CHAPTER III

EXPERIMENTAL

3.1 Materials

3.1.1 Aniline (C_6H_7N) was obtained from Merck.

3.1.2 2-Chloroaniline (C_6H_6ClN) was obtained from Merck.

3.1.3 3-Chloroaniline (C_6H_6ClN) was obtained from Merck.

3.1.4 4-Chloroaniline (C_6H_6ClN) was obtained from Merck.

3.1.5 *o*-Nitroaniline ($C_6H_6N_2O_2$) was obtained from Fluka.

3.1.6 *m*-Nitroaniline ($C_6H_6N_2O_2$) was obtained from Fluka.

3.1.7 *p*-Nitroaniline ($C_6H_6N_2O_2$) was obtained from Fluka.

3.1.8 *o*-Toluidine (C_7H_9N) was obtained from HM.

3.1.9 *m*-Toluidine (C_7H_9N) was obtained from Fluka.

3.1.10 *p*- Toluidine (C_7H_9N) was obtained from Merck.

3.1.11 2-Chloro-4-nitroaniline ($C_6H_5ClN_2O_2$) was obtained from Fluka.

3.1.12 Toluene (C_7H_8) was obtained from Mallinckrodt.

3.1.13 Xylene (C_8H_{10}) was obtained from J.T.Baker.

3.1.14 Chloroform (CH_3Cl) was obtained from BDH.

3.1.15 Methanol (CH_4O) was obtained from J.T.Baker.

3.1.16 Ethylene glycol ($C_2H_6O_2$) was obtained from Carlo Erba.

3.1.17 1-Hexanol ($C_6H_{14}O$) was obtained from Fluka.

3.1.18 Sulfuric acid (H_2SO_4) was obtained from J.T.Baker.

3.1.19 Hydrochloric acid (HCl) was obtained from BDH.

3.1.20 Sodium nitrite ($NaNO_2$) was obtained from Univar.

3.1.21 Sodium carbonate ($NaCO_3$) was obtained from BDH.

3.1.22 Potassium Iodide (KI) was obtained from Merck.

3.1.23 Potassium hydroxide (KOH) was obtained from Merck.

3.1.24 Ethyl-2-aminobenzoate was obtained from Merck.

3.1.25 3-Aminobenzoic acid was obtained from Merck.

3.1.26 Starch was obtained from Merck.

3.2 Instruments and apparatuses

3.2.1 FT-IR Spectrophotometer

The FT-IR Spectrophotometer model 2000 series (Double Beam) from Perkin Elmer was used for characterization at the working range of 400-4000 cm^{-1}.

3.2.2 UV/VIS-Spectrophotometer

The UV/VIS-Spectrophotometer model Lamda-2 from Perkin Elmer was used to quantify at the maximum wavelength of each marker dye.

3.2.3 Nuclear Magnetic Resonance Spectrometer

The FT-NMR spectrometer model JNM-A500 from JEOL Japan was used to characterize the products at 125.65 MHz for ^{13}C-NMR and 500 MHz for ^{1}H-NMR.

3.2.4 Digital Melting Point Apparatus

The digital melting point apparatus from Electrothermal was used to determine the melting point of solid azo dyes.

3.2.5 Petrochemical tintometer

The Petrochemical tintometer model PFX 990/P from Lovibond was used to determine the color by ASTM D1500 method.

3.2.6 CHNS/O Analyzer

The CHNS/O Analyzer model PE 2400 series II from Perkin Elmer was used to determine the elements such as carbon, hydrogen and nitrogen.

3.3 Experimental procedures

3.3.1 Syntheses

3.3.1.1 Esterification of 3-aminobenzoic acid with 1-hexanol [10]

The mixture of 20.57 g. (0.15 mole) of 3-aminobenzoic acid, 229.90 g. (2.25 mole) of 1-hexanol and 3.7 ml (3 percent weight of 1-hexanol) of concentrated sulfuric acid in a 1-litre round-bottomed flask was refluxed for 4 hours. The hot reaction mixture was pour into excess of water, then added sodium carbonate until it was neutral to litmus paper. The mixture solution was transferred into separation funnel and discarded the lower aqueous layer. The upper layer was washed with water several times and distilled which the fractions of lower boiling point (containing water and unreacted 1-hexanol) passed over first until the temperature of residual ester reached 160 ^{o}C, then cooled down to room temperature .The yield of hexyl-3-aminobenzoate was 22 g. (69%).

