

CHAPTER III

EXPERIMENTAL

3.1 Apparatus

Elemental analyses were measured with a Perkin Elmer Elemental Analyzer 2400 CHN.

The FTIR spectra were recorded on a Nicolet Impact 410.

The $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectra were obtained from a Bruker Model ACF 200 spectrometer and JNM-A500, respectively.

Hewlett Packard Thinkjet and Hewlett Packard 8452A Diode Array Spectrophotometer were used for recording absorption spectra and for routine measurement, respectively, with match quartz 1 cm cell.

A pH-meter (Horiba pH meter F-11, Japan)

A magnetic stirrer (Thermolyne, U.S.A)

Glass sinter filter funnel No 4.

Graduated cylinder 50, and 100 mL

Graduated pipettes 1.00, 2.00, 5.00, and 10.00 mL

Volumetric pipettes 5.0, 10.0, 20.0, and 25.0 mL

Beaker 50, 100, 250, 500, and 1000 mL

Burette 50.00 mL

Volumetric Flask 25, 50, 100, 500, and 1000 mL

Starch Iodide paper (Merck, Germany)

TLC (Aluminium Sheet, 20 × 20 cm silica gel 60 F₂₅₄, Merck, Germany)

3.2 Reagents

2-Amino-4-nitrophenol 90% (4NAP), 2-amino-5-nitrophenol 90-95% (5NAP), chromotropic acid disodium salt dihydrate (CTA), sodium nitrate,

sodium hydroxide, pyridine, calcium chloride dihydrate, and bismuth(III) nitrate pentahydrate were of analytical grade and purchased from Fluka chemical company, Switzerland.

2-Amino-3-nitrophenol (3NAP) (analytical reagent grade) was purchased from Aldrich, U.S.A.

Picramic acid 55% (commercial reagent grade) was provided by Matangi Dyestuff Industries Ltd., Thailand.

Lithium hydroxide, calcium oxide, sulphamic acid, *n*-propanol, isobutanol, methyl ethyl ketone, nitric acid 65%, and ammonia solution 25% were of analytical grade and purchased from Carlo Erba, Italy.

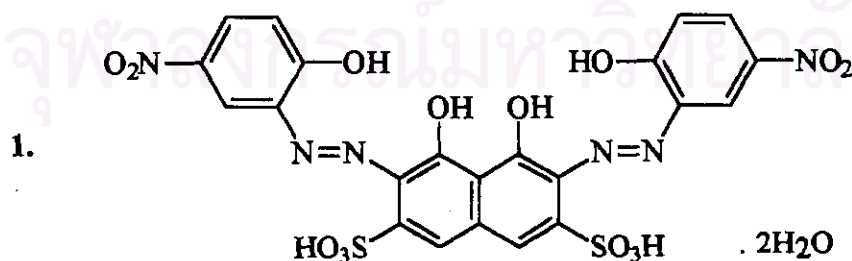
Ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA), xylenol orange, ethanol, glacial acetic acid, sodium acetate trihydrate, ammonium chloride, and hydrochloric acid 37% were of analytical grade and purchased from Merck, Germany.

n-Butanol, toluene, and formic acid were analytical grade and purchased from J.T. Baker chemical company, Holland.

All solutions were prepared by using deionized water.

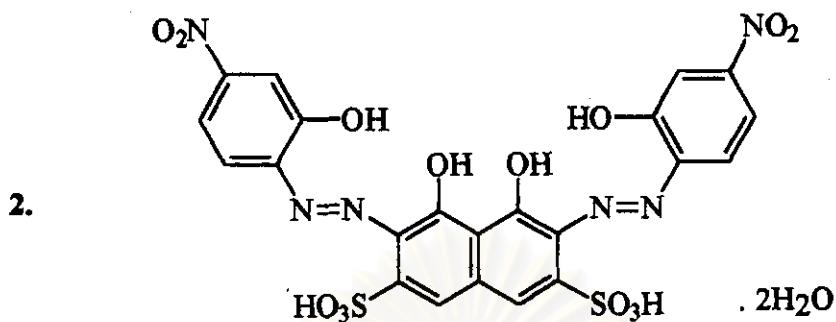
3.3 Proposed Bisazo Dyes to be Synthesized

The followings are some bis[(*O,O'*-dihydroxy-nitrophenyl)azo] derivatives of the chromotropic synthesized in this study.



