

## CHAPTER III

### EXPERIMENTAL SECTION

#### 3.1 Materials

Cashew nut shells were obtained from Phuket province. Hexane, methanol, formaldehyde, aniline, and toluidines were purchased from Merck. Ethylenediamine, diethylenetriamine, sodium nitrite, nitroanilines, chloroanilines, 2-methoxy-4-nitroaniline, Fast Blue B salt, Fast Blue BB salt, and Fast Red RC salt were from Fluka. Potassium hydroxide and ethylene glycol were obtained from Carlo Erba. Hydrochloric acid was purchased from BDH.

#### 3.2 General methods

The infrared spectra were recorded on Nicolet (Impact 410) FT-IR spectrophotometer. The  $^1\text{H}$ -NMR and  $^{13}\text{C}$ -NMR spectra were recorded on Bruker (DRX 400) NMR spectrometer, operating at 400 MHz for  $^1\text{H}$ -NMR and 100 MHz for  $^{13}\text{C}$ -NMR. The quantities of marker dyes in fuel oils were measured using a Perkin Elmer (Lambda 2) UV/VIS spectrophotometer. The kinematic viscosities of dyed and undyed diesel fuels were obtained from Cannon automatic viscosity. Flash points (Pensky-Martens) of dyed and undyed diesel fuels were measured using an ISL (PMFP93) automatic flash point, while the pour points of dyed and undyed diesel fuels were recorded on an ISL (CPP92) automatic pour point.

Sulfur contents in dyed and undyed diesel fuels were determined using an Outokumpu (X-MET820) automatic sulfur content. The distillation of dyed and undyed diesel fuels were carried out with an Herzog (MP626) automatic distillation apparatus, while distillation of dyed and undyed gasoline were performed with an ISL (AD865G) automatic distillation apparatus.

Total acidities of dyed and undyed fuel oils were measured using a Metrohm 665 automatic total acid number, whilst colors of dyed and undyed fuel oils were observed using a Lovibond (PFX990/P) petrochemical tintometer. The octane numbers of dyed and undyed gasoline were recorded on a CFR automatic research octane number, and Reid vapor pressures of dyed and undyed gasoline were obtained from a Grabner Ins automatic Reid vapor pressure.

### **3.3 Experimental procedures**

#### **3.3.1 Synthesis**

##### **3.3.1.1 Decarboxylation of CNSL [13]**

Cashew nut shell (500 g) were extracted with hexane (1 l). The extract was evaporated to dryness to yield a brown viscous liquid of CNSL (215 g).

Natural CNSL contained mainly anacardic acid, which was subsequently decarboxylated by heat to yield cardanol. The CNSL (100 g) was mixed with toluene (200 ml) in a 1,000 ml round bottom flask, and this flask was then fitted with a condenser. This mixture was heated until boiling under reflux for 3 hrs.

CNSL and decarboxylated CNSL were characterized using spectroscopic techniques including FT-IR,  $^1\text{H-NMR}$ , and  $^{13}\text{C-NMR}$ .

### 3.3.1.2 Purification of cardanol [15]

Decarboxylated CNSL (60 g) and methanol (200 ml) were placed in a 500 ml round bottom flask. Then, 17.96 ml of 40% formaldehyde solution and 2.71 ml of diethylenetriamine were added into the solution. This flask was fitted with a condenser, and heated until boiling under reflux for 2 hrs. After standing the solution at room temperature, a phase separation occurred; a slightly reddish upper solution, and a lower phase, which was a dark brown solidified layer. The upper phase was decanted, and treated with distilled water (40 ml) followed by petroleum ether. The petroleum ether layer was evaporated to dryness, leaving a reddish residue.

Cardanol obtained was characterized by spectroscopic techniques (FT-IR,  $^1\text{H-NMR}$ , and  $^{13}\text{C-NMR}$ ).

### 3.3.1.3 Diazotization of aniline and aniline derivatives

#### 3.3.1.3.1 Diazotization of aniline

Aniline (0.93 ml, 0.01 mole) was added to a mixture of 3 ml of concentrated hydrochloric acid, and 3 ml of water. The mixture was stirred vigorously while cooling down to  $-2$  to  $0^\circ\text{C}$ . When the solution was cold, a solution of sodium nitrite (0.69 g, 0.01 mole) was added dropwise to the

reaction mixture, while the temperature of the reaction was kept below 0 °C. Pale yellow solution of aniline diazonium salt was left at this temperature before coupling with cardanol.