3.3.1.2 Diazotization of aniline and aniline derivatives [21]

Procedures

a) Aniline

A solution of 0.93 g. (0.01 mole) of aniline (purified by redistillation if necessary), 2.5 ml of water and 2.5 ml (0.03 mole) of concentrated hydrochloric acid was cooled down to 0-5 °C. A solution of 0.69 g. (0.01 mole) of sodium nitrite in 2.5 ml of water was cooled down to 0-5 °C and then was added dropwise to aniline hydrochloride solution with continuous stirring. The solution should be clear. The mixture was stirred for about 10 minutes after completing the addition of nitrite and was then tested to ensure that it gave a weak blue test with starch-iodine paper.* If the mixture did not give the weak blue test for nitrite, more nitrite solution was added dropwise until a positive test was obtained and persisted for a few minutes. If, on the other hand, a strong blue test for nitrite was obtained, a few drop of a dilute solution of aniline hydrochloride was added until the nitrite test nearly disappears. Aniline diazonium salt solution was obtained and should be used immediately.

* Pure starch (1g.) was rubbed with a small amount of water, and 100 ml of boiling water was added with stirring. After the solution was cooled, 0.2 g. of potassium iodide was added and sheets of clean filter paper were soaked in it and allowed to dry in a clean place.

b) p-Nitroaniline

A mixture of 1.38 g. (0.01 mole) of p-nitroaniline, 2.5 ml of water and 3.3 ml (0.04 mole) of concentrated hydrochloric acid was heated until solution was

complete. The solution was cooled to room temperature with shaking under tap water and cooled down to 0-5 °C. A solution of 0.69 g. (0.01 mole) of sodium nitrite in 2.5 ml of water was cooled down to 0-5 °C and then was added dropwise to p-nitroaniline hydrochloride solution with continuous stirring for about 10 minutes after completing the addition of nitrite and was then tested for nitrite with starch-iodine paper as mentioned above. Any residual undissolved material was removed by filtration. The residue should be very small; the filtrate should be clear and almost colorless and no cloudiness should reappear. The solution of p-nitroaniline diazonium salt was obtained.

c) o-Nitroaniline

The procedure in b) was repeated except that 1.38g. (0.01 mole) of o-nitroaniline was used. The solution of o-nitroaniline diazonium salt was obtained.

d) m-Nitroaniline

The procedure in b) was repeated except that 1.38g. (0.01 mole) of m-nitroaniline was used. The solution of m-nitroaniline diazonium salt was obtained.

e) 2-Chloroaniline

The procedure in b) was repeated except that 1.28g. (0.01 mole) of 2-chloroaniline was used. The solution of 2-chloroaniline diazonium salt was obtained.

f) 3-Chloroaniline

The procedure in b) was repeated except that 1.28g. (0.01 mole) of 3-chloroaniline was used. The solution of 3-chloroaniline diazonium salt was obtained.

g) 4-Chloroaniline

The procedure in b) was repeated except that 1.28g. (0.01 mole) of 4-chloroaniline was used. The solution of 4-chloroaniline diazonium salt was obtained.

h) 2-Chloro-4-nitroaniline

The procedure in b) was repeated except that 1.73g. (0.01 mole) of 2-chloro-4-nitroaniline was used. The solution of 2-chloro-4-nitroaniline diazonium salt was obtained.

i) o-Toluidine

The procedure in a) was repeated except that 1.07g. (0.01 mole) of o-toluidine was used. The solution of o-toluidine diazonium salt was obtained.

j) m-Toluidine

The procedure in a) was repeated except that 1.07g. (0.01 mole) of m-toluidine was used. The solution of m-toluidine diazonium salt was obtained.

k) p-Toluidine

The procedure in a) was repeated except that 1.07g. (0.01 mole) of p-toluidine was used. The solution of p-toluidine diazonium salt was obtained.

3.3.1.3 Coupling reaction of diazonium salts and ethyl-2-amino-benzoate

a) Aniline

Dissolved 1.65 g. (0.01 mole) of ethyl-2-aminobenzoate in 20 ml of methanol containing 0.82 g. (0.01 mole) of sodium acetate. The solution was added

slowly to a good stirred aniline diazoium salt solution, obtained from 3.3.1.2.*a*, held at 0-5 °C. Each drop of the solution added should immediately produce the dye, separated out from aqueous phase. When the addition had been completed. Stirring was continued for 30 minutes. The oily mixture was poured into separating funnel and washed 2-3 times with distilled water. The upper oil phase of ethyl-2-aminobenzoate phenyl azo was collected.

