Chapter 3 Experimental

The procedure is summarized in Fig. 3.1.

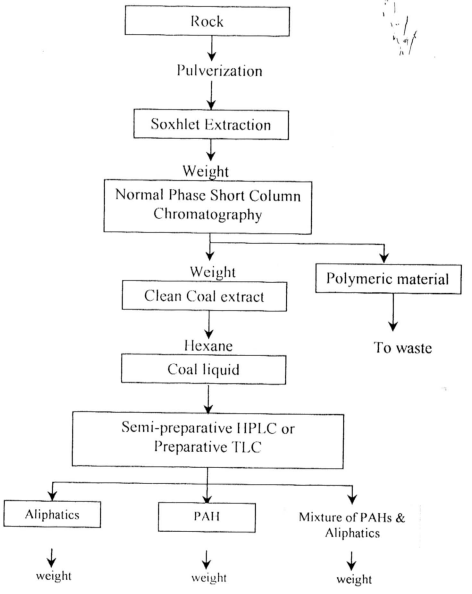

Fig. 3.1 *Flow diagram of experimental procedure.*

3.1 CHEMICALS

Normal grade PAHs (such as naphthalene, 1-naphthol, 2-naphthol, anthracene, phenanthrene, flourene, acenaphthene, benz(b)anthracene, flouranthene, chrysene, pyrene and benzo(e)pyrene) and *n*-alkane (*n*-C_{12}, *n*-C_{14}, *n*-C_{16}, *n*-C_{18}, *n*-C_{20}, *n*-C_{22}, *n*-C_{24}, *n*-C_{26}, *n*-C_{28}, *n*-C_{30}, *n*-C_{32}, *n*-C_{34} and *n*-C_{36}) compound were obtained as standard. HPLC grade Hexane was used as solvents in sample preparation. (Refer Fig. 3.2 and for structure of PAHs)

All solvent mixtures used as mobile phase were prepared volumetrically. HPLC-grade hexane (95% *n*-hexane), ethyl acetate, propan-2-ol, dichloromethane were purchase from Fisher Scientific and J.T. Baker.

The 5-μm *Apex* silica (S/N9021907) normal phase and 4-μm *Genesis* silica (S/N0032201) normal phase was from Jones chromatography. The 6-μm Prep *Nova* silica *PrepPak*(R) catridge was from Waters.

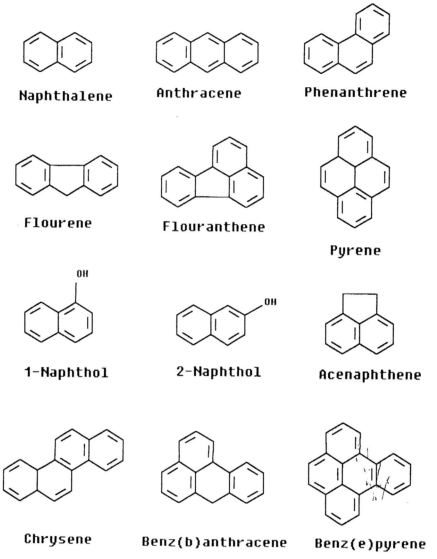

Fig. 3.2 *Structure of some reference Polyaromatic hydrocarbons (PAHs) standard*

3.2 High Performance Liquid Chromatography (HPLC)

HPLC was performed with a Waters[1M] 600 Controller (Millipore, Milford, MA, USA) Model 600E (S/N SX5NM0584M) with Pump (S/N MX5PM8941M)and a U6K injection valve and 50µL sampling loop coupled with three types of column each time:

1. Jones Chromatography APEX® silica 5µm, 25 cm x 4.6 mm ID (S/N9021907)
2. Jones Chromatography Genesis® silica 4µm, 25 cm x 4.6 mm ID(S/N0032201)
3. Waters PepPak® Catridge Prep Nova-silica 6 µm, 10 cm x 2.5cm ID

Eluate was passed through a Waters Model 486 Tunable Absorbance Detector (S/N MXDM2173M) UV-Vis variable-wavelength spectrophotometer and Waters 410 Differential Refractometer (S/N MX5PM9361M) RI detector and data acquisition was performed on a Servogor 120 chart recorder.

In total, 9 different HPLC conditions were tested on to obtain the best separation of the *n*-alkane and PAHs. The HPLC conditions are shown in *Appendix A*.

3.3 PREPARATION OF STANDARD SOLUTIONS

The polyaromatic hydrocarbon (PAHs) standards and *n*-alkane were prepared for qualitative analysis.