#### **3.3.1.3.2 Diazotization of *p*-nitroaniline**

The procedure of 3.3.1.3.1 was repeated except that 1.38 g (0.01 mole) of *p*-nitroaniline was used instead of aniline. Pale yellow solution of *p*-nitroaniline diazonium salt was obtained.

#### **3.3.1.3.3 Diazotization of *m*-nitroaniline**

The procedure of 3.3.1.3.1 was repeated except that 1.38 g (0.01 mole) of *m*-nitroaniline was used instead of aniline. Pale yellow solution of *m*-nitroaniline diazonium salt was obtained.

#### **3.3.1.3.4 Diazotization of *o*-nitroaniline**

The procedure of 3.3.1.3.1 was repeated except that 1.38 g (0.01 mole) of *o*-nitroaniline was used instead of aniline. Pale yellow solution of *o*-nitroaniline diazonium salt was obtained.

#### **3.3.1.3.5 Diazotization of *p*-chloroaniline**

The procedure of 3.3.1.3.1 was repeated except that 1.28 g (0.01 mole) of *p*-chloroaniline was used instead of aniline. Pale yellow solution of *p*-chloroaniline diazonium salt was obtained.

#### **3.3.1.3.6 Diazotization of *m*-chloroaniline**

The procedure of 3.3.1.3.1 was repeated except that 1.05 ml (0.01 mole) of *m*-chloroaniline was used instead of aniline. Pale yellow solution of *m*-chloroaniline diazonium salt was obtained.

#### **3.3.1.3.7 Diazotization of *o*-chloroaniline**

The procedure of 3.3.1.3.1 was repeated except that 1.05 ml (0.01 mole) of *o*-chloroaniline was used instead of aniline. Pale yellow solution of *o*-chloroaniline diazonium salt was obtained.

#### **3.3.1.3.8 Diazotization of 2-chloro-4-nitroaniline**

The procedure of 3.3.1.3.1 was repeated except that 1.72 g (0.01 mole) of 2-chloro-4-nitroaniline was used instead of aniline. Yellow solution of 2-chloro-4-nitroaniline diazonium salt was obtained.

#### **3.3.1.3.9 Diazotization of 2-chloro-5-nitroaniline**

The procedure of 3.3.1.3.1 was repeated except that 1.72 g (0.01 mole) of 2-chloro-5-nitroaniline was used instead of aniline. Pale yellow solution of 2-chloro-5-nitroaniline diazonium salt was obtained.

#### **3.3.1.3.10 Diazotization of 4-chloro-2-nitroaniline**

The procedure of 3.3.1.3.1 was repeated except that 1.72 g (0.01 mole) of 4-chloro-2-nitroaniline was used instead of aniline. Pale yellow solution of 4-chloro-2-nitroaniline diazonium salt was obtained.

#### **3.3.1.3.11 Diazotization of 4-chloro-3-nitroaniline**

The procedure of 3.3.1.3.1 was repeated except that 1.72 g (0.01 mole) of 4-chloro-3-nitroaniline was used instead of aniline. Pale yellow solution of 4-chloro-3-nitroaniline diazonium salt was obtained.

#### **3.3.1.3.12 Diazotization of *p*-toluidine**

The procedure of 3.3.1.3.1 was repeated except that 1.07 g (0.01 mole) of *p*-toluidine was used instead of aniline. Pale yellow solution of *p*-toluidine diazonium salt was obtained.

#### **3.3.1.3.13 Diazotization of *m*-toluidine**

The procedure of 3.3.1.3.1 was repeated except that 1.08 ml (0.01 mole) of *m*-toluidine was used instead of aniline. Pale yellow solution of *m*-toluidine diazonium salt was obtained.

#### **3.3.1.3.14 Diazotization of *o*-toluidine**

The procedure of 3.3.1.3.1 was repeated except that 1.06 ml (0.01 mole) of *o*-toluidine was used instead of aniline. Pale yellow solution of *o*-toluidine diazonium salt was obtained.

#### **3.3.1.3.15 Diazotization of 2-methoxy-4-nitroaniline**

The procedure of 3.3.1.3.1 was repeated except that 1.68 g (0.01 mole) of 2-methoxy-4-nitroaniline was used instead of aniline. Pale yellow solution of 2-methoxy-4-nitroaniline diazonium salt was obtained.

