
Chapter – 3

**GENERAL EXPERIMENTAL
PROCEDURES**

Zero order Spectrophotometry

a. Absorption spectra of 2-AINH and its metal complexes

4 ml of buffer solution of suitable pH, appropriate volume of DMF and the reagent were mixed in a 10 ml volumetric flask and made up to the mark with distilled water. In another 10 ml volumetric flask known volumes of pH and reagent were taken along with a suitable volume of metal solution of appropriate concentration and diluted to the volume with distilled water. The absorbances of the two solutions were measured in the suitable wavelength region against the suitable blanks. Plots were drawn between the absorbance values and wave length which gave the wavelength at which the analysis can be made.

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

A known aliquot of metal ion and appropriate volumes of DMF and reagent solutions are taken in different 10 ml volumetric flasks each containing 4 ml of buffer solution of different pH values. The contents of each flask are made up to the mark with distilled water and the absorbance of these solutions is measured against the corresponding reagent blank at the analytical wavelength. A plot is then made between absorbance and pH, from which the working pH is chosen.

c. Effect of the reagent concentration

In a series of 10 ml volumetric flasks containing 4 ml buffer solution of desired pH, appropriate volume of DMF, different known aliquots of the reagent solution, a known aliquot of metal ion solution is taken and the contents are brought up to the mark with distilled water. The absorbance of the solution in each flask is

measured against the corresponding reagent blank at the analytical wavelength, from which the required molar excess of the reagent necessary for maximum colour formation is ascertained.

d. Effect of time on the colour development and on the stability of the colour

4 ml of buffer solution, required volume of DMF, an aliquot of metal ion and the reagent solutions are taken in a 10 ml volumetric flask and made up to the mark with distilled water. The absorbance of the solution is measured at different time intervals at the selected wavelength against the reagent blank from which the time interval required to be allowed after mixing various components of the reaction mixture and before measuring the absorbance is known.

e. Adherence of the systems to Beer's law¹

To ascertain the sensitivity of the colour reactions and to explore the possibility of determining micro amounts of metal ions, the following procedure is adopted.

Varying known aliquots of metal ion solutions are added to a set of 10 ml volumetric flasks, each containing 4 ml of buffer solution of desired pH, known volume of DMF and the necessary excess of the reagent solution. The contents of the flasks are brought up to the mark with distilled water and the absorbance of the solutions are measured at the analytical wavelength against the reagent blank. A plot of absorbance and amount of metal ion ($\mu\text{g mL}^{-1}$) is constructed. The slope and the intercept of the plot are computed. The molar absorptivity is calculated from the slope of the curve.

f. 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 found associated with the test metal ions in various real samples on the absorbance of the reaction mixture is studied by adopting the following procedure.

To different 10 ml volumetric flasks, each containing an interfering ion of known amount and desired volume of DMF, 4 ml buffer solution of desired pH, known aliquots of metal ion and the reagent solutions are added. The contents are brought up to the mark with distilled water. The absorbance is measured at the analytical wavelength against reagent blank from which the tolerance limit of the foreign ion is determined. The amount of foreign ion which brings about a change in absorbance by $\pm 2\%$ is taken as its tolerance limit.

Composition and stability constant of the complex species

The composition of complex species is determined by Job's² and molar ratio³ methods.

g. Job's method

To a series of 10 ml volumetric flasks, each containing 4 ml of buffer solution of desired pH and known volume of DMF, equimolar solutions of metal ion and the reagent are added in different volume proportions, such that the total volume of the mixture is held constant. The absorbance of each solution is measured at the wavelength of maximum absorbance or at an appropriate wavelength against buffer blank. A plot between mole fraction of the metal ion ($V_M / V_M + V_L$) or

$(C_M / C_M + C_L)$ and the absorbance is made from which the composition of the complex is computed.

The spectrophotometric data obtained in the study of complexes using Job's method is used to calculate the stability constants of the complex species under investigation.

For the complex formation reaction



The stability constant ' β ' in terms of experimentally obtained absorbance value is given by the general equation.

$$\beta = \frac{A/A_m}{m^m n^n \left[\left(1 - A/A_m \right) \right]^{m+n} [C]^{m+n-1}} \quad \dots \quad 3.2$$

where

A_m = absorbance corresponding to the point of intersection of the extrapolated lines

A = observed 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 metal ion corresponding to the point of intersection

β = stability constant of the complex

h. Molar ratio method

4 ml of buffer solution and known volumes of DMF is taken in a series of 10 ml volumetric flasks and known volume of metal ion solution and varying volumes of the reagent solution are added. The contents of each flask are brought up to the mark with distilled water. The absorbance of each solution is measured at the

selected wavelength against the corresponding reagent blank or a blank containing 4 ml of buffer solution and required volume of DMF. From the plot between the absorbance and the volume of the reagent, the composition of the complex is ascertained.

