Chapter 4 Results and Discussion

4.1 SAMPLING INFORMATION

Information on the location of sampling stations is given in Fig. 1.2 (Page 3). Rock samples were obtained from Batu Arang coal mine on 9 March 2000. 11 samples were sampled layer by layer. The rock were knocked and cracked out using a spade. The sampling record is given in Table 3.1.

In the following discussion, all the PAHs and *n*-alkane are represented by the numeric notation as in Table 4.1.

Table 4.1 *Numeric notation of all PAHs and n-alkanes standard used.*

Standard no	Sample name
1	Naphthalene
2	1-Naphthol
3	2-Naphthol
4	Anthracene
5	Phenanthrene
6	Flourene
7	Acenaphthene
8	Benz(b)anthracene
9	Flouranthene
10	Chrysene
11	Pyrene
12	Benzo(e)pyrene
13	Hexane
14	*n*-C_{34}
15	*n*-C_{30}, *n*-C_{34}, *n*-C_{36}
16	*n*-C_{26},*n*-C_{28}, *n*-C_{29}, *n*-C_{30},*n*-C_{32}
17	*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}

4.2 DEVELOPMENT OF METHOD

In this project, normal phase silica high-performance liquid chromatography (HPLC) was explored and to investigate the separation of polyaromatic hydrocarbon and aliphatic hydrocarbons in-groups in coal extract.

It was found that there is an advantage in HPLC which is not available other methods of separation is the variety of mobile phases and absorption wavelength of the UV detector for optimisation of separation conditions. This degree of freedom also adds more than its shares to the difficulty in finding a good starting point. The preliminary work carried out was to study the various aspects regarding the use of HPLC

4.2.1 HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

4.2.1 (a) CHOICE OF UV DETECTOR CONDITION

❖ **WAVELENGTH**

Two different wavelengths were studied in the early stage, i.e. 254nm and 280nm. From the chromatogram, it can be seen that UV detection at wavelength of 254nm has better performance than 280nm in this single wavelength detect method as shown in Fig.4.1 (a) & (b) and Fig. 4.2 (a) & (b). Here phenantrene has a very weak absorption and the anthracene peak was totally not observed. Whereas at λ=254nm, both the compound peaks were observed. Therefore, 254nm was chosen as the operational wavelength for the detection of the polyaromatic hydrocarbons using the HPLC-UV detection.

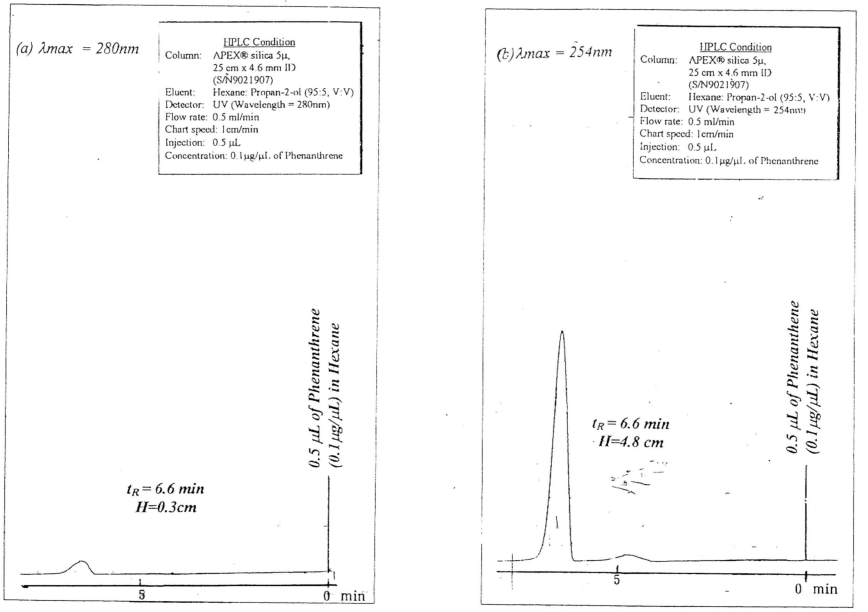
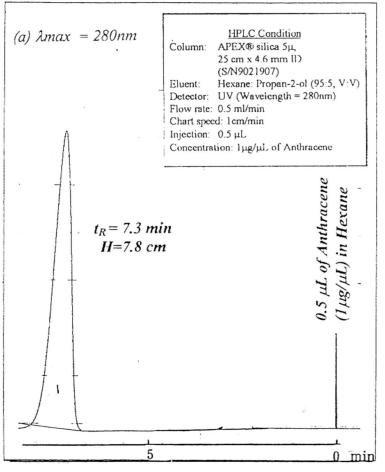


Fig. 4.1 *HPLC analysis of standard Phenanthrene (Optimisation of UV detector) (a) λ_{max} = 280nm and (b)λ_{max} = 254nm*



