Chapter II

Experiment

2.1 Chemicals and reagents

Carmoisine, Sunset Yellow FCF, Orange G, Orange RN, Tartrazine and Green S are certified food color grade. Sunset Yellow FCF, Orange RN, Tartrazine and Green S were kindly donated by the Department of Midical Sciences, the Ministry of Public Health.

Metal ion solutions, buffer solutions, acid and base solutions were prepared from analar grade of metal salts and reagents. Silica gel-G 737 was from E. Merck, Darmstadt., Ltd.

Double distilled water was used through out this research work.

2.2 Instruments used

Visible and UV spectra were firstly obtained with Varian Techtron Model 635 equipped with a Varian Techtron Model 7040A and lately with Shimadzu UV-Visible Recording Spectrophotometer UV-240.

Concentrations of metal ions in the solutions were determined by a Shimadzu Double-Beam Digital Atomic Absorption/Flame Spectrophotometer AA-650.

Infrared spectra of dyes were obtained with a Shimadzu Infrared Spectrophotometer IR-440.

The pH values were measured with a Radiometer Copenhegen PHM 83 Autocal pH Meter.

All measurements in this research work were made at room temperature.

2.3 Procedure

2.3.1 Paper chromatographic technique

2.3.1.1 Developing solvent systems

Three solvent systems were used in the paper chromatographic technique for testing the purity of dyes. System I was the mixture of 1-butanol, water and acetic acid in the ratio of 20:12:5, respectively. System II was prepared by mixing 99 cm^3 water with 1 cm^3 ammonia solution (specific gravity 0.91), and system III was 2.5% aqueous NaCl. Azorubine, Sunset Yellow FCF and Orange RN were tested by using systems I and III. Tartrazine, Orange G and Green S were tested by using systems I and II.

2.3.1.2 Dye solutions

The dye solution was prepared by dissolving a few milligrams of the solid dye in 1 cm^3 of the double distilled water.

2.3.1.3 Chromatographic chamber

A glass cylinder with 28 cm high and 18 cm in diameter containing about 20 cm^3 of the developing solvent served as a chromatographic chamber.

2.3.1.4 Paper chromatograms

A strip of Whatman chromatographic paper number 1 (14.0 cm x 23.0 cm) was spotted with a drop of each dye solution at the point where is 2 cm above the bottom edge of the

paper by using a capillary. The dye spots were dried in air at room temperature. Then the paper was folded in a cylindrical shape, clipped with a staple and immersed into the chromatographic chamber. The chamber was closed with a glass plate and the developing solvent system was allowed to ascend to a premarked line (solvent front). The paper was then removed from the chamber and was dried in air. The developed spots were circled with a pencil and their Rf values were determined.

2.3.2 Thin layer chromatography

2.3.2.1 Developing solvent systems

Five solvent systems were used in the thin layer chromatographic technique. System I and II are the mixture of 2-propanol, ammonia solution (25%) and water in the ratio of 7:2:1 and 10:1:1, respectively. System III was prepared by mixing 2-propanol and ammonia solution (25%) in the ratio of 4:1, respectively. System IV is the mixture of 2-butanol, ethanol, water and ammonia solution (25%) in the ratio of 10:20:10:1, respectively. System V was prepared by mixing 1-butanol, acetic acid and water in the ratio of 10:5:6, respectively.

2.3.2.2 Dye solutions

The dye solution was prepared by dissolving a few milligrams of the solid dye in 1 cm^3 of the double distilled water

2.3.2.3 Chromatographic chamber

A glass cylinder with 28 cm high and 18 cm in diameter containing about 20.0 cm^3 of the developing solvent

served as a chromatographic chamber.

2.3.2.4 Chromatoplate

Thin layer chromatography was carried out by using silica gel-G 737, coated on glass plates (5 cm x 20 cm). Chromatoplates were prepared by using Desaga spreader with the thickness of 0.25 mm. The plates were activated at 110°C for one hour.

2.3.2.5 Thin layer chromatograms

A drop of the dye was spotted on the chromatoplate at the point where is 2 cm above the bottom edge. The chromatoplate was immersed into the chromatographic chamber. The chamber was closed with a glass plate and the developing solvent was allowed to ascend to a solvent front. The chromatogram was then removed from the chamber and was dried in air. The developed spots were circled with a pencil and their Rf values were determined.