3.3.1 PREPARATION OF STOCK SOLUTIONS

Stock solutions of naphthalene (1), 1-naphthol (2), 2-naphthol (3), anthracene (4), phenanthrene (5), flourene (6), acenaphthene (7), benz(b)anthracene (8), flouranthene (9), chrysene (10) and pyrene (11) were prepared. The PAHs standard were dissolved in hexane

For the preparation of 1μg/μL (1000ppm) stock, 0.0010g of each standard was dissolved in 1mL of hexane

All the other *n*-alkane stock of *n*-C_{12}, *n*-C_{14}, *n*-C_{16}, *n*-C_{18}, *n*-C_{20}, *n*-C_{22}, *n*-C_{24}, *n*-C_{26}, *n*-C_{28}, *n*-C_{30}, *n*-C_{32}, *n*-C_{34} and *n*-C_{36} was donated by *Professor C.J.W. Brooks* (Chemistry Department, Glasgow University, Scotland, UK)

3.3.2 PREPARATION OF STANDARD SOLUTIONS OF VARIOUS CONCENTRATIONS.

The PAHs standard solutions of various concentrations were prepared by diluting the stock standard solution according to the simple formula stated below:

$$M_S V_S = M_I V_I \qquad (3.3)$$

Where M_S = Concentration of the stock solution (1000ppm).

V_S = Volume (in mL) of the stock solution used.

M_I = Concentration of the standard solution prepared.

V_I = Volume (in mL) of the standard solution prepared

3.4 DETECTION LIMIT

Determination of the detection limit for each standard was carried out by injecting a series of standard solutions of low concentrations, which gave a signal-to-noise (S/N) ratio response of not less than 3.

3.5 SAMPLING

In this study, 11 organic-rich Tertiary sediments, consisting of two shale, a coaly sandstone, a sandy base, three intercalation of coal with shale, and four coal samples, were examined by organic petrological method.

3.5.1 SAMPLING LOCATION

The 10 (BA1-BA10) samples were from station B (Fig. 1.2) while the remaining one (BA11) sample is from station A. Details of the sampling localities are given in Table 3.1 and in Fig.3.3 (a) - (d).

3.5.2 GENERAL PROCEDURE FOR SAMPLING

The rock samples were knocked out from sampling ground and kept in a clean plastic bag. To maintain the integrity of the rocks, the rocks must be stored properly. It is clean with wire brush and penknife to remove surface contamination. After that the samples are wrapped in aluminium foil until further process.

Table 3.1 *Physical description of sediments collected at Station B and Sample BA11 at Station A.*

Sample No	Layer depth (cm)	Description of sediments
BA1	20	Pure coal
BA2	20	Mixture of shale and coal
BA3	10:10	Coal going up to sandy base
BA4	5	Sandy base
BA5	20	Coal
BA6	8	Prominent coal
BA7	15	Shale
BA8	18	Repetition of coal and shale layer
BA9	25	Pure coal
BA10	30	Intercalation of coal and shale
BA11	[a]	Massive grey shale

[a] - collected in form of rock.

Fig. 3.3 (a) *Photo taken shows stratigraphic layers of BA1, BA2 and BA3.*

Fig. 3.3 (b) *Photo taken shows stratigraphic layers of BA2, BA3 and BA4.*

Fig. 3.3 (c) *Photo taken shows stratigraphic layers of BA5, BA6 and BA7.*

Fig. 3.3 (d) *Photo taken shows stratigraphic layers of BA8, BA9 and BA10.*

3.5.3 ROCK SAMPLE EXTRACTION

Approximately 15 g of powdered rock samples was weight in a thimble and capped using cotton wool. Insert into Soxhlet. 2-3 anti-bumping granules and copper tinnings were added to round bottom flask. 200mL azeotropic mixture of dichloromethane: methanol, 93:7. (V:V) was used as the extracting solvent. Then, the heating mantle was turned and this will carry on for 72 hours in the Soxhlet apparatus. The raw extract was then evaporated to almost dryness by a rotary evaporator [44]

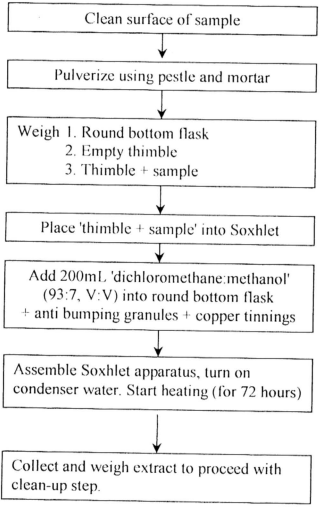

Fig. 3.4 *Flow diagram of extraction procedure*

3.6 SAMPLE CLEAN-UP

The sample extract was then clean-up by normal phase short column chromatography. The glass column (45 cm x 10 mm i.d.) was slurry packed with approximately 10 cm of normal phase silica. The column was pre-eluted with azeotropic mixture of hexane: ethyl acetate (95:5, V:V).

Prior to clean-up procedure, the raw coal extracts were shaken with 10mL of hexane and the asphaltene (high molecular weight material) precipitate filtered. The filtrate were transferred quantitatively to the normal phase column with 10 ml of the hexane: ethyl acetate (95:5, V:V) and eluted with 150mL of the solvent system. The collected elute were evaporated to almost dryness with rotary evaporator. The round bottom flask was then rinsed with high purity hexane and clean extracts were collected in vials.