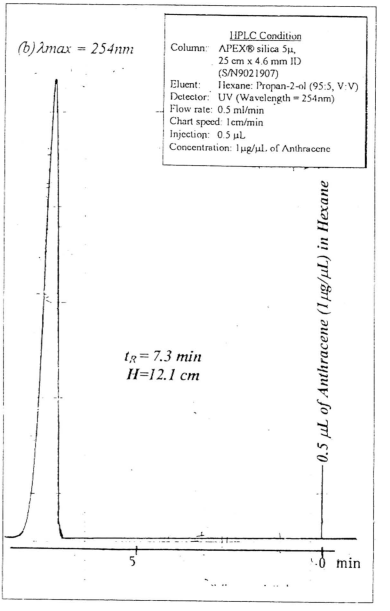


Fig.4.2 *HPLC analysis of standard Anthracene (Optimisation of UV detector) (a) λ_{max} = 280nm and (b) λ_{max} = 254nm*

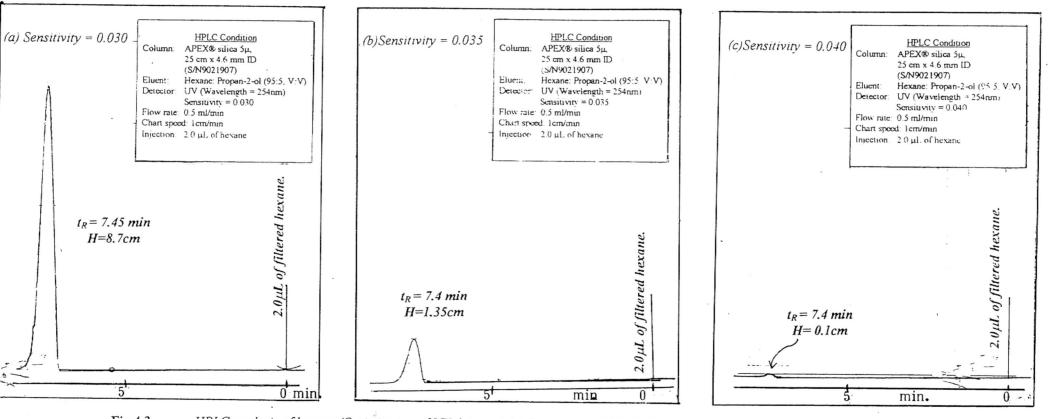


Fig.4.3 *HPLC analysis of hexane (Optimisation of UV detector) (a) Sensitivity = 0.03, (b)Sensitivity = 0.035 and (c)Sensitivity = 0.040*

❖ **SENSITIVITY**

Three different sensitivity were studied, i.e. 0.03, 0.035 and 0.04. As shown in Fig.4.3 (a), (b) and (c), UV detection at sensitivity 0.03 gives the highest peak for n-hexane. While utilizing sensitivity of 0.35, *n*-hexane has a very weak absorption and the peak was totally not observed at sensitivity 0.04. Therefore sensitivity of 0.03 was chosen as the best UV detection for this study.

Actually, hexane is an aliphatic hydrocarbon and is not UV active. The detection here may be due to the UV active contaminants (i.e. PAHs) in hexane.

4.2.1 (b) RI DETECTOR

Three different sensitivity were studied, i.e. 4, 8 and 16. As shown in Fig.4.4 (a), (b) and (c), RI detection at sensitivity 16 gives the highest peak for naphthalene. However the baseline at sensitivity 16 is not as stable as at sensitivity 8. While utilizing sensitivity of 4 naphthalene has a weaker absorption. Therefore sensitivity 8 is chosen as the best RI detection for this study