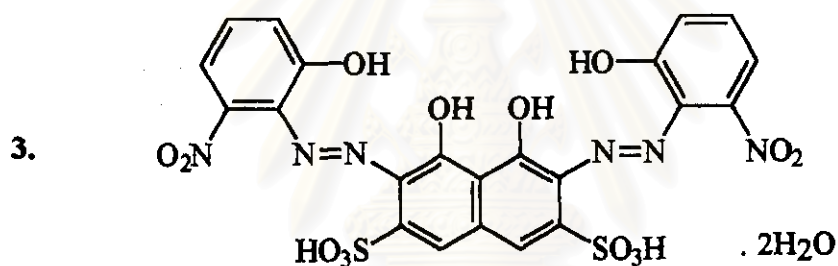
(C₂₂H₁₄N₆O₁₄S₂.2H₂O, M.W.= 686.54)

2,7-Bis[(2-hydroxy-5-nitrophenyl)azo]-1,8-dihydroxy-3,6-naphthalenedisulfonic acid
(assigned as 5N-dye)



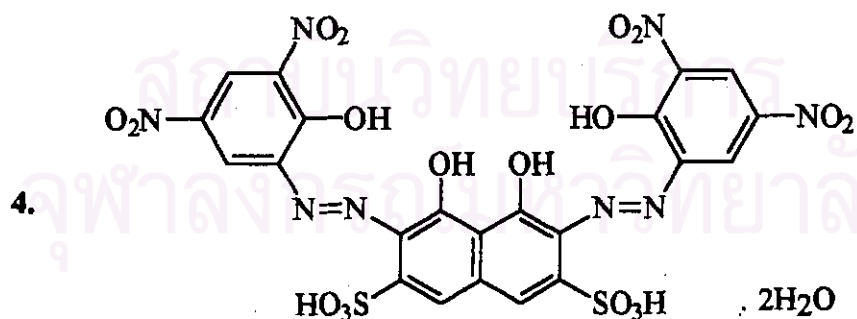
(C₂₂H₁₄N₆O₁₄S₂.2H₂O, M.W.= 686.54)

2,7-Bis[(2-hydroxy-4-nitrophenyl)azo]-1,8-dihydroxy-3,6-naphthalenedisulfonic acid
(assigned as 4N-dye)



(C₂₂H₁₄N₆O₁₄S₂.2H₂O, M.W.= 686.54)

2,7-Bis[(2-hydroxy-6-nitrophenyl)azo]-1,8-dihydroxy-3,6-naphthalenedisulfonic acid
(assigned as 6N-dye)



(C₂₂H₁₄N₆O₁₄S₂.2H₂O, M.W.= 776.55)

2,7-Bis[(2-hydroxy-5-nitrophenyl)azo]-1,8-dihydroxy-3,6-naphthalenedisulfonic acid
(assigned as 3,5N-dye)

3.4 Attempts to Prepare the Proposed Bisazo Dyes

Method 1(34)

In diazotization step, the normal hydrochloric acid-sodium nitrite procedure is used. The coupling reaction is performed under an alkaline condition using sodium hydroxide.

(a) **2,7-Bis[(2-hydroxy-5-nitrophenyl)azo]-1,8-dihydroxy-3,6-naphthalene disulfonic acid (5N-dye):**

The diazotization: 4NAP 17.10 g (0.10 mole) was mixed well with 150.00 mL water and 21.00 mL 37% hydrochloric acid. A solution was cooled to 12 °C and then diazotized by adding dropwise a cold solution of sodium nitrite 7.00 g (0.10 mole) in 20.00 mL. The addition should be completed within 20 minutes. A solution was checked for the excess of nitrite with SI-paper (instantaneously bluing) and stirred for 1 hour under the excess nitrite condition to ensure the complete reaction. After this, the excess of nitrite was removed and destroyed by sulfamic acid.

The coupling reaction: A solution containing 20.00 g (0.05 mole) of CTA in 50.00 mL of water was stirred to dissolve, cooled to 5-10 °C, adjusted pH to 8.00-8.50 with 5% sodium hydroxide. This solution was added dropwise by diazotized solution, with constant stirring and maintained the pH to 8.00-8.50. After completing the addition, stirring was continued for about 2-3 hours. The pH was then adjusted to 5.00-6.00 with dilute hydrochloric acid, then about 15.00 g of sodium acetate was added for isolating dye. The content was stored in a refrigerator overnight. After filtering through a sintered glass funnel No.4, the precipitate was rinsed with 10.00 mL of (3:1) ethanol:water and dried at 70 °C for 24 hours.