b) p-Nitroaniline

Dissolved 1.65 g. (0.01 mole) of ethyl-2-aminobenzoate in 20 ml of methanol containing 1.64 g. (0.02 mole) of sodium acetate. The solution was added slowly to a good stirred p-nitroaniline diazoium salt solution, obtained from 3.3.1.2 *b*, held at 0-5 °C. Each drop of the solution added should immediately produce the dye. Stirring was continued for 30 minutes and then the dye, which was completely precipitated, was filtered off with suction and washed thoroughly with distilled water, dried upon filter paper in the air. The orange cake of azo dye was obtained.

c) m-Nitroaniline

The procedure in b) was repeated except that 0.01 mole of m-nitroaniline diazonium salt, obtained from 3.3.1.2.*c*. The yellow cake of azo dye was obtained.

d) o-Nitroaniline

The procedure in b) was repeated except that 0.01 mole of o-nitroaniline diazonium salt, obtained from 3.3.1.2.*d*. The yellow cake of azo dye was obtained.

e) 2-Chloroaniline

The procedure in b) was repeated except that 0.01 mole of 2-chloroaniline diazonium salt, obtained from 3.3.1.2.e. The yellow cake of azo dye was obtained.

f) 3-Chloroaniline

The procedure in b) was repeated except that 0.01 mole of 3-chloroaniline diazonium salt, obtained from 3.3.1.2. *f*. The yellow cake of azo dye was obtained.

g) 4-Chloroaniline

The procedure in b) was repeated except that 0.01 mole of 4-chloroaniline diazonium salt, obtained from 3.3.1.2.g. The yellow cake of azo dye was obtained.

h) 2-Chloro-4-nitroaniline

The procedure in b) was repeated except that 0.01 mole of 2-chloro-4-nitroaniline diazonium salt, obtained from 3.3.1.2.*h*. The orange cake of azo dye was obtained.

i) o -Toluidine

The procedure in a) was repeated except that 0.01 mole of o-toluidine diazonium salt, obtained from 3.3.1.2.*i*. The upper oil phase of ethyl-2-aminobenzoate-2-methylphenyl azo was obtained.

j) m -Toluidine

The procedure in a) was repeated except that 0.01 mole of m-toluidine diazonium salt, obtained from 3.3.1.2.*j*. The upper oil phase of ethyl-2-aminobenzoate-3-methylphenyl azo was obtained.

k) p –Toluidine

The procedure in a) was repeated except that 0.01 mole of p-toluidine diazonium salt, obtained from 3.3.1.2. *k*. The upper oil phase of ethyl-2-aminobenzoate-4-methylphenyl azo was obtained.

3.3.1.4 Coupling reaction of diazonium salts and hexyl-3-amino-benzoate

a) Aniline

Dissolved 2.21 g. (0.01 mole) of hexyl-3-aminobenzoate, obtained from 3.3.1.1. in 20 ml of methanol containing 0.82 g. (0.01 mole) of sodium acetate. The solution was added slowly to a good stirred aniline diazoium salt solution, obtained from 3.3.1.2.*a*., held at 0-5 °C. Each drop of the solution added should immediately produce the dye, separated out from aqueous phase. When the addition had been completed. Stirring was continued for 30 minutes. The oily mixture was poured into separating funnel and washed 2-3 times with distilled water. The upper oil phase of hexyl-3-aminobenzoate phenyl azo was collected.

b) p-Nitroaniline

Dissolved 2.21 g. (0.01 mole) of hexyl-3-aminobenzoate in 20 ml of methanol containing 1.64 g. (0.02 mole) of sodium acetate. The solution was added slowly to a good stirred p-nitroaniline diazoium salt solution, obtained from 3.3.1.2.*b*., held at 0-5 °C. Each drop of the solution added should immediately produce the dye. Stirring was continued for 30 minutes and then the dye, which was completely precipitated, was filtered off with suction and washed thoroughly with distilled water, dried upon filter paper in the air. The orange cake of hexyl-3-aminobenzoate-4-nitrophenyl azo was obtained.

c) m-Nitroaniline

The procedure in b) was repeated except that 0.01 mole of m-nitroaniline diazonium salt, obtained from 3.3.1.2.*c*. The yellow cake of hexyl-3-aminobenzoate-3-nitrophenyl azo was obtained.

d) o-Nitroaniline

The procedure in b) was repeated except that 0.01 mole of o-nitroaniline diazonium salt, obtained from 3.3.1.2.*d*. The yellow cake of hexyl-3-aminobenzoate-2-nitrophenyl azo was obtained.

e) 2-Chloroaniline

The procedure in b) was repeated except that 0.01 mole of 2-chloroaniline diazonium salt ,obtained from 3.3.1.2.*e*. The yellow cake of hexyl-3-aminobenzoate-2-chlorophenyl azo was obtained.