#### **3.3.1.3.16 Diazotization of Fast Blue B salt**

The procedure of 3.3.1.3.1 was repeated except that 4.72 g (0.01 mole) of Fast Blue B salt was used instead of aniline. Yellow solution of Fast Blue B diazonium salt was obtained.

#### **3.3.1.3.17 Diazotization of Fast Blue BB salt**

The procedure of 3.3.1.3.1 was repeated except that 8.32 g (0.01 mole) of Fast Blue BB salt was used instead of aniline. Yellow solution of Fast Blue BB diazonium salt was obtained.

### 3.3.1.3.18 Diazotization of Fast Red RC salt

The procedure of 3.3.1.3.1 was repeated except that 5.46 g (0.01 mole) of Fast Red RC salt was used instead of aniline. Pale yellow solution of Fast Red RC diazonium salt was obtained.

### 3.3.1.4 Preparation of alkyl phenolate ions

Potassium hydroxide (0.56 g, 0.01 mole) was dissolved in 5.0 ml of methanol. The solution was cooled to 0 °C, and 3.00 g of cardanol (Section 3.3.1.2) was added, while continuously stirring, into the solution to give a reddish-brown oil.

### 3.3.1.5 Preparation of marker dyes

#### 3.3.1.5.1 Aniline diazonium salt

Pale yellow solution of aniline diazonium salt (Section 3.3.1.3.1) was added dropwise into alkyl phenolate ions solution (Section 3.3.1.4), while the temperature of the reaction mixture was controlled to below 0 °C. The reaction mixture was left stirring at the temperature below 0 °C for 1 hr. The marker dye product was extracted from the reaction mixture by treating with methylenechloride. The methylenechloride layer was washed repeatedly, each with 20 ml of distilled water (3-4 times), then evaporated to dryness, yielding a reddish-brown oil of cardanol-phenyl azo.

#### **3.3.1.5.2 *p*-nitroaniline diazonium salt**

The procedure of 3.3.1.5.1 was repeated except that solution of *p*-nitroaniline diazonium salt was used instead of aniline diazonium salt.

#### **3.3.1.5.3 *m*-nitroaniline diazonium salt**

The procedure of 3.3.1.5.1 was repeated except that solution of *m*-nitroaniline diazonium salt was used instead of aniline diazonium salt.

#### **3.3.1.5.4 *o*-nitroaniline diazonium salt**

The procedure of 3.3.1.5.1 was repeated except that solution of *o*-nitroaniline diazonium salt was used instead of aniline diazonium salt.

#### **3.3.1.5.5 *p*-chloroaniline diazonium salt**

The procedure of 3.3.1.5.1 was repeated except that solution of *p*-chloroaniline diazonium salt was used instead of aniline diazonium salt.

#### **3.3.1.5.6 *m*-chloroaniline diazonium salt**

The procedure of 3.3.1.5.1 was repeated except that solution of *m*-chloroaniline diazonium salt was used instead of aniline diazonium salt.

#### **3.3.1.5.7 *o*-chloroaniline diazonium salt**

The procedure of 3.3.1.5.1 was repeated except that solution of *o*-chloroaniline diazonium salt was used instead of aniline diazonium salt.

#### **3.3.1.5.8 2-chloro-4-nitroaniline diazonium salt**

The procedure of 3.3.1.5.1 was repeated except that solution of 2-chloro-4-nitroaniline diazonium salt was used instead of aniline diazonium salt.



**3.3.1.5.9 2-chloro-5-nitroaniline diazonium salt**

The procedure of 3.3.1.5.1 was repeated except that solution of 2-chloro-5-nitroaniline diazonium salt was used instead of aniline diazonium salt.

**3.3.1.5.10 4-chloro-2-nitroaniline diazonium salt**

The procedure of 3.3.1.5.1 was repeated except that solution of 4-chloro-2-nitroaniline diazonium salt was used instead of aniline diazonium salt.

**3.3.1.5.11 4-chloro-3-nitroaniline diazonium salt**

The procedure of 3.3.1.5.1 was repeated except that solution of 4-chloro-3-nitroaniline diazonium salt was used instead of aniline diazonium salt.

**3.3.1.5.12 *p*-toluidine diazonium salt**

The procedure of 3.3.1.5.1 was repeated except that solution of *p*-toluidine diazonium salt was used instead of aniline diazonium salt.