(b) **2,7-Bis[(2-hydroxy-4-nitrophenyl)azo]-1,8-dihydroxy-3,6-naphthalene disulfonic acid (4N-dye):**

For the preparation, the detailed experiment procedure was exactly the same as in the case of 5N-dye except 5NAP was used in place of 4NAP.

(c) 2,7-Bis[2-hydroxy-3,5-dinitrophenyl]azo]-1,8-dihydroxy-3,6 - naphthalene disulfonic acid (3,5N-dye):

The diazotization: picramic acid 20.09 g (0.05 mole) was mixed well with 85.00 mL water and 12.00 mL 37% hydrochloric acid. A solution was cooled to 12 °C and then diazotized by adding dropwise a cold solution of sodium nitrite 4.10 g (0.059 mole) in 20.00 mL. The addition should be completed within 20 minutes. A solution was checked for the excess of nitrite with SI-paper (instantaneously bluing) and stirred for 1 hour under the excess nitrite condition to ensure the complete reaction. After this, the excess of nitrite was removed and destroyed by sulfamic acid.

The coupling reaction: A solution containing 10.00 g (0.025 mole) of CTA in 25.00 mL of water was stirred to dissolve, cooled to 5-10 °C, adjusted pH to 8.00-8.50 with 5% sodium hydroxide. This solution was added dropwise by the diazotized solution, with constant stirring and maintained pH to 8.00-8.50. After completing the addition, stirring was continued for about 2-3 hours. The pH was then adjusted to 5.00-6.00 with dilute hydrochloric acid, then about 6.00 g of sodium acetate was added for isolating dye. The content was stored in a refrigerator overnight. After filtering through a sintered glass funnel No.4, the precipitate was rinsed with 10.00 mL of (3:1) ethanol:water and dried at 70 °C for 24 hours.

(d) 2,7-Bis[2-hydroxy-6-dinitrophenyl]azo]-1,8-dihydroxy-3,6-naphthalene disulfonic acid (6N-dye) :

The diazotization: 3NAP 4.00 g (0.025 mole) was mixed well with 35.00 mL water and 5.50 mL 37% hydrochloric acid. A solution was cooled to 12°C and then diazotized by adding dropwise a cold solution of sodium nitrite 1.73 g (0.025 mole) in 10.00 mL. The addition should be completed within 20 minutes. A solution was checked for the excess of nitrite with SI-paper (instantaneously bluing) and stirred for 1 hour under the excess nitrite condition to ensure the complete reaction. After this, the excess of nitrite was removed and destroyed by sulfamic acid.

The coupling reaction: A solution containing 4.00 g (0.01 mole) of CTA in 15.00 mL of water was stirred to dissolve, cooled to 5-10 °C, adjusted pH to 8.00-8.50 with 5% sodium hydroxide. This solution was added dropwise by the diazotized solution, with constant stirring and maintained the pH to 8.00-8.50. After completing the addition, stirring was continued for about 2-3 hours. The pH was then adjusted to 5.00-6.00 with dilute hydrochloric acid, then about 5.00 g of sodium acetate was added for isolating dye. The content was stored in a refrigerator overnight. After filtering through a sintered glass funnel No.4, the precipitate was rinsed with 10.00 mL of (3:1) ethanol:water and dried at 70 °C for 24 hours.

Method 2 (4)

In diazotization step, the normal hydrochloric acid-sodium nitrite procedure is used. The coupling reaction is performed under a condition using sodium hydroxide and calcium chloride.

(a) 2,7-Bis[(2-hydroxy-5-nitrophenyl)azo]-1,8-dihydroxy-3,6-naphthalene disulfonic acid (5N-dye):

The diazotization: 4NAP 3.40 g (0.022 mole) was mixed well with 50.00 mL water and 4.20 mL 37% hydrochloric acid. A solution was cooled to 12°C and then diazotized by adding dropwise a cold solution of sodium nitrite 1.50 g (0.022 mole) in 10.00 mL. The addition should be completed within 20 minutes. A solution was checked for the excess of nitrite with SI-paper (instantaneously bluing) and stirred for 1 hour under the excess nitrite condition to ensure the complete reaction. After this, the excess of nitrite was removed and destroyed by sulfamic acid.

