CHAPTER III

EXPERIMENT

3.1 Chemicals and reagents

Amaranth, Ponceau 4R, Orange G, Orange RN and Sunset Yellow FCF are certified food color grade. Amaranth and Ponceau 4R were donated by East Asiatic Co.Ltd., Orange RN was donated by the Department of Medical Sciences, Ministry of Public Health . Orange G and Sunset Yellow FCF were donated by D.F. Anstead Ltd. (England).

Buffer and electrolyte solutions used were prepared from the analar grade reagent except tetraethylammonium chloride solution. Tetraethylammonium chloride, from Eastman Kodak Co., was twice recrystallized from the mixed solvents of benzene and methyl alcohol in the ratio 69.5:30.5 (azeotropic composition) (31).

The double deionized water used through out this study was prepared by passing the single distilled water through a series of three columns (3.5cm, I.D.and 55 cm, long). Amberite IR-45 (OH), anionic resins were packed in the first column, Amberite IR-120 (H), cationic resins were in the second column and the last column was one half filled with the anionic resins and another half with the cationic resins (22).

Triply distilled mercury (distilled from the BDH Analar grade Hg) which had been tested for purity by differential pulse polarographic (DPP) analysis was used throughout this study.

3.2 Apparatus

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

Visible and UV spectra were obtained with a Varian Techtron Spectrophotometer Model 635 equipped with a Varian Techtron Recorder Model 7040A.

Polarograms were obtained with a Princeton Applied Research (PAR) Polarographic Analyzer Model 174A equipped with a PAR Model 174/70 drop timer, and a Hewlett Packard Recorder Model 7040A.

The cell employed in all measurements was a jacketed compartment (Radiometer Copenhagen Model V519).

The reference electrode was a saturated calomel electrode, SCE (Radiometer Electrode Model K501).

All potentials in this work were measured against SCE.

In order to purify nitrogen gas before being used to deaerate the test solution, three bubbling towers were placed in the gas line. The first tower contained amalgamated zinc metal in a Cr(III) solution, for reduction of any contaminated oxygen gas. The second tower contained 4% sodium hydroxide for neutralization of the nitrogen gas. The last one was a trapped tower for preventing any solution from the second tower to flow into the test solution.

All measurements in this work were made at $30.0^{\circ} \pm 0.1^{\circ}C$ by means of a circulating constant temperature bath (Laudathermostat Type K2).

3.3 Procedure

3.3.1 Paper Chromatographic technique

3.3.1.1 Developing solvent systems

Three solvent systems were used in the paper chromatographic test of dyes (32): system I is the solution of 2% NaCl in 50% ethanol, system II is the mixture of 2-methyl propan-1-ol, ethanol and water in the ratio of 1:2:1, respectively, and system III is 2.5% NaCl aqueous solution.

3.3.1.2 Dye solutions

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

3.3.1.3 Chromatographic chamber

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

3.3.1.4 Paper chromatography

A strip of whatman filter paper number 1 (15.0 cm x 20.0 cm) was spotted with a drop of each dye solution at the point which is 2 cm above the bottom edge of the paper by means of a 1 mm capillary. Five spots of the dyes were performed in each paper strip. The dye spots were dried in air at room temperature. Then the paper was folded in a cylindrical shape, fastened with adhesive tape and inserted in 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 chromatogram was developed and completed in about half an hour at room temperature. The paper was then removed from the chamber and was dried in air. The developed spots were circled with a pencil and their R_f values were determined.

3.3.2 Spectrophotometric technique

The 2.5×10^{-5} M dye solutions of Amaranth, Ponceau 4R, Orange G, Orange RN and Sunset Yellow FCF were prepared in 0.1 M HCl and 0.1 M NaOH solution.

3.3.3 Polarographic technique

3.3.3.1 Stock solutions of dyes

The stock solution of 1.0×10^{-3}-5.0×10^{-3} M of the dye desired was freshly prepared by dissolving the dye in the double deionized water.

3.3.3.2 Buffer solutions

The buffer solution of pH about 1.5 was prepared by mixing 48.5 cm^3 and 25.0 cm^3 of 0.2 M HCl and 0.2 M KCl solutions.

McIlvaine buffer solutions were prepared by mixing the appropriate volumes of 0.1 M citric acid and 0.2 M disodium hydrogen phosphate solution for the pH range of 2.0-7.2.

Michaelis borate buffer solutions were prepared by mixing the appropriate volumes of 0.1 M sodium hydroxide or 0.1 M hydrochloric acid with borax solution for the pH range of 8.0-12.3.

3.3.3.3 Electrolyte solutions

The 0.2 M solutions of potassium chloride, potassium nitrate and tetraethylammonium chloride were prepared in the double deionized water.

3.3.3.4 Test solutions

The test solution was prepared in

a 50.0 cm^3 volumetric flask by mixing 5.0 cm^3 of the stock solution desired with 25.0cm^3 of the electrolyte solution and 20.0 cm^3 of the buffer solution to give a final volume of 50.0cm^3.

Before the test solution was placed in the polarographic cell, the test compartment was washed two or three times with the double deionized water. Five drops of 1% gelatin solution were added for preventing polarographic maxima, and the deaeration of the test solution with purified nitrogen gas was performed for ten minutes by means of a disposable capillary. During the polarographic performance, a stream of the purified nitrogen gas was maintained over the solution surface to prevent the redissolution of oxygen. The desired potential range, current sensitivity, the height of Hg reservoir, scan rate and polarity were set on the instrument and the polarogram was recorded.

3.3.4 Standard addition technique

A series of the standard dye solutions;0.00, 0.10, 0.20, 0.30, and 0.40 cm^3 of 5.0×10^{-3}M was added to 10.0 cm^3 of the beverage sample solution, followed by 25.0 cm^3 tetraethylammonium chloride and the volume of the solution was made up to 50.0 cm^3 with the buffer of pH 7.0. The final pH of the solution was measured and the polarographic analysis was performed.