f) 3-Chloroaniline

The procedure in b) was repeated except that 0.01 mole of 3-chloroaniline diazonium salt, obtained from 3.3.1.2.*f*. The yellow cake of hexyl-3-aminobenzoate-3-chlorophenyl azo was obtained.

g) 4-Chloroaniline

The procedure in b) was repeated except that 0.01 mole of 4-chloroaniline diazonium salt, obtained from 3.3.1.2.*g*. The yellow cake of hexyl-3-aminobenzoate-3-chlorophenyl azo was obtained.

h) 2-Chloro-4-nitroaniline

The procedure in b) was repeated except that 0.01 mole of 2-chloro-4-nitroaniline diazonium salt, obtained from 3.3.1.2.*h*. The orange cake of hexyl-3-aminobenzoate-2-chloro-4-nitrophenyl azo was obtained.

i) o -Toluidine

The procedure in a) was repeated except that 0.01 mole of o-toluidine diazonium salt, obtained from 3.3.1.2.*i*. The upper oil phase of hexyl-3-aminobenzoate-2-methylphenyl azo was obtained.

j) m -Toluidine

The procedure in a) was repeated except that 0.01 mole of m-toluidine diazonium salt, obtained from 3.3.1.2.*j*. The upper oil phase of hexyl-3-aminobenzoate-3-methylphenyl azo was obtained.

k) p -Toluidine

The procedure in a) was repeated except that 0.01 mole of p-toluidine diazonium salt, obtained from 3.3.1.2.*k*. The upper oil phase of hexyl-3-aminobenzoate-4-methylphenyl azo was obtained.

3.3.2 Treatment of marker dyes

3.3.2.1 Preparation of stock solutions of maker dyes

0.02 g. of each synthesized azo dyes were dissolved in 20 ml of xylene except that ethyl-2-aminobenzoate-2-chlorophenyl azo and ethyl-2-aminobenzoate-2-chloro-4-nitrophenyl azo dye were dissolved in 20 ml of toluene. The stock solutions of 1000 ppm were obtained.

3.3.2.2 Preparation of marked HSD

Pipetted 1 ml of stock solution 1000 ppm of each synthesized azo dyes into 100 ml volumetric flask, then diluted and made up to the volume with commercial HSD. The 10 ppm marked HSD was obtained.

3.3.3 Detection of marker dyes

The method for detection of marker dyes added into HSD in this thesis was the extraction with petroleum - immiscible solvent. During extraction, marker gave a chromophoric reaction, producing a color of marker in extracted phase.

3.3.3.1 Suitable extraction solutions

Pipetted 20 ml of each marked HSD prepared in 3.3.2.2 and 5 ml of each of the extraction solutions into 30 ml screw cap vial, capped and shaken for 30 seconds then, allowed the mixture to separate into two-phase. The marker was dissolved into the lower phase. Suitable extraction solution should develop a different and a stronger color, which was visible to the naked eye in the extracted lower phase. The various extraction solution systems were shown in Table 3-1.

Table 3-1 Extraction solution systems

Acidic extraction solutions	Basic extraction solutions
10% HCl in MeOH	7% KOH in MeOH
10% HCl in EG	5% KOH in EG
---	7% KOH in H_2O
---	10% KOH in 90% MeOH - EG

3.3.3.2 Suitable concentration of potassium hydroxide

To obtain the suitable extraction solution for individual marker dye, three concentration of potassium hydroxide in each of the suitable extraction solution systems were prepared as shown in Table 3-2. Pipetted 20 ml of each marked HSD and 5 ml of each concentration of potassium hydroxide in suitable extraction solution for individual marker into 30 ml screw cap vial, capped and shaken for 30 seconds, then allowed the mixture to separate into two-phase. The lower phase of extraction solution containing the color of marked dye and the absorbance was determined at maximum absorption wavelength in the range of 350-750 nm using US/VIS spectrophotometer. The concentration of potassium hydroxide in each extraction systems, which gave highest absorbance at maximum absorption wavelength was suitable to be used in further experiment.

Table 3-2 The various concentrations of potassium hydroxide in extraction systems

System I	System II	System III
5% KOH in MeOH	8% KOH in 90% MeOH - EG	3% KOH in EG
6% KOH in MeOH	9% KOH in 90% MeOH - EG	4% KOH in EG
7% KOH in MeOH	10%KOH in 90%MeOH - EG	5% KOH in EG

3.3.3.3 Quantitative determination

A relatively quantitative determination of marker added in HSD could be made by using UV/VIS spectroscopy technique.