The coupling reaction: A solution containing 2.00 g (0.005mole) of CTA in 15.00 mL of water was mixed with a solution of calcium chloride. Prepared by dissolving 3.00 g of calcium chloride ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) in 10.00 mL of water, the whole was added to 20.00 mL of 10 F sodium hydroxide and cooled to 0°C. Into this solution was added dropwise, with constant stirring the previously

prepared diazonium salt solution. The temperature was kept 5 °C. After completing the addition, stirring was continued for about 1 hour the solution was then added to 20.00 mL of 37% hydrochloric acid and stored in a refrigerator overnight. After filtering through a sintered glass funnel, the precipitate was rinsed with of 6 F hydrochloric acid and dried at 70 °C for 24 hours.

(b) 2,7-Bis[(2-hydroxy-4-nitrophenyl)azo]-1,8-dihydroxy-3,6-naphthalene disulfonic acid (4N-dye):

For the preparation, the detailed experiment procedure was exactly the same as in the case of 5N-dye except 5NAP was used in place of 4NAP.

Method 3 (34)

In diazotization step, the normal hydrochloric acid-sodium nitrite procedure is used. The coupling reaction is performed under the pH adjusted condition between 2 and 10 using hydrochloric acid and sodium hydroxide.

2,7-Bis[(2-hydroxy-5-nitrophenyl)azo]-1,8-dihydroxy-3,6-naphthalene disulfonic acid (5N-dye):

The diazotization: 4NAP 1.71 g (0.01 mole) was mixed well with 15.00 mL water and 2.10 mL 37% hydrochloric acid. A solution was cooled to 12°C and then diazotized by adding dropwise a cold solution of sodium nitrite 0.70 g (0.01 mole) in 5.00 mL. The addition should be completed within 20 minutes. A solution was checked for the excess of nitrite with SI-paper (instantaneously bluing) and stirred for 1 hour under the excess nitrite condition to ensure the complete reaction. After this, the excess of nitrite was removed and destroyed by sulfamic acid.

The coupling reaction: A solution containing 4.00 g (0.01 mole) of CTA in 15.00 mL of water was stirred to dissolve, cooled to 5-10 °C, adjusted pH to 8.00-8.50 with 5% sodium hydroxide. This solution was added dropwise by the above diazotized solution with constant stirring and maintained at pH the 8.00-

8.50. After completing the addition, stirring was continued for about 2 hours. At this stage, only monoazo derivative was stoichiometrically obtained.

Another diazotized solution was prepared using exactly the same condition as described above. The monoazo solution was then added dropwise by the diazotized solution and the pH was maintain in the range 8.00-8.50 and kept temperature 5-10 °C. After completing the addition, the solution was adjusted the pH to 10.00 and stirred for 1 hour. The solution was adjusted the pH to 2.00 using dilute hydrochloric acid and stirred for 15 minutes, and then slowly increased the pH to 8.00-10.00 and stirred for 2 hours. This process of pH adjustment was repeated for seven times. The content was adjusted the pH to 5.00-6.00 with 5% hydrochloric acid and then added sodium acetate 10.00 g for isolating dye. The content was stored in a refrigerator overnight. After filtering through a sintered glass funnel No.4, the precipitate was rinsed with 10.00 mL of (3:1) ethanol:water and dried at 70 °C for 24 hours.

Method 4. (34)

In diazotization step, the normal hydrochloric acid-sodium nitrite procedure is used. The coupling reaction is performed under condition using sodium hydroxide and pyridine.

(a) 2,7-Bis[(2-hydroxy-5-nitrophenyl)azo]-1,8-dihydroxy-3,6-naphthalene disulfonic acid (5N-dye):

The diazotization was performed in the same manner of the method 3.

The coupling reaction: chromotropic acid 4.00 g (0.01 mole) was dissolved in 15.00 mL of water, adjusted pH to 8.00-8.50 and added 2.00 mL of pyridine. This solution was added dropwise by the diazotized solution with constant stirring, kept temperature 5-10 °C and maintained the pH to 8.00-8.50. After completing the addition, stirring was continued for about 2 hours. At this stage, only monoazo derivative was stoichiometrically obtained. And then added 2.00 mL of pyridine.