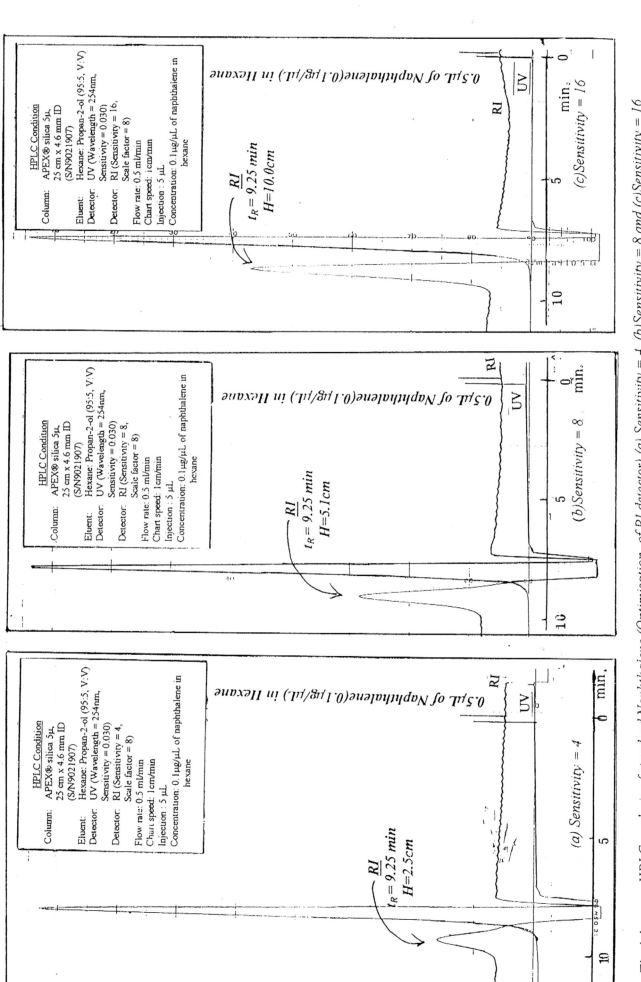


Fig.4.4 *HPLC analysis of standard Naphthalene (Optimisation of RI detector) (a) Sensitivity = 4, (b)Sensitivity = 8 and (c)Sensitivity = 16*

4.2.1 (c) CHOICE OF MOBILE PHASE AND FLOW RATE

❖ **Jones Chromatography APEX® silica 4µm, 25 cm x 4.6 mm ID (S/N9021907) column**

Four types of different ratio/type of mobile phase were tested on Jones Chromatography APEX® silica, 25cm x 4.6 mm ID (S/N9021907) column. The mobile phases are as follows:

1. hexane: propan-2-ol (95:5, V:V)
2. dichloromethane: ethyl acetate (95:5, V:V)
3. hexane: ethyl acetate (95:5, V:V)
4. hexane: ethyl acetate (98:2, V:V)

A mixture of the HPLC grade hexane: propan-2-ol, hexane: ethyl acetate and dichloromethane: ethyl acetate was used as the mobile phase. To achieve a proper balance between analysis time and the separation of mixtures, it is important to match the polarity of the samples with that of the stationary phase and to select an appropriate mobile phase of suitable polarity.

In this study, hexane was used as a reference point. Hexane is the choice because it is used as to dissolve all standards and rock sample extract. A suitable mobile phase is said to have obtained when the *n*-alkane (mixture) elutes out before hexane, and followed by the PAHs (mixture).

Preliminary study was carried out by using a mixture of hexane: propan-2-ol with composition of 95:5 (V/V) as the mobile phase to obtain from the RI chromatogram the retention time, tr of the PAHs and *n*-alkanes standards. The tr obtained is tabulated in Table 4.2 and the chromatograms are at Appendix B.

From the results, clearly this mixture is not a suitable mobile system as both PAHs and *n*-alkane standards except (1-naphthol) elute out before hexane. Therefore no separation is achieved.

The second attempt is the mixture of 95% of dichloromethane in 5% propan-2-ol. This is a very unsuitable mixture; it is very difficult to get a stable RI baseline with this system. A very 'noisy' chromatogram spectra was obtained. Another obstacle was the back-pressure; the pressure was running back and forth fluctuating when this solvent system was flowing. There was no presentable tr data available from this dichloromethane: propan-2-ol (95:5) as it was difficult to get a stable baseline.

A new mixture 95% hexane and 5 % ethyl acetate was then tested to obtain from the RI chromatogram the retention time, tr of the PAHs and *n*-alkanes standards as mentioned earlier. The tr obtained is tabulated in Table 4.3 and the chromatograms are at Appendix C. It was found that n-alkane standards elute out before the hexane and the PAHs standards elute out after the hexane.

The flow rate of the mobile phase also plays a part in obtaining a good analysis. A flow rate of 0.5mL/min was tried. At this rate, the analysis time is about 7 - 9 minutes for each standard. The peak obtained are quite sharp and it was decided that the flow rate is maintained at 0.5mL/min. Flow rate of less that 0.5mL/min will give very broad peaks and low resolution. At a higher rate, the compounds will be flushed out very quickly and a narrow peak will be obtained.

The last solvent system tested on this Jones Chromatography APEX® silica column is hexane: ethyl acetate (98:2, V:V). This is to test further for a better solvent system. From Table 4.4, both the PAHs and *n*-alkane standards eluted out before the hexane. This system is not a good separation system.

After setting the solvent mixture to hexane: ethyl acetate, the next thing to test on is the Jones Chromatography Genesis® silica 4µ, 25cm x 4.6 mm ID (S/N0032201) column.

