Chapter 2 Methodology

2.1 LITERATURE REVIEW

There are several methods establish for the study of coal analysis. Among them is a method suggested by Michalcyk, (1985) [32]. Michalcyk suggested a high-performance liquid chromatography separation for the cleaned up saturate-aromatic fraction of the oil or bitumen is separated using a HPLC pump equipped with a silica column (9.4 mm I.D. x 50cm). Here, the eluent is divided into three cuts (saturates; *mono-*, *di-*, and *tri*aromatics; and polar compounds) by using a programmable fraction collector and 400 ml glass bottles. Under "normal flow" conditions (Fig. 2.1, Lower left), the saturates (2 ml/minute for 15.4 minutes) and aromatics (9 ml/minutes for 26.5 minutes) are eluted with hexane while methylene chloride is used for the polar compounds (7 ml/minute for 20 minutes). Before the polars are eluted from the column with methylene chloride, the backflush valve is switched, resulting in passage of the eluent from the injection port to the main column and back through the guard column. In this "reversed flow" valve position (Fig 2.1 lower right), the direction of flow through the main column remains the same, but flow through the guard column is reversed (backflushed). The columns are partially reequilibrated with hexane (7 ml/minute for 12 minutes) before the next separation.

A UV detector (254nm; HPLC flow cell) and an RI detector monitor the fractions using a two pen recorder. The cut point between saturates and aromatics are determined based on retention times of cholestane and monoaromatic steroid standards. The cut between troaromatics and polar is made immediately after elution of dimethyl phenanthrene.

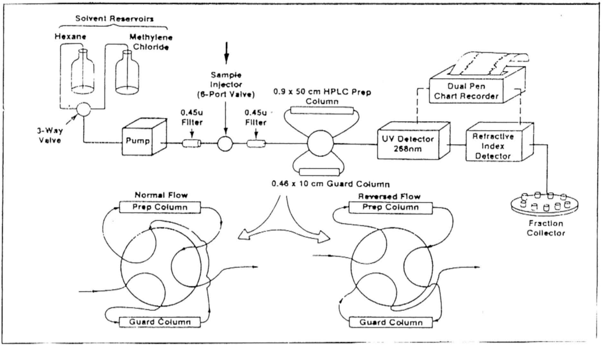


Fig. 2.1 *Configuration of the high-performance liquid chromatography (HPLC) for automated separation of saturate, aromatic, and polar fractions from petroleum samples. Except for the sample inlet valve, all are automated using microprocessor in the pump. (Adapted from [32])*

Ralph and Catcheside (1996) suggested size-exclusion chromatography of solubilised low rank coal [33]. Here, the solubilised brown coal is a polydisperse and chemically heterogeneous polyanion. The parameters appropriate for estimation of its molecular mass (M_r) by size-exclusion chromatography (SEC) using a *stationary phase comprising glyceryl-propyl silica* were investigated.

Hartgers, Sinnighe Damste and de Leeuw (1992) suggested identification of C_2-C_4 alkylated benzenes in flash pyrolysates of kerogens, coals and asphaltenes [34]. In their study, Pseudo-Kovats indices were determined for all positional isomers of C_2-C_4 alkylated benzenes using a *non-polar GC capillary column* (CP SIL-5). These indices were applied in combination with mass spectral data to identify these compounds in complex mixture of a relatively non-polar nature generated upon flash pyrolysis of macromolecular sedimentary organic matter (kerogens, asphaltenes and coals).

An earlier work by John W, Haas III and Peter C. Uden (1991), worked on chemical class separation of coal liquids by high-performance liquid chromatography on semi-preparative, phosphoric acid-modified amino-bonded-phase column [35]. An *amino-bonded stationary phase, modified by treatment with phosphoric acid* to form an amino phosphate, permits the high-performance liquid chromatographic separation of aliphatic, non-polar aromatic neutral/acidic polar aromatic and basic polar aromatic hydrocarbon chemical classes. It was employed to fractionate coal liquid samples on a *semi-preparative scale*. Separation of model compounds added to a coal liquid sample diluted with hexane with GC analysis of the HPLC fraction for a more concentrated coal liquid/hexane sample demonstrated that compounds were eluted according to chemical class with little or no overlap between classes. The modified column uniquely separates the polar acids and bases into distinct fraction while demonstrating the same capability as other polar bonded phases for separating the non-polar hydrocarbon classes. The method could be scaled up to full preparative size and modified further (e.g. by using gradual gradient program) to fractionate very complex samples more extensively, including synthetic and natural fuels or environment samples requiring individual compound identification.

Moliner, Lazaro, Fernandez and Ibarra. (1993) worked on conversion of alkanes into aromatic compounds during coal pyrolysis and the evidence are analyzed and obtained by gas chromatography-mass spectrometry [36]. Analysis by high-resolution gas chromatography interfaced to mass selective detector of tars has shown that tar composition depends on pyrolysis conditions. Low-temperature, low-pressure tars have higher *n*-alkanes concentrations. Evidence of conversion of *n*-alkanes into aromatic compounds mainly alkyl derivatives of naphthalene and phenanthrene, at high temperature and pressure has been presented. Sulphur released from coal during pyrolysis is involved in cyclisation reactions of *n*-alkanes, yielding heterocyclic sulphur compounds, mainly alkyl derivatives of benzothiophene.