Another diazotized solution was prepared using exactly the same condition as described above. The monoazo solution was then drop-wisely added by the diazotized solution and the pH was maintain in the range 8.00-8.50 and kept temperature 5-10 °C. After completing the addition, stirring was continued for about 2-3 hours. The pH was then adjusted to 5.00-6.00 with dilute hydrochloric acid, then about 6.00 g of sodium acetate was added for isolating dye. The content was stored in a refrigerator overnight. After filtering through a sintered glass funnel No.4, the precipitate was rinsed with 10.00 mL of (3:1) ethanol:water and dried at 70 °C for 24 hours.

(b) 2,7-Bis[(2-hydroxy-4-nitrophenyl)azo]-1,8-dihydroxy-3,6-naphthalene disulfonic acid (4N-dye):

For the preparation, the detailed experiment procedure was exactly the same as in the case of 5N-dye except 5NAP was used in place of 4NAP.

Method 5. (35)

In diazotization step, the normal hydrochloric acid-sodium nitrite procedure is used. The coupling reaction is performed under an alkaline condition using calcium oxide and pyridine.

2,7-Bis[(2-hydroxy-5-nitrophenyl)azo]-1,8-dihydroxy-3,6-naphthalene disulfonic acid (5N-dye):

The diazotization was performed in the same manner of the method 3. The coupling reaction: A solution containing 2.00 g (0.005 mole) of CTA in 20.00 mL water and 5.00 mL pyridine was added dropwise with agitation a solution into a solution containing CaO 2.00 g in 30.00 mL water and 5.00 mL pyridine. The mix solution was stirred for reaction completes about 1 hour. This solution was added dropwise by diazotized solution, stirred reaction for completes about 2-3 hours. After the solution was cooled and added a cold solution of 20.00 mL concentrated hydrochloric acid. The content was stored in a refrigerator

overnight. The precipitate was filtered through a sintered glass funnel No.4, rinsed with 2 F hydrochloric acid and dried at 70 °C.

Method 6. (34)

In diazotization step, the normal hydrochloric acid-sodium nitrite procedure is used. In this method a mono-2-hydroxy-nitrophenylazo of chromotropic acid is synthesized first. This mono derivative is then coupled with a diazonium salt (prepared by the usual method) under an alkaline condition using lithium hydroxide.

(a) 2,7-Bis[(2-hydroxy-5-nitrophenyl)azo]-1,8-dihydroxy-3,6-naphthalene disulfonic acid (5N-dye):

The diazotization: 4NAP 1.40 g (0.008 mole) was mixed well with 10.00 mL water and 1.70 mL 37% hydrochloric acid. A solution was cooled to 12°C and then diazotized by adding dropwise a cold solution of sodium nitrite 0.56 g (0.008 mole) in 5.00 mL. The addition should be completed within 20 minutes. A solution was checked for the excess of nitrite with SI-paper (instantaneously bluing) and stirred for 1 hour under the excess nitrite condition to ensure the complete reaction. After this, the excess of nitrite was removed and destroyed by sulfamic acid.

The coupling reaction: A solution containing 2.26 g (0.004 mole) of monoazo (2-[(2-hydroxy-5-nitrophenyl)azo]-1,8-dihydroxy-3,6-naphthalenedisulfonic acid obtained from method 1(a)) in 20.00 mL water containing 5.00 g lithium hydroxide was stirred to dissolve, cooled to 0-5 °C, and adjusted pH 9.00-10.00 with a 10% lithium hydroxide solution. This solution above was added dropwise by the diazotized solution, with constant stirring and maintained pH 9.00-10.00. After completing the addition, stirring was continued for about 1 hour. The pH was then adjusted to 5.00-6.00 with dilute hydrochloric acid, then about 6.00 g of sodium acetate was added for isolating dye. The content was stored in a refrigerator overnight. After filtering through a sintered glass funnel No.4, the precipitate was rinsed with 10.00 mL of 2F hydrochloric acid and dried at 70 °C.

(b) 2,7-Bis[(2-hydroxy-4-nitrophenyl)azo]-1,8-dihydroxy-3,6-naphthalene disulfonic acid (4N-dye):

For the preparation, the detailed experiment procedure was exactly the same as in the case of 5N-dye except 2-[(2-hydroxy-4-nitrophenyl)azo]-1,8-dihydroxy-3,6-naphthalene disulfonic acid was used in place of 2-[(2-hydroxy-5-nitrophenyl)azo]-1,8-dihydroxy-3,6-naphthalene disulfonic acid.