Table 4.2 *HPLC retention time, tr (RI detector) of standards from utilizing hexane: propan-2-ol (95:5, V V) mobile phase on APEX(R) silica column (HPLC Condition 1) and injection volume of 5 µL.*

Standard no	Compound	Retention time, tr/ minute
1	Naphthalene	8.4
2	1-Naphthol	10.6
4	Anthracene	8.35
13	Hexane	8.4
14	*n*-C_{34}	7.5
15	*n*-C_{30}, *n*-C_{34}, *n*-C_{36}	7.6
16	*n*-C_{26},*n*-C_{28}, *n*-C_{29}, *n*-C_{30}, *n*-C_{32}	7.9
17	*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}	7.6

Table 4.3 *HPLC retention time, tr (RI detector) of standards from utilizing Hexane: Ethyl Acetate (95:5, V V) mobile phase on APEX® silica column (HPLC Condition 3) and injection volume of 5 µl..*

Standard no	Compound	Retention time, tr/ Minute
1	Naphthalene	9.35
2	1-Naphthol	9.35
3	2-Naphthol	9.35
4	Anthracene	9.50
5	Phenanthrene	9.40
6	Flourene	9.40
7	Acenaphthene	9.30
8	Benz(b)anthracene	9.40
9	Flouranthene	9.75
10	Chrysene	9.40
11	Pyrene	9.20
12	Benzo(e)pyrene	9.30
13	Hexane	9.30
14	n-C_{34}	7.55
15	n-C_{30}, n-C_{34}, n-C_{36}	7.55
16	n-C_{26},n-C_{28}, n-C_{29}, n-C_{30}, n-C_{32}	8.00
17	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}	7.60

Table 4.4 *HPLC retention time, tr (RI detector) of standards from utilizing Hexane: Ethyl acetate (98:2, V V) mobile phase on APEX® silica column (HPLC Condition 4) and injection volume of 5 µL.*

Standard no	Compound	Retention time, tr/ Minute
1	Naphthalene	8.10
13	Hexane	9.80
14	*n*-C_{34}	8.10
15	*n*-C_{30}, *n*-C_{34}, *n*-C_{36}	8.00

❖ **Jones Chromatography Genesis® silica 4µ, 25cm x 4.6 mm ID (S/N0032201) column.**

A mixture hexane: ethyl acetate (95:5, V:V) was then tested to obtain from the HPLC-RI chromatogram the retention time, tr of the PAHs and *n*-alkanes standards. The tr obtained is tabulated in Table 4.5. From Table 4.5, both the PAHs and *n*-alkane standards eluted out before hexane (tr = 10.2 minutes). Retention time for naphthalene, anthracene and phenanthrene is at 8.45, 8.55 and 8.4 respectively. Whereas tr for *n*-C_{34} is at 8.4 minutes and *n*-alkane mixture of *n*-C_{30}, *n*-C_{34}, *n*-C_{36} eluted out at 7.7 and 8.4 minutes. This system is not a suitable separation system, from the data obtained, there is an overlap between the aliphatic hydrocarbons and PAHs.

Table 4.5 *HPLC retention time, tr (RI detector) of standards from utilizing Hexane: Ethyl Acetate (95:5, V:V) mobile phase on Genesis® silica column (HPLC condition 5) and injection volume of 5 µL.*

Standard no	Compound	Retention time, tr/ Minute
1	Naphthalene	8.45
4	Anthracene	8.55
5	Phenanthrene	8.40
13	Hexane	10.20
14	*n*-C_{34}	8.40
15	*n*-C_{30}, *n*-C_{34}, *n*-C_{36}	8.40 & 7.70

❖ **Waters PrepPak® Catridge, Prep Nova-silica 6 µm, 10 cm x 2.5cm ID Preparative column**

From the preliminary work done on the HPLC analytical columns, the best separation was obtained from HPLC condition no 3 (Refer Appendix A). This condition was hoped to be adopted for the Waters PrepPak® Catridge, Prep Nova-silica 6 µm. However, the anticipated results were not observed. Therefore other types of conditions were tested out to obtain the best solvent system utilizing this normal phase semi-preparative column.

Four types of different ratio/type of mobile phase were tested on Waters PrepPak®, Catridge Prep Nova-silica 6 µm, 10 cm x 2.5cm ID prep column. The mobile phases are as follows:

1. hexane: ethyl acetate (95:5, V:V)
2. hexane: ethyl acetate (97:3, V:V)
3. hexane: ethyl acetate (98:2, V:V)
4. hexane: propan-2-ol (95:5, V:V)

The results of the HPLC-RI retention time of each standard PAHs and *n*-alkane are presented in Table 4.6, Table 4.7 and Table 4.8.

From the data in Table 4.6, there is a slight separation of *n*-alkane and PAH when utilizing hexane: ethyl acetate (95:5, V:V) solvent system. The *n*-alkanes elute out at after 15 minute. Whereas, the PAH elutes out at after 16 minutes. However the hexane peak only appears at 18.7 minutes, after the elution of *n*-alkane and PAHs standards.