Alexander and Hazai (1981) suggested a method of chromatographic fingerprinting of coal extract [37]. Coal samples of different ranks were examined by solvent extraction, liquid chromatographic (LC) separation of the extract and analysis of the aliphatic and aromatic hydrocarbon LC fraction by high-resolution gas chromatography. The LC fraction distribution is characteristic of the coal. The gas chromatograms of the two hydrocarbons LC fractions show strong similarities for related samples and can serve as "fingerprints" of the coals. For the *n*-alkanes, parameters such as "carbon preference index" can be calculated. For other aliphatic and aromatic hydrocarbons, respectively, the retention and "PAH indices" are presented. LC method can separate the extracts of different coals into various fractions, and the weight distribution of these differs according to the type and rank of coal. The GC analysis of the more or less volatile aliphatic and aromatic fractions resulted in chromatograms, which are useful in the fingerprint characterization of the coals. There are striking similarities between the gas chromatograms of related coals. Group of peaks at retention indices of 2000 and 3000 in the aliphatic gas chromatograms can be attributed to geochemical fossils of cyclic di- and triterpanes respectively. The *n*-alkane in the chromatograms can be used to calculate certain parameters (CPI) related to coal rank. The PAH indices of the aromatic peaks and their dependence on the programming rate may be used to reveal their hydroaromatic character.

Michael J. Sepaniak and Edwards S.Yeung (1981) suggested high-performance liquid chromatographic studies of coal liquids by laser-based detector [38]. Four solvent-refined coals were studied by isolating the asphaltene fractions, using reversed-phase liquid chromatography and detecting with three independent spectroscopic detectors. Simultaneous chromatograms from a UV absorption detector, a visible. fluorescence detector, and a two-photon excited fluorescence detector provide an abundance of information, even though baseline chromatographic resolution is not achieved. The chromatograms were to be good fingerprints of the individual solvents-refined coals.

Alan A. Herod and Rafael Kandiyoti (1995) suggested a fractionation method by thin layer chromatography [40]. In their study, the fractionation of coal tar pitch by planar chromatography for characterization by size-exclusion chromatography, UV, fluorescence and direct-probe mass spectrometry, A coal tar pitch, extensively studied by other techniques, has been fractionated by planar chromatography using successive development in tetrahydrofuran, chloroform-methanol (4:1, v/v), toluene and pentane. Pitch fractions were distinguished by relative retention. The fractions were characterised in solution by size-exclusion chromatography, UV-fluorescence emission spectroscopy and as the solid on silica, by direct solid-probe mass spectrometry. The method has led to structural information not readily available by direct characterisation of the original mixture itself.

2.2 HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

High-performance liquid chromatography (HPLC) is a form of liquid chromatography to separate compounds that are dissolved in solution. HPLC instruments consist of a reservoir of mobile phase, a pump, an injector, a separation column, and a detector. Compounds are separated by injecting an amount of the sample mixture onto the column. The different components in the mixture pass through the column at different rates due to differences in their partitioning behavior between the mobile liquid phase and the stationary phase.

Instrumentation of HPLC can be found at Fig.2.2. Solvents must be degassed to eliminate formation of bubbles. The pumps provide a steady high pressure with no pulsating, and can be programmed to vary the composition of the solvent during the course of the separation. The liquid sample is introduced into a sample loop of an injector with a syringe. When the loop is filled, the injector can be injecting the sample into the stream by placing the sample loop in line with the mobile phase tubing. The different types of HPLC columns are described in a separate document. The presence of analytes in the column effluent is recorded by detecting a change in refractive index, UV-VIS absorption at a set wavelength, fluorescence after excitation with a suitable wavelength, or electrochemical response. Mass spectrometers can also be interfaced with liquid chromatography to provide structural information and help identify the separated analytes.