(c) 2,7-Bis[(2-hydroxy-3,5-dinitrophenyl)azo]-1,8-dihydroxy-3,6-naphthalene disulfonic acid (3,5N-dye):

The diazotization: picramic acid 3.20 g (0.008 mole) was mixed well with 10.00 mL water and 1.70 mL 37% hydrochloric acid. A solution was cooled to 12 °C and then diazotized by adding dropwise a cold solution of sodium nitrite 0.59 g (0.0086 mole) in 5.00 mL. The addition should be completed within 20 minutes. A solution was checked for the excess of nitrite with SI-paper (instantaneously bluing) and stirred for 1 hour under the excess nitrite condition to ensure the complete reaction. After this, the excess of nitrite was removed and destroyed by sulfamic acid.

The coupling reaction: A solution containing 2.58 g (0.004 mole) of monoazo (2-[(2-hydroxy-3,5-nitrophenyl)azo]-1,8-dihydroxy-3,6-naphthalenedisulfonic acid obtained from method 1(c)) in 20.00 mL water containing 5.00 g lithium hydroxide was stirred to dissolve, cooled to 0-5 °C, and adjusted pH 9.00-10.00 with a 10% lithium hydroxide solution. This solution was added dropwise by the diazotized solution, with constant stirring and maintained pH 9.00-10.00. After completing the addition, stirring was continued for about 1 hour. The pH was then adjusted to 5.00-6.00 with dilute hydrochloric acid, then about 6.00 g of sodium acetate was added for isolating dye. The content was stored in a refrigerator overnight. After filtering through a sintered glass funnel No.4, the precipitate was rinsed with 10.00 mL of 2F hydrochloric acid and dried at 70 °C.

3.5 Isolation of the Dyes

Some impurities due to starting material and the derivative of dye caused by the side reaction were expected to present in the crude dye. It is consequently necessary that the following isolation and purification of dyes to be carried out.

All Monoazo Dyes Derivatives of Chromotropic Acid were Obtained from Method 1

The crude dye of monoazo derivatives were recrystallized from a mixture of ethanol and water (3:1) and dried at 70 °C in the oven for 24 hours. Recrystallizations were carried out 3 times.

The 5N-Dye Prepared from Method 6

The crude dye was dissolved in a small amount of deionized water, and cooled in an ice bath, then transferred drop-wisely in a cold solution of about 20.00 mL of 10 F hydrochloric acid with constant stirring (by using a magnetic stirrer) and stored in the refrigerator overnight. The precipitate was filtered through a sintered glass funnel No.4, and rinsed with some aliquot of 2 F hydrochloric acid. The residue was dried at 70 °C in the oven for 24 hours.

The procedure was recycled and repeated for three times. The purity of dye was checked by TLC with suitable solvent system. Detailed experiment and result are described in the following section.

3.6 Thin-Layer Chromatography Studies of the Dyes

Various composition of mixed solvent systems were investigated by the ascending thin-layer chromatography in order to determine the optimal condition for separating the dye from its impurity. Various composition of mixed solvent systems were shown in Table 3.1.

Table 3.1 The various composition of mixed solvent systems

System No.	Solvent	Composition	Ref.
1	Toluene : Ethanol	1 : 1	34
2	1M HCl : Pyridine	8 : 2	36
3	<i>n</i> -Propanol : 25% NH ₃	8 : 2	34
4	<i>n</i> -Propanol : Formic acid	8 : 2	34
5	<i>n</i> -Butanol : Acetic acid : H ₂ O	4 : 3 : 3	34
6	<i>n</i> -Butanol : Acetic acid : H ₂ O	4 : 3 : 1	34
7	<i>n</i> -Propanol : 25% NH ₃ : Pyridine	8 : 4 : 1	36
8	<i>n</i> -Propanol : 25% NH ₃ : Methyl Ethyl Ketone	4 : 2 : 3	34
9	<i>n</i> -Propanol : 25% NH ₃ : Methyl Ethyl Ketone : H ₂ O	4 : 2 : 3 : 1	34
10	<i>n</i> -Propanol : 25% NH ₃ : Ethyl Acetate : H ₂ O	10 : 3 : 8 : 4	34
11	<i>n</i> -Propanol : Isobutane : Ethyl Acetate : H ₂ O	4 : 2 : 1 : 3	34

Procedure

A few milligrams of dye was dissolved in 5.00 mL of water and 3-5 drops of acetone. A small amount of the solution was spotted on TLC alumina sheet at the lower edge. Two lines were drawn on each plate. The first one was 1.00 cm from the lower edge, the another line about 5.00-8.00 cm above and paralleled to the first line. The dried TLC plate was immersed into a developing solution contained in an enclosed container. Chromatogram were developed and completed at room temperature. The TLC plate was then remove from the chamber and dried in air.