**3.3.1.5.13 *m*-toluidine diazonium salt**

The procedure of 3.3.1.5.1 was repeated except that solution of *m*-toluidine diazonium salt was used instead of aniline diazonium salt.

**3.3.1.5.14 *o*-toluidine diazonium salt**

The procedure of 3.3.1.5.1 was repeated except that solution of *o*-toluidine diazonium salt was used instead of aniline diazonium salt.

**3.3.1.5.15 2-methoxy-4-nitroaniline diazonium salt**

The procedure of 3.3.1.5.1 was repeated except that solution of 2-methoxy-4-nitroaniline diazonium salt was used instead of aniline diazonium salt.

#### **3.3.1.5.16 Fast Blue B diazonium salt**

The procedure of 3.3.1.5.1 was repeated except that solution of Fast Blue B diazonium salt was used instead of aniline diazonium salt.

#### **3.3.1.5.17 Fast Blue BB diazonium salt**

The procedure of 3.3.1.5.1 was repeated except that solution of Fast Blue BB diazonium salt was used instead of aniline diazonium salt.

#### **3.3.1.5.18 Fast Red RC diazonium salt**

The procedure of 3.3.1.5.1 was repeated except that solution of Fast Red RC diazonium salt was used instead of aniline diazonium salt.

### **3.3.2 Characterization of marker dyes**

Marker dyes synthesized in Section 3.3.1.5 were characterized by spectroscopic techniques (FT-IR,  $^1\text{H}$ -NMR, and  $^{13}\text{C}$ -NMR).

### **3.3.3 Treatment of marker dyes in fuel oils**

#### **3.3.3.1 Preparation of stock marker dye solution**

##### **3.3.3.1.1 Preparation of 50,000 ppm stock marker dye solutions**

Each 50,000 ppm stock marker dye solution was prepared by dissolving 2.50 g of each marker dye (Section 3.3.1.5) with toluene, and made up to 5 ml in a volumetric flask.

### 3.3.3.1.2 Preparation of 1,000 ppm stock marker dye solutions

Each 1,000 ppm stock marker dye solution was prepared by pipetting 1 ml of 50,000 ppm stock marker dye solution into a 50 ml volumetric flask, and made up with toluene.

### 3.3.3.2 Suitable extraction system for the detection of marker dyes in fuel oils

Since marker dye synthesized in Section 3.3.1.5 were phenyl azo compounds that have weakly basic character. This type of markers can be detected in petroleum products by extraction into an appropriate alkaline aqueous solution that reacts with a marker dye, and produces color in an aqueous phase.

In this research, the extraction systems were divided into 3 major systems, on the basis of the type of bases used, which were potassium hydroxide, ethylenediamine, and diethylenetriamine.

Gasoline containing 2 ppm of 2-chloro-4-nitrophenyl azo was used for the determination of suitable extraction system. This dyed gasoline was prepared by pipetting 1 ml of 1,000 ppm stock marker dye solutions into a 500 ml volumetric flask, and made up with undyed gasoline.

Extraction system, which gave the highest absorption at its  $\lambda_{\max}$ , was chosen as the suitable extraction system.

#### 3.3.3.2.1 Extraction system A

The dyed gasoline was pipetted (30 ml) into a 50 ml screw cap vial. Then, 6 ml of 1% potassium hydroxide in a solution of 1:1 ethylene glycol

and methanol was added into the vial, capped, and shaken for 30 seconds. The mixture was left at room temperature until two phases were observed. The lower phase, which developed color, was drawn off for recording the maximum absorption in the visible region from 350 to 750 nm using an UV/VIS spectrophotometer. The procedure was repeated except that 2%, 3%, 4%, and 5% potassium hydroxide in a solution of 1:1 ethylene glycol and methanol were individually used.

#### **3.3.3.2.2 Extraction system B**

The procedure of 3.3.3.2.1 was repeated except that 10%, 20%, 30%, 40%, and 50% ethylenediamine in a solution of 1:1 ethylene glycol and methanol was individually used instead of potassium hydroxide in the same solution.

#### **3.3.3.2.3 Extraction system C**

The procedure of 3.3.3.2.1 was repeated except that 10%, 20%, 30%, 40%, and 50% diethylenetriamine in a solution of 1:1 ethylene glycol and methanol was separately used instead of potassium hydroxide in the same solution.

