CHAPTER 2

EXPERIMENTAL

2.1 Chemicals and reagents

Amaranth, Erythrosine and Ponceau 4R are certified food color grade and were kindly donated by the Department of Medical Science, Ministry of Public Health.

Metal ion solutions, buffer, acid, and base solutions were prepared from the analar grade of metal salts and reagents.

Thrice deionized water used through out this study were prepared as described in reference(16).

2.2 Apparatus

Concentrations of metal ions and dyes in the solutions were determined by spectrophotometric techniques with a Varian Techtron Atomic Absorption Spectrophotometer Model AA-5 and a Perkin-Elmer Double Beam Spectrophotometer Model 124.

Infrared spectra of dyes and compounds formed between dyes and metal ions were obtained with a Pye Unicam Sp 200 G Grating Infrared Spectrophotometer.

The pH values were measured with a pH meter (Radiometer Copenhagen type P.H.M. 28)

All measurements in this work were carried out at room temperature.

2.3 Procedure

2.3.1 Paper Chromatographic technique.

2.3.1.1 Developing solvent systems.

Solvent systems used for performing paper chromatography of dyes are system I : the mixture of n-butanol, 70% ethanol, water and ammonia solution in the ratio of 100:20:44:1, respectively, and system II : the 2% NaCl in 50% ethanol.

2.3.1.2 Dye solutions.

Dye solution was prepared by dissolving a few milligrams of the solid dye in 1 cm^3 of the solvent system; Amaranth in the solvent system I, as well as Ponceau 4R and Erythrosine in the solvent system II.

2.3.1.3 Chromatographic chamber.

A 1 dm^3-beaker containing about 20 cm^3 of the developing solvent system served as a chromatographic chamber.

2.3.1.4 Paper chromatography.

A strip of Whatman filter paper number 1 (10.0 in. x 5.5 in.) was spotted with a drop of the dye solution at each point where was 2 cm above the bottom edge of the paper by using a 1 mm (id) capillary, 4 spots for each strip. The dye spots were dried in the air at the room temperature. Then the paper was fold in a cylindrical shape, fastened with adhesive tape and inserted in the chromotographic 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 chromatogram was developed and completed in about half an hour at the room temperature. The paper was then removed from the chamber and was dried in the air. The developed spots were circled with a pencil and their R_F values were measured.

2.3.2 Spectrophotometric technique

2.3.2.1 Stock solutions of dyes.

A 1.00×10^{-2}M dye solution was prepared by dissolving the appropriate amount of the dye desired with the deionized water in a 250 cm^3 volumetric flask.

2.3.2.2 Stock solutions of metal ions.

A 1.00×10^{-2} M solution of the metal ion desired was prepared from the analar grade of its salt, Hg(II) ion (from $Hg(NO_3)_2$) in 0.01 MHNO_3 solution, Fe(II) ion (from $(NH_4)_2SO_4 \cdot FeSO_4 \cdot 6H_2O$) in 0.02 M H_2SO_4 solution, Fe(III) ion (from $NH_4Fe(SO_4)_2 \cdot 12H_2O$) in 0.01 M H_2SO_4 solution, as well as Pb(II) ion (from $Pb(NO_3)_2$) and Cd(II) ion (from $Cd(NO_3)_2$) in the deionized water.

2.3.2.3 Buffer solutions.

McIlvaine buffer solutions were prepared from 0.1 M citric acid and 0.2 M disodium hydrogen phosphate for pH 3.4, 6.4 and 7.6.

Phosphate buffer solutions were prepared from 0.200 M disodium hydrogen phosphate and 0.0667 M potassium dihydrogen phosphate for pH 6.4 and 7.4.

Acetate buffer solutions of pH 4.4 and 6.0 were prepared from 0.2 M acetic acid and 0.2 M sodium acetate. For the dye solution of pH3, glacial acetic acid was used for the adjustment.

For the dye solutions of pH 12.5, diethylamine was used to adjust the Amaranth and Ponceau 4 R solutions as well as the mixture of 2.5 cm^3 of 0.2 M potassium chloride and 2.04 cm^3 of 0.2 M sodium hydroxide was added to the Erythrosine solution.