The monoazo dyes

It was found that the solvent system of No.9 and No.11 appeared to be most suitable for the monoazo dye.

After the third recrystallization of the dyes the chromatogram of the monoazo dyes that the dye sample consisted of the major component had purity more than 95% and average R_f value are concluded in the Table 3.2 below

Table 3.2 Average R_f of the dyes

The monoazo dyes	Average R_f		See Appendix
	Solvent No.9	Solvent No.11	
2-[(2-hydroxy-5-nitrophenyl)azo]-1,8-dihydroxy-3,6-naphthalenedisulfonic acid	0.36	0.52	Figure A-2
2-[(2-hydroxy-4-nitrophenyl)azo]-1,8-dihydroxy-3,6-naphthalenedisulfonic acid	0.41	0.64	Figure A-6
2-[(2-hydroxy-3,5-nitrophenyl)azo]-1,8-dihydroxy-3,6-naphthalenedisulfonic acid	0.53	0.48	Figure A-9

The 5N-dye

It was found that the solvent system of No. 5 appeared to be most suitable for 5N-dye. (see appendix Figure A-5)

After the third recrystallization of the dye the chromatogram of 5N-dye showed that the dye sample consisted of the major component had a purity more than 95% and an average R_f value is 0.40.

3.7 Solutions Used in Preliminary Studies and Visible Spectrometric studies

5N-Dye 0.5%: Dissolve 0.2500 g of 5N-dye in 50 mL of water.

Xylenol Orange 0.5%: Dissolve 0.2500 g of xylenol orange in 50 mL of water.

Stock 5N-dye solution: A 10^{-2} F dye was prepared by dissolving 0.6870 g of the purified dyestuff in 100 mL of deionized water. 10^{-3} F and 10^{-4} F of the dye solutions were prepared by further dilution of 10^{-2} F dye solution. The solutions were kept in the absence of light in order to minimize the photochemical reaction of the dye.

Standard Bismuth Solution 0.05F: A standard bismuth solution was prepared by dissolving 6.0600 g of bismuth nitrate pentahydrate in deionized water, 10.0 mL of 65% nitric acid and solution was diluted to 250 mL with deionized water. The concentration of bismuth solution was 0.05F. The concentration of solution was standardized by 0.05F EDTA solution and xylenol orange as indicator(37). This standard stock solution was then further diluted to the concentration of 10^{-2} F and 10^{-3} F with deionized water. The 10^{-4} F solution was freshly prepared frequently to minimize the possible change in concentration due to adsorption caused by ionic exchange with glass container *etc.*

Diverse ion solutions, 10^{-2} F: Stock solution of diverse ions were prepared from most analytical grade and some laboratory grade of various metal salts of nitrate, chloride and sulfate. Sodium salts were preferably employed in the case of anions. Dilute nitric acid was added in these stock solutions whenever necessary. The concentrations were of 10^{-2} F, and further dilution of these solutions were made when required. Deionized water was used in preparation of all solutions.

Standard EDTA solution 0.05F: Dissolve 18.6130 g of A.R. ethylenediaminetetraacetic acid disodium salt dihydrate in deionized water and dilute to 1000 mL in a volumetric flask with deionized water.

10⁻²F EDTA Solution: 0.9320 g of ethylenediaminetetraacetic acid disodium salt dihydrate was dissolved in deionized water and then diluted to 250 mL.

Solution for pH adjustment : Six solutions were prepared as follows :

Table 3.3 Solutions for pH adjustment

pH	Solutions
2	2F Hydrochloric acid or 2F Nitric acid
4	acetate buffer (250 mL of glacial acetic acid + 137.0 g of sodium acetate trihydrate made up to 1 liter with distilled water
7	sodium acetate trihydrate solution (20% w/v)
10	ammonia-ammonium chloride solution (NH ₄ Cl 70.0 g + NH ₃ (sp.gr 0.880) 568.0 mL make up to 1 liter with distilled water)
11.5	ammonia solution (NH ₃ sp.gr 0.880): distilled water = 1:1
12	2 F sodium hydroxide solution

For the adjustment of other pH values ammonia solution (0.01, 0.10 and 1.0 F) and / or nitric acid (0.01, 0.10 and 1.0 F) were employed.