### **3.3.3.3 Effects of marker dye on the physical properties of dyed fuel oils**

#### **3.3.3.3.1 Effects of marker dye on the physical properties of dyed gasoline**

Gasoline was dyed with 2 ppm of cardanol-2-chloro-4-nitrophenyl azo, which was prepared by pipetting 4 ml of 1,000 ppm stock marker dye solution into a 2,000 ml volumetric flask, and made up with undyed gasoline. Physical properties of dyed and undyed gasoline were studied using the ASTM method described in Table 3-1.

Table 3-1: The ASTM testing methods of dyed and undyed gasoline.

Test items	Test methods ASTM
API gravity @ 60 °F	D 1298
Specific gravity @ 15.6 / 15.6 °C	D 1298
Octane number	
Research method (RON)	D 2699
Reid vapor pressure @ 37.8 °C, kPa	D 5191
Copper strip corrosion (3 hrs. 50 °C)	D 130
Distillation	D 86
Total acid number, mg KOH/g	D 974
Color	D 1500

### 3.3.3.3.2 Effects of marker dye on physical properties of dyed diesel fuel

Diesel was dyed with 2 ppm of cardanol-2-chloro-4-nitrophenyl azo, which was prepared by pipetting 4 ml of 1,000 ppm stock marker dye solution into a 2,000 ml volumetric flask, and made up with undyed diesel fuel.

Physical properties of dyed and undyed diesel fuels were studied employing the ASTM method described in Table 3-2.

Table 3-2: The ASTM testing methods of dyed and undyed diesel fuels.

Test items	Test methods ASTM
API gravity @ 60 °F	D 1298
Specific gravity @ 15.6 / 15.6 °C	D 1298
Calculated cetane index	D 976
Kinematic viscosity @ 40 °C, cSt	D 445
Pour point, °C	D 97
Flash point, °C	D 93
Sulfur content, %wt	D 4294
Copper strip corrosion (3 hrs, 50 °C)	D 130
Distillation	D 86
Total acid number, mg KOH/g	D 974
Color	D 1500

### 3.3.3.4 Quantitative determination of marker dyes in dyed fuel oils

#### 3.3.3.4.1 Calibration curve of cardanol-phenyl azo in gasoline

The standard calibration curve of cardanol-phenyl azo in gasoline was prepared at concentrations from 0 to 8 ppm of marker dye by the following procedure.

The stock marker dye solution (1,000 ppm) was pipetted into a 50 ml volumetric flask, and made up with undyed gasoline. The volume of the stock solution for each concentration is shown in Table 3-3.

Table 3-3: The volume of the stock solution (1,000 ppm) for each 0-8 ppm concentration.

Concentration (ppm)	Volume of 1,000 ppm stock solution (ml)
0	0
2	0.10
4	0.20
6	0.30
8	0.40

The dyed gasoline (30 ml) of each concentration was pipetted into a 50 ml screw cap vial, and 6 ml of 50% ethylenediamine in a solution of 1:1 ethylene glycol and methanol was added. Then, a vial was capped, and shaken for 30 seconds. When two phases were observed, the lower phase was drawn off for recording the UV/VIS absorption at its  $\lambda_{\max}$ . The calibration curve was a plot between absorbance and the concentration of marker dye in gasoline.

#### 3.3.3.4.2 Calibration curve of cardanol-phenyl azo in diesel fuel

The standard calibration curve of cardanol-phenyl azo in diesel fuel was prepared at concentrations from 0 to 8 ppm of marker dye by the following procedure.

The stock marker dye solution (1,000 ppm) was pipetted into a 50 ml volumetric flask, and made up with undyed diesel fuel. The volume of the stock solution for each concentration is shown in Table 3-3.

The dyed diesel fuel (30 ml) of each concentration was pipetted into a 50 ml screw cap vial, and 6 ml of 50% ethylenediamine in a solution of 1:1 ethylene glycol and methanol was added. Then, a vial was capped, and shaken for 30 seconds. When two phases were observed, the lower phase was drawn off for recording the UV/VIS absorption at its  $\lambda_{\text{max}}$ . The calibration curve was a plot between absorbance and the concentration of marker dye in gasoline.

#### **3.3.3.4.3 Calibration curve of cardanol-*p*-nitrophenyl azo in gasoline**

The standard calibration curve of cardanol-*p*-nitroaniline azo in gasoline was prepared at concentrations from 0 to 4 ppm of marker dye by the following procedure.

