upto the mark with distilled water. The absorbance of each solution was measured at the wavelength of maximum absorbance or at an appropriate
wavelength against reagent blank. From the plot of absorbance versus volume of metal ion or reagent, the composition of the complex was ascertained.

g) Molar ratio method

A series of solutions were prepared by keeping the volume of metal ion solution constant and varying the volumes of reagent solution. 5ml of buffer solution of suitable pH were added to each flask and the contents were made up to the mark with distilled water. The absorbance of each solution was measured at the selected wavelength against the reagent blank. From the plot between absorbance and the volume of reagent, the composition of the complex was determined.

h) Determination of stability constant of the metal complexes

The spectrophotometric data obtained in the study of complexes using job’s method were used to calculate the stability constant of the complexes.

For the complex formation reaction

\[ mM + nL \rightarrow M_{mL_n} \] (3.1.1)

The stability constant \( \beta \), in terms of experimentally obtained absorbance values is given by the general equation.

\[
\beta = \frac{A}{\frac{m}{m+n}} n m \left[ 1 - \frac{A}{A_m} \right]^{-\frac{m}{m+n}} (C)^{m+n-1} \] (3.1.2)

where

\[ A_m = \text{Absorbance corresponding to the point of intersection of the tangents of the inverted parabola curve} \]
A = measured maximum absorbance at concentration ‘C’

m = number of moles of metal ion involved in complexation

n = number of moles of ligand involved in complexation

C = concentration of variable corresponding to the point of intersection of the tangents

β = stability constant
Section 2: Derivative Spectrophotometry

a) First, Second, Third and Fourth order derivative spectra of the solution containing the complex species

In a 10ml volumetric flask, 5ml of buffer solution of desired pH and desired volumes of metal ion and the reagent solution are taken. The contents of the flasks were made up to the mark with distilled water. The 1st, 2nd, 3rd and 4th order derivative curves were recorded for these solutions in the appropriate wavelength regions. The amplitudes of the derivative curves were measured by following Peak-base line method.

b) Determination of metal ion by derivative spectrophotometry (calibration plot)

Various known aliquots of metal ion solutions and reagent solutions were taken in various 10ml volumetric flasks each containing 5ml buffer solution of the selected pH. The contents of each flask were made up to the mark with distilled water and the derivative spectra of these solutions were recorded against reagent blank in suitable wavelength range with scan speed fast and with suitable degrees of freedom. Derivative amplitudes were measured at appropriate wavelengths and plotted against the concentration of metal ion. The slope and intercept of the plots and Beer’s law range were evaluated.

(c) Effect of foreign ions

In different 10ml volumetric flask each containing an interfering ion of known amount and 5ml of buffer solution of the selected pH, known aliquots of metal ion and the reagent solutions were taken. The contents of each flask were brought up to the mark with distilled water. The derivative spectra were recorded against the reagent
blank and the derivative amplitudes were measured at the selected wavelengths from which the tolerance limits of foreign ions were determined. The amount of foreign ion which brings about change in the derivative amplitude by ± 2% was taken as its tolerance limit.
REFERENCES

