GENERAL EXPERIMENTAL PROCEDURES
Second order derivative spectrophotometric procedures:

a) Derivative spectrum of the solution containing the complex species

In a 10 mL volumetric flask, 5 mL of buffer solution of desired pH and appropriate volumes of DMF, metal ion solution and reagent solution are taken. The contents of flask are brought to the mark with distilled water. Its absorbance spectrum is measured in a suitable wavelength region against the reagent blank prepared under identical conditions. Then, the second order derivative spectrum is recorded. From this the analytical wavelength is selected.

b) Effect of pH on the derivative amplitude of the solution containing complex species

A known aliquot of metal ion & appropriate volumes of DMF & reagent solutions are taken in different 10 mL volumetric flasks each containing 5 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 spectra of these solutions are recorded against the corresponding reagent blank. The second order derivative spectra of these solutions are then recorded. The derivative amplitudes at the analytical wavelength is then measured. A plot is then made between amplitude and pH, from which the working pH is chosen.

c) Effect of the reagent concentration

In a series of 10ml volumetric flasks containing 5 ml buffer solution of desired pH, appropriate volume of DMF, different known aliquots of the reagent solution, known aliquot of metal ion solution, is then added and the contents are brought up to the mark with distilled water. The derivative amplitude 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) **Adherence of the systems to Beer’s law (Calibration Plot)**

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 10ml volumetric flasks, each containing 5 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 derivative amplitudes of the solutions are measured at the analytical wavelength against the reagent blank. A plots of derivative amplitude and amount of metal ion ($\mu$ gmL$^{-1}$) is constructed. The slope and the intercept of the plot are computed.

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

To different 10ml volumetric flasks, each containing an interfering ion of known amount and desired volume of DMF, 5ml 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 derivative amplitude are measured at the analytical wavelength from which the tolerance limit of the foreign ion is determined. The amount of foreign ion which brings about a change in derivative amplitude by $\pm 2\%$ is taken as its tolerance limit.
f) **Preparation of alloy, steel, flyash and industrial samples**

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 5 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.

g) **Preparation of plant and other biological material samples**

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 method according to the procedure given in the literature

h) **Wet ashing**

Weighed quantity of finely powdered sample is transferred into a 100ml beaker. To it, 20 ml mixture 1:2:5 of H₂SO₄, H₃PO₄ and HNO₃ are added. The contents are heated until sulfurious acid fumes are evolved and the volume is reduced to about 5 ml. A little distilled water is added filtered through an acid washed Whatman filter paper No.41 into a volumetric flask and made up to the mark.

i) **Dry ashing**

A known quantity of the powdered sample is taken in a silica crucible and heated to oxidize 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 filtered paper into a volumetric flask and the residue is washed with water. The washings are also collected into volumetric flask and finally made up to the mark with distilled water. The solution is then diluted according to the requirement.
j) **Preparation of soil samples**

An accurately weight (0.100 g) of the soil sample was dried at 110°C and treated with 3 ml of conc. nitric acid and 6 ml of conc. hydrochloric acid until the evolution of brown oxides of nitrogen had ceased. The mixture was evaporated to dryness. The residue was decomposed by heating with 20 ml of 0.1 M sulphuric acid. The insoluble material was then filtered and washed. The filtrate and washings were collected in a beaker, and then reduced to a small volume. The solution was neutralized with 0.1M sodium hydroxide solution and then transferred into a 10 ml volumetric flask. Then a known aliquot of the sample was analysed by the general procedure.
REFERENCES