2.3.3 Column chromatography

A chromatographic column used is a glass column with 60 cm long and 2.5 cm in diameter. A 70.0 gm of silica gel-G 737 was mixed thoroughly with 150 cm^3 of the mixed solvent (2-propanol:ammonia (25%): water = 7:2:1). The mixture was stirred to a slurry and was poured into the column which was plugged with a small piece of cotton wool. The liquid was allowed to drain to the upper surface of the silica, then the mixed solvent was added to the column and allowed to drain until the level of the liquid was about 2 cm above the upper surface of the silica. The dye was mixed with a small amount of silica and then added to the packed column. The side of the column was carefully washed with a few

cm^3 of the mixed solvent. The column was eluted with the mixed solvent and the effluent was collected, 20 cm^3 for each fraction. The purity of each fraction was monitored by thin layer chromatography, using solvent system I. The pure fractions were evaporated by a rota evaporator until it almost dried. The solid dye obtained was dried in the dessicator. The impure fractions were repurified in the same manner.

2.3.4 Determination of the dye content in the certified grade dye

2.3.4.1 Titanium trichloride method (30)

2.3.4.1.1 Reagents used

The 1.00×10^{-2} M of the certified grade dye was prepared by dissolving the dye in the double distilled water.

Titanium trichloride solution was prepared by adding 75.0 cm^3 concentrated hydrochloric acid to 60.0 cm^3 25% (m/v) titanium trichloride solution and the solution was diluted to 1000.0 cm^3 with the double distilled water so as to make the solution approximately 0.1 N. This solution was preserved in the atmosphere of hydrogen and allowed to stand for two days before use.

The 0.0167 M potassium dichromate standard solution was prepared by dissolving 1.2258 gm of the analar grade of potassium dichromate in the double distilled water and the volume of this solution was made up to 250.00 cm^3.

2.3.4.1.2 Standardization of titanium trichloride solution

A 1.5000 gm ammonium ferrous sulfate $[(NH_4)_2SO_4.FeSO_4.6H_2O]$ was dissolved in 25 cm^3 freshly boiled water and 12.5 cm^3 40% sulfuric acid in 250 cm^3 flask. Nitrogen gas was passed through the flask continuously until the end of the determination. A 20.00 cm^3 of 0.0167 M standard potassium dichromate solution was rapidly added without interrupting the current of nitrogen gas. This mixture was then titrated with titanium trichloride solution until the calculated end point nearly reached. Then 2.50 gm ammonium thiocyanate (NH_4SCN) were quickly added and the titration was continued until the red color was discharged and the green solution appeared. The concentration of titanium trichloride solution was calculated.

2.3.4.1.3 Determination of the dye content in Azorubine, Sunset Yellow FCF, Orange G, Tartrazine and Green S.

A 15.00 cm^3 of the certified grade dye solution was pipetted into a 250 cm^3 flask. A 4.5 gm sodium hydrogen tartrate (or sodium citrate for Sunset Yellow FCF) was added. The solution was diluted with the double distilled water to a volume of 60-100 cm^3, heated to boil. The mixture was then titrated with standard titanium trichloride solution, while the nitrogen gas was continuously passing through the mixture during the titration.

2.3.4.2 Spectrophotometric method

The stock solutions of 1.00×10^{-2} M of the standard dyes and the certified grade dyes were prepared by dissolving the dye in the double distilled water.

2.3.4.2.1 The linearity of the dye concentration and absorbance

The 1.00×10^{-5} M - 8.00×10^{-5} M of the standard Sunset Yellow FCF and Tartrazine were prepared from the 1.00×10^{-2} M stock solutions. Absorbances of both dyes were measured at the maximum wavelengths (482 nm for Sunset Yellow FCF, 432 nm for Tartrazine). The calibration curves of standard dyes were obtained by plotting the absorbances vs concentrations.

2.3.4.2.2 Determination of the dye contents in Sunset Yellow FCF and Tartrazine.

A 2.00×10^{-5} M sample solution of Sunset Yellow FCF or Tartrazine was prepared from the 1.00×10^{-2} M of the certified grade dye and the absorbance was measured.

The mixture of 2.00×10^{-5} M sample solution of Sunset Yellow FCF or Tartrazine and 1.00×10^{-5} M standard dye was also prepared. The absorbance of this solution was measured at the maximum wavelength. The exact concentration of the sample was calculated from the equation below.

$$\frac{A'}{A} = \frac{C_{sample} + C_{std}}{C_{sample}}$$

where A = absorbance of the sample solution

A´ = absorbance of the standard solution + sample

C = concentration

Then the dye content of the sample can be calculated.

2.3.4.3 Kjeldahl method (From nitrogen content) (30)

2.3.4.3.1 Indicator solution

A 0.10 gm methyl red was dissolved in 20 cm^3 ethanol and the solution was diluted to 33 cm^3 with the double distilled water.

A 0.066 gm methylene blue was dissolved in 33 cm^3 50% ethanol and this solution was added to methyl red solution.