Derivative spectrophotometry

i. First and second order derivative spectra of the solution containing the complex species

In a 10 ml volumetric flask, 4 ml of buffer solution of desired pH and desired volumes of DMF, metal ion and the reagent solutions are taken. The contents of the flask are made up to the mark with distilled water. Its absorbance in the suitable wavelength region is recorded with scan speed fast (nearly 2400 nm/min) against the reagent blank prepared under identical conditions. From this the first order derivative spectra are scanned (with appropriate degrees of freedom) from which the analytical wavelength is selected. The first and second order derivative amplitude is measured by following either the peak-zero or valley to peak methods.

j. Determination of metal ion by derivative spectrophotometry (Calibration plot)

Various known aliquots of metal ion solution, appropriate volumes of DMF and the reagent solution are taken in various 10 ml volumetric flasks each containing 4 ml buffer solution of the selected pH. The contents of each flask are made up to the mark with distilled water and the derivative spectra of these solutions are recorded against reagent blank in suitable wavelength range with scan speed fast and with suitable degrees of freedom. Derivative amplitude is measured by peak-zero or valley peak methods. A plot between derivative amplitude or peak height and the amount of metal ion ($\mu\text{g/mL}$) is prepared. The slope and intercept of the plot are evaluated.

k. Effect of foreign ions

In different 10 ml volumetric flasks each containing an interfering ion of known amount and required volume of DMF, 4 ml of buffer solution of the selected pH, known aliquot of metal ion and the reagent solutions are taken. The contents of each flask are brought up to the mark with distilled water. The first derivative spectrum is recorded for each solution against the reagent blank and the derivative amplitude is measured at the selected wavelength from which the tolerance limits of foreign ions are determined. The amount of foreign ion which brings about change in the derivative amplitude by $\pm 2\%$ is taken as the tolerance limit.

Preparation of sample solutions

l. Preparation of an alloy steel samples, fly ash and industrial samples⁴

A 0.1 - 0.5 g of the sample is dissolved in a mixture of 2 ml HCl and 10 ml HNO₃. The resulting solution is evaporated to a small volume. To this 4 ml of 1:1 H₂O : H₂SO₄ mixture is added and evaporated to dryness. The residue is dissolved in 15 ml of distilled water and filtered through Whatman filter paper No.41. The filtrate is collected in a 100 ml volumetric flask and made up to the mark with distilled water. The solution is further diluted as required.

m. Preparation of plant and other biological materials⁵⁻⁹

Freshly collected samples are cleaned and dried for one hour in open air protecting from mineral contamination. The dried sample is powdered in a mortar for the purpose of analysis. The powdered material is brought into solution either by wet ashing or dry ashing method according to the procedures given in the literature¹⁰.

n. Wet ashing

Weighed quantity of finely powdered sample is transferred into a 100 ml beaker. To it, 20 ml mixture of 1:2:5 of H_2SO_4 , H_3PO_4 and HNO_3 are added. The contents are heated until sulfurous acid fumes are evolved and the volume is reduced to about 4 ml. A little distilled water is added and filtered through an acid washed Whatman filter paper No.41 into a volumetric flask and made up to the mark.

o. Dry ashing

A known quantity of the powdered sample is taken in a silica crucible and heated to oxidise organic matter. It is ashed at 550°C by keeping for 3-4 hours in a muffle furnace. The ash is dissolved in 10 ml of 1:1 HCl by heating, filtered through an acid washed filter paper into a volumetric flask and the residue is washed with hot water. The washings are also collected into volumetric flask finally made up to the mark with distilled water. The solution is then diluted according to the requirement.

p. Pharmaceutical sample solutions¹¹

Finely ground iron tablets of known weight were treated with 5 ml of concentrated nitric acid and the resulting mixture were evaporated to dryness. The residue was leached with 5 ml of 0.5 M sulfuric acid. The solution was boiled with dilute H_2O_2 for 10 minutes. The heating was continued for further 5 minutes to boil off any excess H_2O_2 . The solution was cooled, neutralized with dilute ammonia and diluted to known volume with distilled water.

q. Soil Samples¹²

The soil sample (5.0g) was weighed into a 250 ml Teflon high pressure microwave acid digestion bomb and 50 ml of aquaregia were added. The bomb was

sealed tightly and then positioned in the carousel of a microwave oven. The system was operated at full power for 30 minutes. The digested material was evaporated to incipient dryness. Then, 50 ml of 5% hydrochloric acid were added and heated close to boiling to leach the residue. After cooling, the solution was filtered and the residue was washed two times with small volumes of 5% hydrochloric acid. The filtrate was quantitatively collected in 250 ml volumetric flask and diluted to the mark with distilled water.

r. Water sample¹³

The water samples were collected from different places of Anantapuramu district (A.P). The water samples (1 lit) were collected in a clean 2 litre beaker and slowly evaporated to about 25 ml. 5ml of H₂O₂ was added and evaporated to dryness⁶. It was then dissolved in 2 ml of water and filtered to remove insoluble substance. The filtrate was collected in 100 ml volumetric flask quantitatively and diluted to the mark with distilled water.

s. Preparation of aluminum based alloy sample

About 0.4g alloy sample was treated with 15 ml of 1:1 HCl. To this, 3 ml of HNO₃ was added and boiled until dissolution was complete. 10 ml of water and 40 ml of ammonium hydroxide solution were added and filtered through a whatman filter paper No. 41. The filtrate was collected into 25 ml volumetric flask and made up to the mark with distilled water.

t. Preparation of vegetable (ground nut) oil sample solution

100g of hydrogenated groundnut oil (edible) was dried in a hot air oven at 100°C and subsequently dissolved in 20 ml mixture of 1 : 2 : 5 H₂SO₄ : H₃PO₄;

HNO₃. The contents were heated until sulphurous fumes were evolved and the volume was reduced to about 5 ml. A little quantity of distilled water was added and filtered through an acid washed Whatman filter paper 41 into a 100 ml volumetric flask and made up to the mark with distilled water.

u. Food stuffs

5g of food or fruit material (dried for about 24hrs at 70°C in an oven) was wet ashed with nitric acid and perchloric acid^{14,15}. The ash was dissolved in 5 ml of hydrochloric acid and evaporated to dryness. This procedure was repeated and the dried residue was dissolved in water. The solution was filtered into a 25 ml standard flask, one or two drops of concentrated hydrochloric acid was added and made up to the mark with distilled water.

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