The stock marker dye solution (1,000 ppm) was pipetted into a 50 ml volumetric flask, and made up with undyed gasoline. The volume of the stock solution for each concentration is shown in Table 3-4.

The dyed gasoline (30 ml) of each concentration was pipetted into a 50 ml screw cap vial, and 6 ml of 50% ethylenediamine in a solution of 1:1 ethylene glycol and methanol was added. Then, a vial was capped, and shaken for 30 seconds. When two phases were observed, the lower phase was drawn off for recording the UV/VIS absorption at its  $\lambda_{\text{max}}$ . The calibration curve was a plot between absorbance and the concentration of marker dye in gasoline.



Table 3-4: The volume of the stock solution (1,000 ppm) for each 0-4 ppm concentration.

Concentration (ppm)	Volume of 1,000 ppm stock solution (ml)
0	0
1	0.05
2	0.10
3	0.15
4	0.20

#### 3.3.3.4.4 Calibration curve of cardanol-*p*-nitrophenyl azo in diesel fuel

The standard calibration curve of cardanol-*p*-nitroaniline azo in diesel fuel was prepared at concentrations from 0 to 4 ppm of marker dye by the following procedure.

The stock marker dye solution (1,000 ppm) was pipetted into a 50 ml volumetric flask, and made up with undyed diesel fuel. The volume of the stock solution for each concentration is shown in Table 3-4.

The dyed diesel fuel (30 ml) of each concentration was pipetted into a 50 ml screw cap vial, and 6 ml of 50% ethylenediamine in a solution of 1:1 ethylene glycol and methanol was added. Then, a vial was capped, and shaken for 30 seconds. When two phases were observed, the lower phase was drawn off for recording the UV/VIS absorption at its  $\lambda_{\max}$ . The calibration curve was a plot between absorbance and the concentration of marker dye in diesel fuel.

#### **3.3.3.4.5 Calibration curve of cardanol-*m*-nitrophenyl azo in gasoline**

The procedure of 3.3.3.4.3 was repeated except that cardanol-*m*-nitrophenyl azo was used instead of cardanol-*p*-nitrophenyl azo.

#### **3.3.3.4.6 Calibration curve of cardanol-*m*-nitrophenyl azo in diesel fuel**

The procedure of 3.3.3.4.4 was repeated except that cardanol-*m*-nitrophenyl azo was used instead of cardanol-*p*-nitrophenyl azo.

#### **3.3.3.4.7 Calibration curve of cardanol-*o*-nitrophenyl azo in gasoline**

The procedure of 3.3.3.4.3 was repeated except that cardanol-*o*-nitrophenyl azo was used instead of cardanol-*p*-nitrophenyl azo.

#### **3.3.3.4.8 Calibration curve of cardanol-*o*-nitrophenyl azo in diesel fuel**

The procedure of 3.3.3.4.4 was repeated except that cardanol-*o*-nitrophenyl azo was used instead of cardanol-*p*-nitrophenyl azo.

#### **3.3.3.4.9 Calibration curve of cardanol-*p*-chlorophenyl azo in gasoline**

The procedure of 3.3.3.4.1 was repeated except that cardanol-*p*-chlorophenyl azo was used instead of cardanol-phenyl azo.

#### **3.3.3.4.10 Calibration curve of cardanol-*p*-chlorophenyl azo in diesel fuel**

The procedure of 3.3.3.4.2 was repeated except that cardanol-*p*-chlorophenyl azo was used instead of cardanol-phenyl azo.

#### **3.3.3.4.11 Calibration curve of cardanol-2-chloro-4-nitrophenyl azo in gasoline**

The procedure of 3.3.3.4.3 was repeated except that cardanol-2-chloro-4-nitrophenyl azo was used instead of cardanol-*p*-nitrophenyl azo.

#### **3.3.3.4.12 Calibration curve of cardanol-2-chloro-4-nitrophenyl azo in diesel fuel**

The procedure of 3.3.3.4.4 was repeated except that cardanol-2-chloro-4-nitrophenyl azo was used instead of cardanol-*p*-nitrophenyl azo.

#### **3.3.3.4.13 Calibration curve of cardanol-*p*-methylphenyl azo in gasoline**

The standard calibration curve of cardanol-*p*-methylphenyl azo in gasoline was prepared at concentrations from 0 to 10 ppm of marker dye by the following procedure.

The stock marker dye solution (1.000 ppm) was pipetted into a 50 ml volumetric flask, and made up with undyed gasoline. The volume of the stock solution for each concentration is shown in Table 3-5.