2.3.4.3.2 Determination of the dye content in Orange RN

A 16.00 milligram of Orange RN was heated with 4.0 cm^3 concentrated sulfuric acid. A 0.6 gm sodium sulfate and 0.1 gm mercuric acetate were added to the cool solution of Orange RN. Then the solution was digested until it was clear. The double distilled water, 5-6 cm^3 50% sodium hydroxide solution and 3 cm^3 of 21% sodium thiosulfate solution were added into the digestion flask. The solution was distilled and the collection of ammonia was performed in 5.00 cm^3 2% boric acid which had 3 drops of indicator solution. Ammonia in boric acid solution was titrated with 0.0206 N standard

sulfuric acid. Nitrogen content of the dye was calculated from the equation below.

1.00 cm^3 0.0206 N sulfuric acid = 0.2884 milligram nitrogen

2.3.5 pH effect on the solubility of dyes, metal ions and their mixtures

A 1.00 cm^3 of 5.00 x 10^{-3} M of the certified grade dye solution was pipetted into a test tube containing the buffer solution at any pH and the mixture was mixed thoroughly.

A series of the test tubes containing 1.00 cm^3 of 5.00 x 10^{-3} M or 1.00 x 10^{-4} M of metal ions in the buffer solution of any pH was also prepared.

A 1.00 cm^3 of 5.00 x 10^{-3} M of the certified grade dye solution was mixed thoroughly with metal ions in the buffer solution of any pH.

The physical properties of these solutions, such as color change or precipitation were observed.

2.3.6 Spectrophotometric studies of dyes and complexes

2.3.6.1 Stock solutions

The stock solutions of 1.00 x 10^{-2} M of certified grade dyes were prepared by dissolving the appropriate amounts of the dye in the double distilled water.

The 4.00 x 10^{-3} M Ti (IV) solution was prepared in 0.5 M sulfuric acid by dissolving 0.0799 gm of TiO_2 in 30.00 cm^3 of concentrated sulfuric acid and vigorously heated until the white fume of SO_3 was disappeared. Then the solution was diluted with the double distilled water to the mark of 250.00

cm^3 volumetric flask.

The 2.50×10^{-4} M Cr (III) solution was prepared by diluting the 1.00 $\mu g/cm^3$ standard $Cr(NO_3)_3$ solution for atomic absorption with the double distilled water.

The 1.00×10^{-2} M Fe (II) solution was prepared by dissolving 0.9802 gm of $(NH_4)_2SO_4.FeSO_4.6H_2O$ and diluting with 0.02 M sulfuric acid in a 250.00 cm^3 volumetric flask.

The 1.00×10^{-2} M Fe (III) solution was prepared by dissolving 1.2054 gm of $NH_4Fe(SO_4)_2.12H_2O$ and diluting with 0.01 M sulfuric acid in a 250.00 cm^3 volumetric flask.

The 1.00×10^{-2} M Mn (II) solution was prepared by dissolving 0.4948 gm of $MnCl_2.4H_2O$ with the double distilled water and the volume of this solution was made up to 250.00 cm^3 with the double distilled water.

The 1.00×10^{-2} M Co (II) solution was prepared by dissolving 0.7276 gm of $Co(NO_3)_2.6H_2O$ with the double distilled water and the volume of this solution was made up to 250.00 cm^3 with the double distilled water.

The 1.00×10^{-2} M Ni (II) solution was prepared by dissolving 0.7270 gm of $Ni(NO_3)_2.6H_2O$ with the double distilled water and the volume of this solution was made up to 250.00 cm^3 with the double distilled water.

The 1.00×10^{-2} M Cu (II) solution was prepared by dissolving 0.6040 gm of $Cu(NO_3)_2.3H_2O$ with the double distilled water and the solution was made up to 250.00 cm^3 with the double distilled water.

The 1.00×10^{-2} M Zn (II) solution was prepared by dissolving 0.7436 gm of $Zn(NO_3)_2 \cdot 6H_2O$ in the double distilled water and the solution was made up to 250.00 cm^3 with the double distilled water.

2.3.6.2 Buffer solutions

McIlvaine buffer solutions were prepared from 0.4 M disodium hydrogen phosphate and 0.2 M citric acid for pH 3.10, 5.12 and 7.45.

Phosphate buffer solutions were prepared from 0.2 M disodium hydrogen phosphate and 0.0667 M potassium dihydrogen phosphate for pH 5.58 and 7.00.

Acetate buffer solutions were prepared from 0.2 M sodium acetate and 0.2 M acetic acid for pH 4.00, 5.10 and 6.10.