The second attempt was a mixture of 97% hexane and 3% ethyl acetate. The results obtained were almost the same as the previous mixture and were tabulated in Table 4.7. Two peaks were observed for the naphthalene at tr = 16.1 & 18.5 minutes, flourene at 16.1 minutes, acenaphthene at 19.6 minutes and benzo(e)pyrene at 16.0 minute. The hexane is elutes out at 20.2 minutes. For the n-alkane standards, tr for *n*-C_{34} is at 20.3 minutes, tr for *n*-C_{30}, *n*-C_{34}, *n*-C_{36} mixture is at 15.2 and 16.3 minutes, tr for *n*-C_{26}, *n*-C_{28}, *n*-C_{29}, *n*-C_{30}, *n*-C_{32} mixture is at 15.9 and 19.9 minutes and tr for *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} mixture is at 15.5 & 17.6 minutes.

Another different ratio of hexane: ethyl acetate mixture was tested The third attempt was the at 98:2 (V:V) This is a very unsuitable mixture; it was very difficult to get a stable RI baseline with this system. A very 'noisy' spectra is obtained. This cannot be rectified even after purging the RI detector cells several times.

The last solvent system tested on the Waters PrepPak® Catridge Prep Nova-silica semi preparative column was the mixture of 95% hexane and 5% propan-2-ol. The results obtained is presented in Table 4.8. This solvent system gives quite a good separation as the PAH standards were eluted out after hexane and *n*-alkanes were eluted out before hexane.

This hexane:propan-2-ol solvent system was used to separate the aliphatic hydrocarbon and PAHs groups in all the rocks.

Table 4.6 *HPLC retention time, tr (RI detector) of standards from utilizing Hexane: ethyl acetate (95:5, V V) mobile phase on Waters PrepPak® Catridge Prep Nova-silica semi preparative column (HPLC Condition 6) and injection volume of 25µl..*

Standard no	Compound	Retention time, tr/ Minute
1	Naphthalene	16.30
4	Anthracene	16.50
5	Phenanthrene	16.35
13	Hexane	18.7
15	n-C_{30}, n-C_{34}, n-C_{36}	15.2 & 16.3
16	n-C_{26},n-C_{28}, n-C_{29}, n-C_{30}, n-C_{32}	15.45

Table 4.7 *HPLC retention time, tr (RI detector) of standards from utilizing Hexane: Ethyl Acetate (97:3, V V) mobile phase on Waters PrepPak® Catridge Prep Nova-silica semi preparative column (HPLC Condition 7) and injection volume of 25µl..*

Standard no	Compound	Retention time, tr/ Minute
1	Naphthalene	16.1 & 18.5
6	Flourene	16.1
7	Acenaphthene	19.6
12	Benzo(e)pyrene	16.0
13	Hexane	20.2
14	n-C_{34}	20.3
15	n-C_{30}, n-C_{34}, n-C_{36}	15.2 & 16.3
16	n-C_{26},n-C_{28}, n-C_{29}, n-C_{30},n-C_{32}	15.9 & 19.9
17	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}	15.5 & 17.6

Table 4.8 *HPLC Retention time, tr (RI detector) of standards from utilizing Hexane: Propan-2-ol (95:5, V/V) mobile phase on Waters PrepPak(R) Cartridge Prep Nova-silica semi preparative column (HPLC Condition 9) and injection volume of 25µl..*

Standard no	Compound	Retention time, tr/ Minute
1	Naphthalene	16.6
4	Anthracene	16.75
5	Phenanthrene	16.65
7	Acenaphthene	16.60
13	Hexane	15.45
14	*n*-C_{34}	15.50
15	*n*-C_{30}, *n*-C_{34}, *n*-C_{36}	15.30
16	*n*-C_{26},*n*-C_{28}, *n*-C_{29}, *n*-C_{30}, *n*-C_{32}	15.45
17	*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}	16.10

4.2.2 RECOVERY

Recovery work is essential in any analytical method. This information not only provides the degree of sensitivity but also the precision of the particular analytical method. The characteristic data obtained from the standardized analytical method, i.e. the recovery percentage can be used as a basis for the applicability of the analytical method on the compounds of interest. The percentage of recovery shows the method efficiency and this value must be taken into consideration during the calculation of quantitative concentration of the compounds. Besides, a recovery of the presence or absence of the interfering substance in a particular determination.

Table 4.9 *The recovery and percentage recovery of the organic matter (aliphatic and polyaromatic hydrocarbon) from the Batu Arang coal rock samples.*

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, %
BA1	16.0017	0.4170	0.0696	25.0
BA2	16.1215	0.2147	0.0348	12.6
BA3	15.4676	0.4965	0.0920	46.1
BA4	15.0071	0.3290	0.0960	40.1
BA5	14.9606	0.3072	0.0703	18.8
BA6	15.5920	0.4293	0.1029	25.2
BA7	15.7911	0.1176	0.0434	21.3
BA8	15.5864	0.3720	0.1121	62.3
BA9	15.2649	0.3752	0.1160	51.6
BA10	15.8093	0.2278	0.0280	15.6
BA11	15.5111	0.075	0.0270	5.1

4.2.3 DETECTION LIMIT

The detection limit can be defined as the smallest quantity or concentration substances that can be quantitatively detected by a single analysis. The detection limits of each individual PAHs by HPLC-UV determination are shown in Table 4.10.