The dyed gasoline (30 ml) of each concentration was pipetted into a 50 ml screw cap vial, and 6 ml of 50% ethylenediamine in a solution of 1:1 ethylene glycol and methanol was added. Then, a vial was capped, and shaken for 30 seconds. When two phases were observed, the lower phase was drawn off for recording the UV/VIS absorption at its  $\lambda_{\max}$ . The calibration curve was a plot between absorbance and the concentration of marker dye in gasoline.

Table 3-5: The volume of the stock solution (1,000 ppm) for each 0-10 ppm concentration.

Concentration (ppm)	Volume of 1,000 ppm stock solution (ml)
0	0
4	0.20
6	0.30
8	0.40
10	0.50

#### 3.3.3.4.14 Calibration curve of cardanol-*p*-methylphenyl azo in diesel fuel

The standard calibration curve of cardanol-*p*-methylphenyl azo in diesel fuel was prepared at concentrations from 0 to 10 ppm of marker dye by the following procedure.

The stock marker dye solution (1,000 ppm) was pipetted into a 50 ml volumetric flask, and made up with undyed diesel fuel. The volume of the stock solution for each concentration is shown in Table 3-5.

The dyed diesel fuel (30 ml) of each concentration was pipetted into a 50 ml screw cap vial, and 6 ml of 50% ethylenediamine in a solution of 1:1 ethylene glycol and methanol was added. Then, a vial was capped, and shaken for 30 seconds. When two phases were observed, the lower phase was drawn off for recording the UV/VIS absorption at its  $\lambda_{\text{max}}$ . The calibration curve was a plot between absorbance and the concentration of marker dye in diesel fuel.

#### **3.3.3.4.15 Calibration curve of cardanol-2-methoxy-4-nitrophenyl azo in gasoline**

The procedure of 3.3.3.4.1 was repeated except that cardanol-2-methoxy-4-nitrophenyl azo was used instead of cardanol-phenyl azo.

#### **3.3.3.4.16 Calibration curve of cardanol-2-methoxy-4-nitrophenyl azo in diesel fuel**

The procedure of 3.3.3.4.2 was repeated except that cardanol-2-methoxy-4-nitrophenyl azo was used instead of cardanol-phenyl azo.

#### **3.3.3.4.17 Calibration curve of cardanol-Fast Blue B azo in gasoline**

The standard calibration curve for cardanol-Fast Blue B azo in gasoline was prepared at concentrations from 0 to 12 ppm of marker dye by the following procedure.

The stock marker dye solution (1.000 ppm) was pipetted into a 50 ml volumetric flask, and made up with undyed gasoline. The volume of the stock solution for each concentration is shown in Table 3-6.

The dyed gasoline (30 ml) of each concentration was pipetted into a 50 ml screw cap vial, and 6 ml of 50% ethylenediamine in a solution of 1:1 ethylene glycol and methanol was added. Then, a vial was capped, and shaken for 30 seconds. When two phases were observed, the lower phase was drawn off for recording the UV/VIS absorption at its  $\lambda_{\text{max}}$ . The calibration curve was a plot between absorbances and concentrations of marker dye in gasoline.

Table 3-6: The volume of the stock solution (1.000 ppm) for each 0-12 ppm concentration.

Concentration (ppm)	Volume of 1.000 ppm stock solution (ml)
0	0
6	0.30
8	0.40
10	0.50
12	0.60

#### 3.3.3.4.18 Calibration curve of cardanol-Fast Blue B azo in diesel fuel

The standard calibration curve of cardanol-Fast Blue B azo in diesel fuel was prepared at concentrations from 0 to 12 ppm of marker dye by the following procedure.

The stock marker dye solution (1,000 ppm) was pipetted into a 50 ml volumetric flask, and made up with undyed diesel fuel. The volume of the stock solution for each concentration is shown in Table 3-6.

The dyed diesel fuel (30 ml) of each concentration was pipetted into a 50 ml screw cap vial, and 6 ml of 50% ethylenediamine in a solution of 1:1 ethylene glycol and methanol was added. Then, a vial was capped, and shaken for 30 seconds. When two phases were observed, the lower phase was drawn off for recording the UV/VIS absorption at its  $\lambda_{\max}$ . The calibration curve was a plot between absorbance and the concentration of marker dye in diesel fuel.

