



CHAPTER III

EXPERIMENT

3.1 Chemicals and reagents

Quinoline Yellow, Tartrazine and Brilliant Blue FCF are certified food color grade. Quinoline Yellow and Tartrazine were donated by D.F Anstead Ltd. (England) and Brilliant Blue FCF was donated by East Asiatic Co. Ltd. (Thailand).

Chemicals and reagents used for preparations of buffer and electrolyte solutions were of analar grade except tetraethylammonium chloride (TEAC) from Eastman Kodak Co. TEAC was twice recrystallized from the mixed solvent of benzene and methyl alcohol in the ratio 69.5 : 30.5 (azeotropic composition) (30).

Purified nitrogen gas, used for deaerating test solutions, was prepared by passing the nitrogen gas through a vanadium (II) chloride solution and then through the double deionized water before entering the cell.

The double deionized water was prepared by passing the single distilled water through a series of three columns (3.5 cm , I.D and 55 cm , long). **Amberlite** IR-45 (OH), anionic resins were packed in the first column; **Amberlite** 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 cationic resins(31).

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

Polarograms were obtained with a Princeton Applied Research (PAR) Polarographic Analyzer Model 174A equipped with a PAR Drop timer Model 174/70, and a Hewlett-Packard X-Y Recorder Model 7040A. The cell employed in all measurements was a jacketed compartment, (Radiometer Copenhagen Model V 519). Three electrodes were used, they were a dropping mercury electrode (DME) as the working electrode, a saturated calomel electrode (SCE) (Radiometer Electrode Model K501) as the reference electrode, and a carbon rod (6mm dia.) as the counter electrode.

All measurements in this work were made at $30.0 \pm 0.1^\circ\text{C}$ by means of a circulating constant temperature bath (Laudathermostat Type K2). The pH measurements were obtained with a pH meter (Radiometer Copenhagen Type PHM 28). All potentials in this work were measured against SCE.

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

Infrared spectra were obtained with a Pye Unicam Sp 2000 Grating Infrared Spectrophotometer.

3.3 Procedure

3.3.1 Paper Chromatographic Technique

3.3.1.1 Developing solvent systems

Four solvent systems were used for testing the purity of Quinoline Yellow, Tartrazine and Brilliant Blue FCF. System I is the

mixture of 1-butanol, water and acetic acid in the ratio 20:12:5, respectively. System II was prepared by mixing 99 cm³ of the mixture contained 2-methyl-1-propanol, ethanol and water in the ratio 3:2:2, respectively, with 1 cm³ of 0.88 ammonia solution. System III is the mixture of 30 g phenol and 20 g water and system IV is the mixture of 2-butanone, 2-propanone, water and 0.88 ammonia solution in the ratio 70:30:30:0.2, respectively (32). Tartrazine was tested by using systems I and II, Quinoline Yellow was tested by systems I and IV and Brilliant Blue FCF was tested by using systems II and III.

3.3.1.2 Dye solutions

The dye solutions were prepared by dissolving a few milligrams of the solid dyes in 1 cm³ of the double deionized water.

3.3.1.3 Chromatographic chamber

A 1 dm³-beaker containing about 20 cm³ of the developing solvent 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 the dye solution at the point where it was 2 cm above the bottom edge of the paper by using a 1 mm (ID) capillary. Four spots of the dyes were performed in each strip. The dye spots were dried in air at room temperature. Then the paper was folded in 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 one 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

3.3.2.1 Visible and UV spectrophotometric study

The 2.5×10^{-5} M dye solutions of Quinoline Yellow, Tartrazine and Brilliant Blue FCF were prepared in 0.1 M HCl and in 0.1 M NaOH.

3.3.2.2 Infrared spectrophotometric study

The dried KBr was well mixed with the dried sample and ground **thoroughly** to achieve homogeneous mixture. The mixture was then transferred evenly into a standard Pye Unicam die (13 mm). The Blockhawk Enerpac Model P-39, was used to press the mixture to be a pellet under the pressure of 2000 psi. The IR spectrum of this pellet was recorded in the range of 4000 cm^{-1} to 650 cm^{-1} .

3.3.3 Polarographic Technique

3.3.3.1 Stock solutions of dyes

The stock solution of 5.0×10^{-3} M of the dye desired was prepared by dissolving the appropriate amounts of 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 of 0.2 M HCl and 25.0 cm^3 of 0.2 M KCl

Mc Ilvaine 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 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 7.6-12.3.

Michaelis phosphate buffer solutions were prepared by mixing the appropriate volumes of M/15 potassium phosphate and M/15 disodium phosphate for the pH range of 4.5-8.3.

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 cm³ volumetric flask by mixing 5.0 cm³ of the stock solution of the dye desired with 25.0 cm³ of the electrolyte solution and 20.0 cm³ of the buffer solution to give a final volume of 50.0 cm³.

Before the test solution was placed in the polarographic cell, the cell compartment was rinsed twice or thrice with the double deionized water and then with the test solution.

Five drops of 1% gelatin solution were added for preventing polarographic maxima, and the deaeration of the test solution with the 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, scan rate, sensitivity, the height of mercury reservoir, polarity and drop time 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 , 0.06 , 0.12 , and 0.18 cm^3 of 5.0×10^{-3} M was added to 5.0 cm^3 of the beverage sample solution , followed with 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 5.6 . The final pH was measured and the polarographic analysis of beverage was performed .