Table 4.10 *Detection limits (μg mL) of PAHs by HPLC-UV analysis.*

Sample name	Detection Limit, (μg/mL)
Naphthalene	0.01
1-Naphthol	1.0
2-Naphthol	1.0
Anthracene	0.05
Phenanthrene	0.05
Flourene	0.05
Acenaphthene	0.01
Benz(b)anthracene	0.05
Flouranthene	0.05
Chrysene	0.02
Pyrene	0.02
Benzo(e)pyrene	0.02

4.3. SAMPLE CLEAN-UP

Rock extracts are normally too and often incompatibles with the chromatographic system to permit analysis by direct injection. As the matter of fact, all rock extract samples require a certain extent of treatment to remove the large quantities of interfering materials and to prevent contamination of the analytical column [45].

In theory, the coal extract should separate into four bands in a normal phase column. Namely, aliphatic (saturated and unsaturated), polyaromatic hydrocarbons (PAHs), hydrocarbon – nitrogen and sulfur containing groups (polar compounds) and resins. The aliphatic having the lowest polarity will elute out first, followed by the PAHs and the polar compound. The 'sticky' highly polar resins are likely to be retained in the column.

In this study, we have to clean-up the rock sample extract by normal phase short column chromatography. There was precipitation when the raw coal extract was dissolved in hexane for short column chromatography. The precipitate clogged up the column and this has made the clean-up step unsuccessful as the column has dried up instead. It was later found out that this precipitate is asphaltene [33]. Asphaltene is a high molecular weight material. The precipitate is then filtered before the short column chromatography step. This will assure a good flow in the clean-up step.

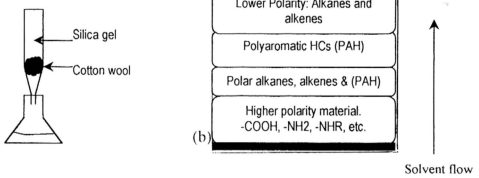


Fig. 4.5 *(a) An illustration if normal phase short column chromatography (b) Separation (development) of coal extract on normal phase TLC plate.*

4.4 PRELIMINARY IDENTIFICATION OF PAHS AND ALIPHATIC HYDROCARBONS.

HPLC chromatogram obtained can be used for preliminary identification purposes. Identification was done solely by comparing retention data of the unknown peaks with that of the standards. A standard of PAHs and *n*-alkanes was injected into HPLC and for analysis prior to each sample analysis. In this analysis, this method was found very useful when standard is tested. However, when the cleaned-up rock extract was injected into the HPLC, the separation is not clear. From *Appendix C, Fig. C(a)-C(d)* shows the spectra of clean coal extract separation by semipreparative HPLC. The HPLC-RI chromatograms obtained show no clear separation of aliphatic hydrocarbon and PAHs.

4.5 INTERFERENCE

4.5.1 METHOD INTERFERENCES

HPLC is a very sensitive analytical instrument in trace analysis. Thus, contaminants from solvent impurities, cleanliness of the glassware and through sample treatment may lead to discrete artifacts or elevated baseline problems in the chromatogram [46]. Precautions have to be taken to ensure that the analysis is free from any interference.

The glassware was soaked in distilled water and then they were rinsed with extraction solvent system or the dissolving solvent, depending on the application and dried in an oven.

In this analysis, HPLC grade hexane, ethyl acetate, propan-2-ol, dichloromethane and methanol were used to minimize the contamination. All solvents and HPLC mobile phase mixture were filtered through a 0.45μm membrane filter prior to being used in the analysis.

4.5.2 MATRIX INTERFERENCE

The matrix interference may be caused by the contaminants present in the rocks, which were also extracted from the rock sample. This type of interference may vary depending upon the nature and also diversity type of rock and what type of vegetation (if any) is present in that area. However, the matrix interference was greatly reduced after extraction and clean-up.

4.6 EVALUATION OF ANALYTICAL DATA

4.6.1 ERRORS

In this project, the analytical errors are exposed to systematic errors and random error.

Systematic errors in this analysis can be traced to instrumental uncertainties, method uncertainties and errors that resulted from physical or psychological limitation of the analyst.

On the other hand, random error is a consequence of the changes, which is not controllable when it reaches the instruments' limitation such as noise and electronic fluctuations.

4.7 DISCUSSION

From the results obtained, this method of separation PAHs from *n*-alkanes is successfully separated in-groups as anticipated (refer Table 4.2 - 4.8). Further work, was done to fine-tune this method of separation so that a clearer separation is obtained. The best results (elution) would have been the *n*-alkane (being the lower polarity) elute out first then comes the hexane (as the dissolving solvent) and last to elute out is the PAHs groups on the APEX® silica analytical column and solvent system of 95% hexane and 5% ethyl acetate.