3.7 IDENTIFICATION OF POLYAROMATIC HYDROCARBONS (PAHs) AND ALIPHATIC HYDROCARBONS IN COAL SAMPLES.

Normal phase silica high-performance liquid chromatography was used to fractionate and preliminary identify PAHs and aliphatic hydrocarbons (i.e. *n*-alkanes) as groups. The PAHs and *n*-alkane identification were carried out by injecting individual standard and standard mixture to obtain the well-resolved chromatograms. The fractionation of clean rock extract was then analyzed using the same conditions. Identification was then done by comparing the retention times (tr) of the sample PAHs with those of standard PAHs and sample aliphatic hydrocarbons with those of standard *n*-alkanes.

3.8 DETERMINATION OF YIELD FROM SOXHLET EXTRACTION.

Table 3.2 *Data for the determination of yield from extraction of rock samples*

Sample no.	Description of sediments	% of yeild recovered
BA1	Pure coal	25.0
BA2	Mixture of shale and coal	12.6
BA3	Coal going up to sandy base	46.1
BA4	Sandy base	40.1
BA5	Coal	18.8
BA6	Prominent coal	25.2
BA7	Shale	21.3
BA8	Repetition of coal and shale layer	62.3
BA9	Pure coal	51.6
BA10	Intercalation of coal and shale	15.6
BA11	Massive grey shale	5.1

Small amount of hydrocarbon maybe lost during solvent extraction process of rock samples, and transferring of solution. The yield of hydrocarbons (raw extract) was 5-62%. The percentage recovery of each hydrocarbon from rock sample was determined to evaluate the amount of hydrocarbon in a particular rock.

The percentage yield of hydrocarbon in rocks was calculated by the formula stated below:

$$\% \text{ yield} = C / (A-B) \times 100\% \qquad (3.4)$$

Where A = Weight of rock sample before extraction lost (g)
B = Weight of rock sample after extraction lost (g)
C = Weight raw extract (g)

Sample no	Weight of rock samples, g	Recovered weight after Soxhlet extraction, g	Recovered weight after Short column clean-up, g	Percentage recovery of organic matter, %	Preparative TLC Recovery		
					Upper band (Aliphatic HC), g	Middle band (PAH), g	Lower band (High polarity HC), g
BA1	16.0017	0.4170	0.0696	25.0	0.0195	0.0893	0.0544
BA2	16.1215	0.2147	0.0348	12.6	0.0136	0.0408	0.0272
BA3	15.4676	0.4965	0.0920	46.1	0.0907	0.0648	0.0389
BA4	15.0071	0.3290	0.0960	40.1	0.0669	0.0223	0.0399
BA5	14.9606	0.3072	0.0703	18.8	0.1169	0.0784	0.0399
BA6	15.5920	0.4293	0.1029	25.2	0.1174	0.0804	0.0435
BA7	15.7911	0.1176	0.0434	21.3	0.0496	0.0339	0.0183
BA8	15.5864	0.3720	0.1121	62.3	0.0775	0.0503	0.0230
BA9	15.2649	0.3752	0.1160	51.6	0.0437	0.0780	0.0343
BA10	15.8093	0.2278	0.0280	15.6	0.0330	0.0219	0.0108
BA11	15.5111	0.0750	0.0270	5.10	0.0151	0.0121	0.0091

Table 3.3 *Percentage of TLC recovery of the organic matter (aliphatic and PAH) from Batu Arang coal rocks.*

3.9 PREPARATIVE THIN-LAYER CHROMATOGRAPHY (TLC).

Typically thin-layer separations are performed on a glass plate that is coated with a thin and adherent layer of finely divided particles; this layer constitutes the stationary phase. In this case, the stationary phase is of course the normal silica gel. The particles are similar to those described in the discussion of normal-phase column chromatography. A thin layer plate is *prepared* by spreading an aqueous slurry of silica gel in water onto the clean surface of glass slide. This plate is then allowed to stand until the layer has set and adheres tightly to the surface; usually these plates are heated in an oven overnight to activate the silica.

Plate development is the process in which a coal rock extract is carried through the stationary phase by a mobile phase of petroleum ether. The process is analogous to elution in liquid chromatography. The most common way of developing a plate is to place a drop of the sample near one edge (the plates have dimension of 20 x 20 cm) and mark its position with a pencil. After the sample solvent has evaporated, the plate is placed in a closed container that is saturated with petroleum ether. One end of the plate is immersed in the developing solvent, with care being taken to avoid direct contact between the sample and the developing solvent. After the developer has traversed one half or two thirds of the length of the plate, the plate is removed from the container and dried.

For preparative TLC, the dried plates are examined under UV light at 254nm; zones of fluorescence are marked using a soft pencil, to permit estimation of relative retention factors and collection of sample laden silica layers for further study. Three bands were observed the UV active band contains PAH (middle band). The band above contains aliphatic HC. Whereas, the band below contains highly polar HC. The surface is scraped onto aluminium foil using a spatula and stored in a freezer until required.

3.10 DETERMINATION OF TLC RECOVERY OF ORGANIC MATTER.

From Table 3.3, Sample BA5 has the highest content of HC and the lowest being Sample BA11. This is accurate as BA5 is a coal layer and BA11 was collected as a form of rock (massive grey shale).