### 3.4 Stability of marker dyes in fuel oils

#### 3.4.1 Stability of cardanol-*p*-nitrophenyl azo in fuel oils

##### 3.4.1.1 Stability of cardanol-*p*-nitrophenyl azo in gasoline

The stock solution (1,000 ppm) of cardanol-*p*-nitrophenyl azo (1 ml) was pipetted into a 500 ml volumetric flask, and made with undyed gasoline. The quantity of marker dye contained in gasoline was determined monthly for 3 months by the following procedure.

The dyed gasoline (30 ml) was pipetted into a vial, and 6 ml of 50% ethylenediamine in a solution of 1:1 ethylene glycol and ~~50%~~ methanol was added. The reaction vial was capped, and shaken for 30 seconds. The UV/VIS absorption (at its  $\lambda_{\max}$ ) of the lower phase led to the determination of the marker dye quantity in gasoline by comparing the measured absorbance with those in the calibration curve (Section 3.3.3.4.3).

##### 3.4.1.2 Stability of cardanol-*p*-nitrophenyl azo in diesel fuel.

The stock solution (1,000 ppm) of cardanol-*p*-nitrophenyl azo (1 ml) was pipetted into a 500 ml volumetric flask, and made up with undyed diesel fuel. The quantity of marker dye contained in diesel fuel was determined monthly for 3 months by the following procedure.

The dyed diesel fuel (30 ml) was pipetted into a vial, and 6 ml of 50% ethylenediamine in a solution of 1:1 ethylene glycol and methanol was added. The reaction vial was capped, and shaken for 30 seconds. The UV/VIS absorption (at its  $\lambda_{\max}$ ) of the lower phase led to the determination of the marker

dye quantity in diesel fuel by comparing the measured absorbance with those in the calibration curve (Section 3.3.3.4.4).

### **3.4.2 Stability of cardanol-2-chloro-4-nitrophenyl azo in fuel oils**

#### **3.4.2.1 Stability of cardanol-2-chloro-4-nitrophenyl azo in gasoline**

The procedure of 3.4.1.1 was repeated except that cardanol-2-chloro-4-nitrophenyl azo was used instead of cardanol-*p*-nitrophenyl azo, and the calibration curve from section 3.3.3.4.11 was applied.

#### **3.4.2.2 Stability of cardanol-2-chloro-4-nitrophenyl azo in diesel fuel**

The procedure of 3.4.1.2 was repeated except that cardanol-2-chloro-4-nitrophenyl azo was used instead of cardanol-*p*-nitrophenyl azo, and the calibration curve from section 3.3.3.4.12 was applied.

### **3.4.3 Stability of cardanol-2-methoxy-4-nitrophenyl azo in fuel oils**

#### **3.4.3.1 Stability of cardanol-2-methoxy-4-nitrophenyl azo in gasoline**

The stock solution (1,000 ppm) of cardanol-2-methoxy-4-nitrophenyl azo (2.5 ml) was pipetted into a 500 ml volumetric flask, and made up with undyed gasoline. The quantity of marker dye contained in gasoline was determined monthly for 3 months by the following procedure.

The dyed gasoline (30 ml) was pipetted into a vial, and 6 ml of 50% ethylenediamine in a solution of 1:1 ethylene glycol and methanol was added. The reaction vial was capped, and shaken for 30 seconds. The UV/VIS absorption (at its  $\lambda_{\max}$ ) of the lower phase led to the determination of the marker



dye quantity in gasoline by comparing the measured absorbance with those in the calibration curve (Section 3.3.3.4.15).

#### **3.4.3.2 Stability of cardanol-2-methoxy-4-nitrophenyl azo in diesel fuel**

The stock solution (1,000 ppm) of cardanol-2-methoxy-4-nitrophenyl azo (2.5 ml) was pipetted into a 500 ml volumetric flask, and made up with undyed diesel fuel. The quantity of marker dye contained in diesel fuel was determined monthly for 3 months by the following procedure.

The dyed diesel fuel (30 ml) was pipetted into a vial, and 6 ml of 50% ethylenediamine in a solution of 1:1 ethylene glycol and methanol was added. The reaction vial was capped, and shaken for 30 seconds. The UV/VIS absorption (at its  $\lambda_{\text{max}}$ ) of the lower phase led to the determination of the marker dye quantity in diesel fuel by comparing the measured absorbance with those in the calibration curve (Section 3.3.3.4.16).