The method developed in this experiment is not as successful as methods discussed in the Methodology Section (Chapter 2). This method is applicable when separating known compound (i.e. standard solution). When a mixture of *n*-alkanes and PAHs was injected into the APEX® analytical column, a separation was obtained. However, when this is applied to rock (coal) extract, the result anticipated is not obtained (refer Appendix D). The consistent result obtained from the HPLC-RI chromatograms was the hexane peak.

The selection of mobile phase at the correct polarity plays an important role in the effectiveness in chromatography. Polarity is a term used in chromatography as an index of ability of compounds to interact with one another in these various ways. It is applied very freely to solutes, stationary phases and mobile phases. The more polar a molecule, the more strongly it can interact with other molecules through the mechanism above. The stationary phase used in this study is the highly polar normal silica, needs a mobile phase with relatively low polarity i.e. hexane and dichloromethane. If high polarity mobile phase was used the silica could be dissolved.

When nonpolar molecules or portion of molecule are introduced into a polar matrix, the nonpolar will orient in a manner leading to maximized polar interaction. This result in nonpolar being pushed together. The net effect is that *likes attract likes* - polar with polar and nonpolar molecules with nonpolar. The higher polarity PAHs will have a longer retention time compared to aliphatic hydrocarbons in this study because the very polar silica stationary phase has higher attraction toward PAHs.

Since the mobile phase is continuously being replaced, each component is washed off at a different rate.

It is helpful in LC to have a quantitative measure of polarity so that, for example, the relative polarity of a solvent or of a mixture of solvent can be expressed a s number. This may be done in a number of ways, none of which are entirely satisfactory but they do allow us to arrange solvents in order of polarity and to estimate the polarity of solvent mixture. One such ways is to use as measure of polarity of a quantity called the solubility parameter (δ), defined by

$$\delta = \left(\frac{\Delta E}{V} \right)^{1/2}$$

Where ΔE = internal energy of vaporization and V = molar volume [47]. Table 4.10 shows δ and P' values for a number of solvent, arranged in order of increasing δ.

Table 4.10 *Polarity index for a range of solvents.*

Solvent	δ, $Pa^{1/2} \times 10^{-3}$
Hexane	7.3
Ethyl acetate	8.6
Dichloromethane	9.6
Propan-2-ol	10.2
Methanol	12.9

In the HPLC condition No.3 (refer Appendix A), where a mixture of 5% ethyl acetate with 95% hexane is used, the retention time tr for PAH is in the range of 9.2-9.75 min. Whereas, when a 2% ethyl acetate (HPLC condition no 4) is used, the tr is at is at 8.1-8.5 minutes. This clearly shows that at a higher polarity of mobile phase, the longer the elution time will be for the PAHs.

The preliminary work for getting a suitable solvent system was quite a challenge. As clearly shown in Table 4.3, a clear-cut separation has been obtained by employing the APEX® analytical column coupled with HPLC condition No.3. However this is not observed in the semipreparative application. When the Waters PrepPak®, Nova-silica semipreparative column was tested with the same solvent system (HPLC condition no.6), there is not clear-cut separation as when the APEX® analytical column was employed. Therefore a few more solvent systems was tested out.

There are a few differences between an analytical column and a preparative column. Therefore, the methodology developed using the APEX® silica column cannot be employed for the Waters Nova-silica preparative column. The obvious difference is the column size, in term of length and diameter. Calculation of flow rate for semi preparative is as Equation 3.2.

$$FR_2 \quad FR_1 X (D_2 D_1)^2 \qquad (3.2)$$

Flow rate used in APEX® column, FR_1 = 0.5ml/min

Diameter of APEX® column, D_1 = 4.6mm

Diameter of Waters preparative column, D_2 = 2.5cm

New flow rate, FR_2 = $FR_1 X (D_2/D_1)^2$

= 0.5 ml/min x $(25mm/4.6mm)^2$

= 14.77ml/min

The calculated flow rate is 14.77ml/min. This rate is too high; there would not be any separation due to the high flow rate. Anyway, this is not cost effective, as too much solvent (mobile phase) will be used in a short period of time. The flow rate of 2.0ml/min is employed instead. This flow rate gives a very stable base line and quite sharp peak.

There is another glaring difference between the analytical column and the semipreparative column used, which is the stationary phase particle size. The particle of APEX® silica is 5μm whereas Nova-silica is 6μm. We have to note that smaller particle size in a column makes it more difficult to drive the mobile phase through. Because of the difference in particle size, the initial HPLC condition developed in the

APEX® silica cannot be directly applied on the Waters PrepPak® Semipreparative column. This is another reason why the flow rate is set at 2ml/min and not 14.77ml/min!