3.3.3.3.1 Calibration curve of ethyl-2-aminobenzoate-2-nitro-phenyl azo

The working standards of 2, 3, 4 and 5 ppm were prepared as the following procedure. Pipetted 1000 ppm stock solution of ethyl-2-aminobenzoate-2-nitrophenyl azo 0.20, 0.30, 0.40 and 0.50 ml into 100ml volumetric flask respectively and made up to the volume with unmarked commercial HSD. Then, pipetted 20 ml of each working standards and 5 ml of suitable extraction solution into 30 ml screw cap vial, capped and shaken for 30 seconds and allowed the mixture to separate into two-phase. The lower extracted phase was filled into UV cuvette cell and the absorbance was measured at maximum absorption wavelength. The measured absorbances were plotted as a function with the concentration to obtain working standards. All working standards were tested according to ASTM D-1500 method.

3.3.3.3.2 Calibration curve of ethyl-2-aminobenzoate-4-nitro-phenyl azo

The procedure in 3.3.3.3.1 was repeated except that the working standards of 0.25, 0.5, 1.0 and 2.0 ppm were prepared as the following procedure. Pipetted 1000 ppm stock solution of ethyl 2-aminobenzoate-4-nitrophenyl azo in the amounts of 0.025, 0.050, 0.10 and 0.20 ml and put into each 100 ml volumetric flask, respectively and made up to the volume with unmarked commercial HSD.

3.3.3.3.3 Calibration curve of ethyl-2-aminobenzoate-2-chloro-4-nitrophenyl azo

The procedure in 3.3.3.3.1 was repeated except that the working standards were prepared as following procedure. Pipetted 0.025, 0.05, 0.10 and 0.20 ml of 1000 ppm stock solution of ethyl-2-aminobenzoate-2-chloro-4-nitrophenyl azo dye and put into each 100 ml volumetric flask, respectively and made up to the volume with unmarked commercial HSD.

3.3.3.3.4 Calibration curve of hexyl-3-aminobenzoate-2-nitro-pheny azo

The procedure in 3.3.3.3.1 was repeated except that the working standards of 4, 5, 6 and 7 ppm were prepared as the following procedure. Pipetted 0.40, 0.50, 0.60 and 0.70 ml of 1000 ppm stock solution of hexyl-3-aminobenzoate-2-nitrophenyl azo and put into each 100 ml volumetric flask, respectively and made up to the volume with unmarked commercial HSD.

3.3.3.3.5 Calibration curve of hexyl-3-aminobenzoate-4-nitro-pheny azo

The procedure in 3.3.3.3.1 was repeated except that the working standards of 0.5, 1, 2 and 3 ppm were prepared as following procedure. Pipetted 0.050, 0.10, 0.20 and 0.30 ml of 1000 ppm stock solution of hexyl-3-aminobenzoate-4-nitrophenyl azo and put into each 100 ml volumetric flask, respectively and made up to the volume with unmarked commercial HSD.

3.3.3.3.6 Calibration curve of hexyl-3-aminobenzoate-2-chloro-4-nitrophenyl azo

The procedure in 3.3.3.3.1 was repeated except that the working standards of 3, 4, 5 and 6 ppm were prepared as the following procedure. Pipetted 0.30, 0.40, 0.50 and 0.60 ml of 1000 ppm stock solution of hexyl-3-aminobenzoate-2-chloro-4-nitrophenyl azo and put into each 100 ml volumetric flask, respectively and made up to the volume with unmarked commercial HSD.

3.3.4 Characterization

Hexyl-3-aminobenzoate and all dyes were characterized using various techniques such as FT-IR, ^{13}C-NMR, ^{1}H-NMR and elemental analysis.

3.3.5 Physical properties of marker dyes

Azo dyes were tested for basic physical properties, for example, melting point and solubility property.

3.3.6 Stability of marker dyes in HSD

The marked HSD at the concentrations from the follwing :

- 4 ppm of ethyl-2-aminobenzoate-2-nitrophenyl azo
- 1 ppm of ethyl-2-aminobenzoate-4-nitrophenyl azo
- 1 ppm of ethyl-2-aminobenzoate-2-chloro-4-nitrophenyl azo
- 6 ppm of hexyl-3-aminobenzoate-2-nitrophenyl azo
- 2 ppm of hexyl-3-aminobenzoate-4-nitrophenyl azo

- 5 ppm of hexyl-3-aminobenzoate-2-chloro-4-nitrophenyl azo

They were prepared and stored in area protected from light. The amount of each marker dyes added in HSD was measured by UV/VIS-spectrophotometer every month for the storage period of three months.