Phosphoric acid, acetic acid, diethylamine were used for the pH adjustment of the dye solutions at pH 1.00, 2.30 and 12.50, respectively.

2.3.6.3 Spectra and calibration curves of dyes

Visible-ultraviolet spectra of the dye solutions of Azorubine, Sunset Yellow FCF, Orange G, Orange RN, Tartrazine and Green S were recorded in the range of wavelengths between 220-760 nm. The calibration curves of dyes were obtained by plotting the absorbances against concentrations in the ranges of 0.40×10^{-5} M - 7.00×10^{-5} M for Azorubine, Sunset Yellow FCF, Orange G, Orange RN, Tartrazine and 0.10×10^{-5} M - 2.60×10^{-5} M for Green S.

2.3.6.4 Dissociation constants of dyes

A series of the dye solutions at various pH and a constant concentration of the dye (3.00×10^{-5} M Azorubine, 2.00×10^{-5} M Sunset Yellow FCF, 4.00×10^{-5} M Orange G, 3.20×10^{-5} M Orange RN, 3.60×10^{-5} M Tartrazine or 8.00×10^{-6} M Green S) was prepared and their absorbances were measured. The absorbance and $\frac{\Delta A}{\Delta pH}$ of each dye solution were plot against pH. The inflection points of the curves were the pKa of the dyes.

2.3.6.5 The influence of time on the absorbance of metal-dye complex

A constant concentration of the dye (4.00×10^{-5} M Azorubine, Sunset Yellow FCF, Orange G or Orange RN) was mixed with Cu (II) ion (4.00×10^{-5} M for Azorubine, 1.20×10^{-3} M for Sunset Yellow FCF, Orange G or Orange RN). The 20.00 cm^3 of the buffer solution was added to ensure the required condition of pH. The volume of the solution was then made up to 50.00 cm^3 with the double distilled water. The absorbances were read at 0, 5, 15, 30 min., etc.

2.3.6.6 Complex formations of dyes and metal ions

2.3.6.6.1 Continuous variation method

A series of the mixture solutions at various mole fractions of the metal ion and the dye, but the sum of the concentrations of the metal ion and the dye was kept constant at 4.00×10^{-5} M, was prepared in the buffer solution. The absorbance of each solution was measured at the wavelength where the complex absorbed against the buffer solution.

2.3.6.6.2 Molar ratio method

Three series of the mixture solutions of the dye and the metal ion were prepared.

The first series of solutions contained a constant concentration of Azorubine (4.00×10^{-5} M), a variation of the concentration of Cu (II) ion (0.40×10^{-5} M - 8.80×10^{-5} M), 10.00 cm^3 phosphate buffer pH 7.00 or 5.85 or acetate buffer pH 6.10 or 5.10. The volumes of these solutions were made up to 25.00 cm^3 with the double distilled water in volumetric flasks. The absorbance was measured at the wavelength where the complex absorbed against the reagent blank.

The second series of solutions contained a constant concentration of Sunset Yellow FCF (2.00×10^{-5} M), Orange G or Orange RN (4.00×10^{-5} M), a variation of the concentration of Cu (II) ion (0.32×10^{-5} M - 16.00×10^{-5} M), and 10.00 cm^3 acetate buffer pH 6.10, 5.10 or 4.00. The final volumes of these solution were 25.00 cm^3. The absorbance was measured against the buffer solution at the wavelength where the dye absorbed.

The third series of solutions contained the constant concentration of the dye (3.20×10^{-5} M Sunset Yellow FCF, 4.00×10^{-5} M Orange G or Orange RN) and a variation of the concentration of Cu (II) ion (4.00×10^{-4} M - 3.20×10^{-3} M), 10.00 cm^3 acetate buffer pH 6.10, 5.10 or 4.00. The final volume was 25.00 cm^3. The absorbance of each solution was measured against the

buffer solution.

2.3.6.7 Atomic absorption spectroscopy .

The calibration curves of metal ions were established by plotting the absorbance of the metal ion solution against its concentration in the range of 3.147×10^{-5} M - 1.574×10^{-4} M for Cu (II) ion.

2.3.6.8 Infrared spectrophotometric study of dyes

Two methods, the KBr pellet and mull techniques, were used for preparing the sample. In the first method, the dried sample was well mixed with dried KBr and grounded thoroughly to achieve homogeneous mixture which was transferred into a standard Pye Unicam die (13 mm). The Blackhand Enerpac Model p-39, was used to press the mixture to a pellet under the pressure of 12,500 pounds/cm^2. For mull technique, the sample was ground very finely with paraffin in an agate mortar. The suspension is then placed on KCl cell. The infrared spectra were recorded in the range of 300 cm^{-1} - 5,000 cm^{-1}.