2.3.2.4 Visible-ultraviolet spectrophotometry

2.3.2.4.1 Spectra and calibration curves of dyes.

Visible-ultraviolet spectra of Amaranth, Erythrosine and Ponceau 4R were recorded in the range of wavelengths of 180-800 nm, using the deionized water as reference. The calibration curves of dyes were obtained by plotting the absorbances against concentrations of the dyes desired in the ranges of concentrations of 0.10×10^{-4}M - 1.50×10^{-4}M for Amaranth and Ponceau 4R and in the range of 1.00×10^{-6} M-2.00×10^{-5}M for Erythrosine at pH 3.3 and 4.4

2.3.2.4.2 Dissociation constants of dyes.

A series of the dye solutions contained a constant concentration of the dye at various pH were prepared. The curve for evaluating the pKa of the dye was established by plotting the absorbance of the dye solution versus its pH.

2.3.2.4.3 Compound formation of dyes and metal ions.

Four series of the mixture solutions of dyes and metal ions studied were prepared.

The first series solutions contained a constant concentration of the dye (Erythrosine 2.00×10^{-5}M, Ponceau 4R 6.00×10^{-5}M or Amaranth 8.00×10^{-5}M), a buffer solution of any pH and a variation of concentrations of metal ion solution desired. The volumes of these solutions were made up to 25.0cm^3 with the deionized water in the 25.0cm^3 volumetric flasks.

The second series solutions contained a constant concentration of the dye (Erythrosine 2.00×10^{-5}M, Ponceau 4R 6.00×10^{-5}M or Amaranth 8.00×10^{-5}M), and a variation of concentrations of the metal ions desired. The volumes of these solutions were made up to 25.0 cm^3 with the deionized water in the volumetric flasks.

The third series solutions contained a constant concentration of Erythrosine (2.00×10^{-5}M), a variation of concentrations of the metal ion, Fe(III) ion (0.60×10^{-5}M - 1.20×10^{-4}M), Hg(II) ion (0.40×10^{-5}M - 1.60×10^{-5}M), and the acetate buffer pH 6.0 or 4.4. The volumes of these solutions were made up to 25.0 cm^3 with the deionized water in the 25.0 cm^3 volumetric flasks.

The last series was the series of the mixture solutions containing various concentrations of Erythrosine (3.00×10^{-6}M-1.20 x

10^{-4} M) and a constant concentration of the metal ion Fe(III) 1.20×10^{-5}M or Pb(II) 1.20×10^{-5}M and the acetate buffer pH 6.0 or 4.4. The volumes of these solutions were made up to 25.0 cm^3 with the deionized water in the 25.0 cm^3 volumetric flasks.

In any mixture solution, if any precipitate occurred, it was filtered off and the absorbance of the filtrate was measured at the wave length where the dye absorbed.

2.3.2.5 Atomic absorption spectrophotometry.

2.3.2.5.1 Calibration curves of metal ions.

The calibration curves of metal ions were established by plotting the absorbance of the metal ion solution against its concentration in the range of 1.50×10^{-4}M - 1.00×10^{-3}M for Hg(II) and 3.60×10^{-5}M - 3.63×10^{-4}M for Fe(III).

2.3.2.5.2 Compounds formation of dyes and metal ions.

Two series of the mixture solutions of dyes and metal ions were prepared. The first series solutions contained a variation of concentrations of the dye, buffer solution of any pH, and a constant concentration of the metal ion (Fe(II) 6.00×10^{-4}M, Fe (III) 6.00×10^{-4}M, Cd(II) 4.00×10^{-5}M, Hg(II) 1.00×10^{-3}M), or Pb(II) 2.00×10^{-4}M). The volumes of these solutions were made up to 25.0 cm^3 with the deionized water in the 25.0 cm^3 volumetric flasks.

The second series was the series of the mixture solutions containing a variation of concentrations of the dye, a constant concentration of the metal ion (Fe(II) 8.00 x 10^{-4}M, Fe(II) 8.00 x 10^{-4}M, Hg(II) 1.00 x 10^{-3}M, or Pb(II) 2.00 x 10^{-4}M), and the acetate buffer pH 6.0 or 4.4. The volumes of these solutions were made up to 25.0 cm^3 with the deionized water in 25.0 cm^3 volumetric flasks.

If any precipitate occurred in the mixture solution, it was filtered off and the percent transmittance of the filtrate was measured at the wavelength where the metal ion absorbed.

2.3.2.6 Infrared spectrophotometric study of Erythrosine and its compound.

The dried KBr was well mixed with the dried sample and ground throughly to achieve homogeneous mixture. The mixture was then transferred evenly into a standard Pye Unicam die (13 mm) The Blackhawk Enerpac Model P-39, was used to press the mixture to be a pellet under the pressure of 1406139 g/cm^2. The IR spectrum of this pellet was recorded in the range of 4000 cm^{-1} to 650 cm^{-1}.