HPLC condition no.2 is worth to look into. Here a mobile phase of dichloromethane 95% and propan-2-ol 5% is being tested on the Jones Chromatography APEX ® silica analytical column. It was very difficult to obtain a 'good' baseline while working with this solvent system. The recorder was recording (for HPLC-RI chromatogram) a very noisy baseline even after purging the RI detector several times [48]. Another problem faced was the back-pressure; it was fluctuating excessively by going high and low. These problems were faced simultaneously. This may be due to the high polarity of dichloromethane and propan-2-ol. The normal phase silica column has very high polarity as well and because "likes attracts likes" the solvent will form a layer of solvent around each particle. Making the increase in particle size. This in turn will cause a high resistance towards the mobile phase making the back-pressure to rise. When the pressure is high enough, the 'flow through' of mobile phase is smooth again and then causing the dive of the back-pressure. The unstable back pressure and 'noisy' base line makes this mixture a non-choice as a solvent system for this study.

The APEX® silica column is a predecessor to theGenesis® silica column. Both these columns are normal phase analytical columns. The Genesis® silica column was purchased to facilitate in method development of this study. APEX® silica is with uniform sized spherical particles, narrow particle size distribution, limited porosity range, and controlled surface area [49]. These properties provide packed beds of maximum efficiency. The Genesis® has all the above and more. It is has an ultra-pure and highly inert HPLC packing material. The high purity silica permits maximum surface coverage, uniform density bonded phases, exceptional pH stability and low secondary activity towards basic and acidic analytes. However, the results obtained shows the opposite. The separation of *n*-alkane and PAHs are not as anticipated. Instead the results shows a poorer separations of *n*-alkane and PAH standard. From Table 4.5, all the standards have almost the same retention time of 8.4-8.55 min; except Hexane which eluted out at 10.2 min.

Rock (Coal) sample extraction.

Solvent extraction has always been one of the most commonly used techniques for studying the composition of coal. Its primary aim was to isolate the material, or materials, from which coal derives its coking properties. In this study, specific extraction method has been adopted. Hydrocarbon extraction of rock (coal) sample should consist of aliphatic hydrocarbons, polyaromatic hydrocarbon (PAHs) and highly polar substituted hydrocarbon (R-COOH, R-O-R, R-S-R, R-S-S-R, >S=O, NH_2 and -SH) [2]. In this study, the focus is on the aliphatic hydrocarbons and PAHs. The substituted hydrocarbon (interference) is eliminated by adding two to three copper tinning to the round bottom flask (extract collector) prior to the heating [44].

HPLC Chromatograms of analysis on coal extract are in Appendix D. All the coal extracts are tested utilising HPLC condition 6 and 9 (refer Appendix A). However only a selected few was tested with HPLC condition 7 and 8. From the HPLC chromatograms, clearly there is not any separation. Solvent mixture of hexane: ethyl acetate gives a better separation than analysis using hexane:propan-2-ol. From all the chromatograms of rock extracts, clearly the PAHs have overwhelmed the aliphatic hydrocarbons as only UV active compound can be detected by the UV detector. The RI detector merely shows the peak of the hexane on the HPLC chromatograms. There are a few factors why this may happen and they are:

(a) The concentration of *n*-alkane in the rock extract is too low.

(b) The extraction method is not an effective one and mostly PAHs are effectively extracted from the rock samples.

(c) The choice of mobile phase in the isocratic short column chromatography clean-up step was not a suitable mixture. The mixture used was hexane: ethyl acetate (95: 5, V:V). This mixture was agreed upon then because the separation method has been developed on the APEX® silica analytical column (refer HPLC condition no 3

(d) The RI detector is not sensitive enough to detect the low concentration of aliphatic hydrocarbons in the rock extract. The RI detector is the closest thing in HPLC to a universal detector, as any solute can be detected as long as there is a difference RI between the solute and the mobile phase. However, the RI detector is he least sensitive detector when it is compared to UV detector.

A few problems was encountered during the utilization of instruments. As seen in most of the HPLC chromatograms, a common problem of UV detector is that the detector register very high or off-scale absorbance readings all the time, i.e. the UV radiation is being absorbed strongly when it should not be. The possible causes are:

(a) The mobile phase contains some UV absorbing component. This can be checked by measuring the absorbance of the mobile phase using another spectrophotometer, but make sure to take the sample directly from the mobile phase to have been made up incorrectly, and if you, make up a fresh sample you might get it right!

(b) There are large air bubbles in the flow cell. These can sometimes be removed by pumping at high flow rate or by disconnecting the column and passing solvent rapidly through the flow cell using a syringe. This can be done with a syringe. This can be done with a syringe of 10-20 cm^3 capacity with a 1/16 union fitted to the end of the needle.

(c) The flow cell may be leaking so that there are drops of solvent on the outside of the end windows, or the end windows may be dirty, or cell may not be properly aligned in the instrument. The alignment is easily checked, but only dismantle and clean the flow cell as a last resort; some types are quite difficult to reassemble. Faults in the detector can be checked by seeing if zero absorbance can be obtained with the flow cell removed; if you can, the detector is probably all right.