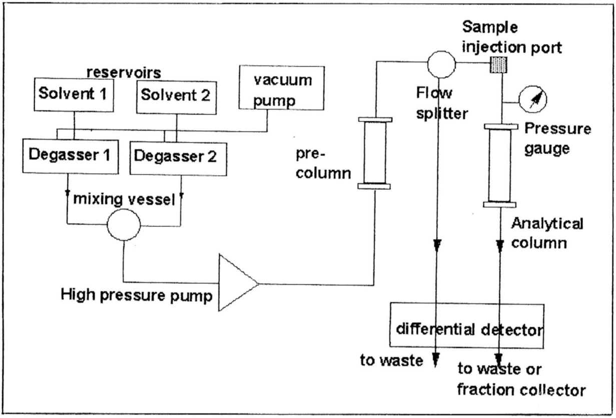


Fig. 2.2 *Schematic diagram of HPLC. (Adapted from [42])*

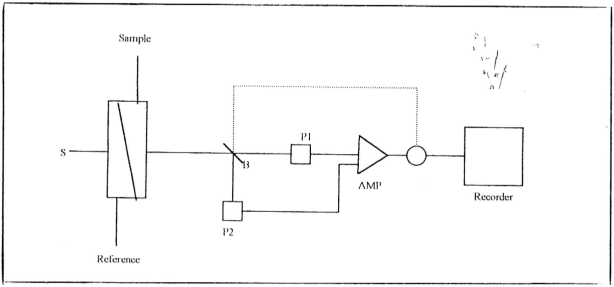


Fig. 2.3 *Refractive index detector (refractometer). (Adapted from [41])*

2.3 HPLC DETECTORS

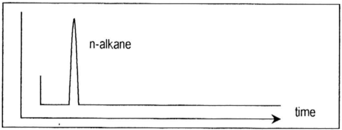
2.3.1 REFRACTIVE INDEX

These detectors sense the difference in refractive index between the column eluent and reference stream of pure mobile phase. They are the closest things in HPLC to a universal detector, as any solute can be detected as long as there is a difference in RI between the solute and the mobile phase.

Refractive index detectors are not as sensitive as UV absorbance detectors. The best noise levels obtainable are about 10^{-7} r.i.u. (Refractive index units), which corresponds to a noise equivalent concentration of about 10^{-6} gcm^{-3} for most solutes. The linear range of most RI detectors is about 10^{4}. If you want to operate them at their highest sensitivity you have very good control of the temperature of the instrument and of the composition of the mobile phase.

Because of their sensitivity to mobile phase composition it is very difficult to do gradient elution work and they are generally held to be unsuitable for this purpose. Several rather different designs of RI detector have been used in HPLC, Fig.2.3 shows the operating principle of one type, the deflection refractometer. Light from the source S is focussed onto the cell, which consists of sample and reference chambers separated by a diagonal sheet of glass. After passing through the cell, the light is diverted by a beam splitter B to two photocells P2 and P2. A change in the RI of the sample stream causes a change in the angle at which the beam strikes the splitter. This causes a change in the relative output. This difference is amplified, giving an error signal at the amplifier output that operates a servomotor, which rotates the beam splitter until the error signal is reduced to zero. The beam splitter movement (proportional to the difference in RI that caused it) is measured by the recorder [41].

In this study, *n*-alkanes standard mixture is injected into the HPLC to verify that the mixture will elute out as a group, all at almost the same retention time, tr (Fig. 2.4). *n*-alkanes can be detected by RI detector. This tr will be used as the reference retention time for *n*-alkanes.



RI detector

Fig. 2.4 *Chromatogram of n-alkane standard mixtures*

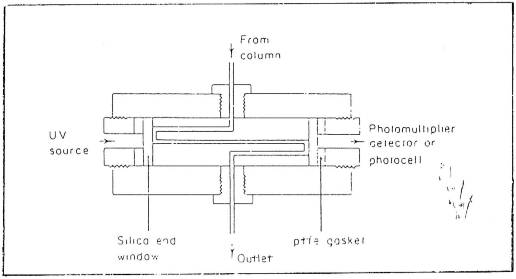


Fig. 2.5 *Flow cell for UV visible absorbance detector.*

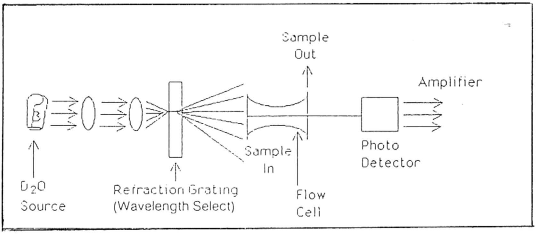


Fig. 2.6 *A variable ultraviolet detector light path.*

2.3.2 UV DETECTOR

These are by far the most popular in HPLC. The principle is that the mobile phase from the column is passed through a small flow cell held in the radiation beam of a UV/visible photometer or spectrophotometer. These detectors are selective in the sense that they will detect only those solutes that absorb UV (or visible) radiation. Such solutes include alkenes, aromatics and compounds having multiple bonds between C and O, N or S. The mobile phase we use, on the other hand, should absorb little or no radiation [41].

Absorption of radiation by solutes as a function of concentration, c, is described by the Beer-Lambert law:

$$A = \varepsilon bc \qquad (3.1)$$

Where A = absorbance, b = path length of the cell and ε = molar absorptivity, which is a constant for a given solute and wavelength

Fig. 2.5 is a diagram of a simple type of UV flow cell. The cell has a 1 mm internal diameter and the optical path is 10mm giving it an internal volume of just under 8 µL. Modern instruments use a 'cassette' type flow cell, which plugs into a holder in the detector; in older detectors there are arrangements for horizontal and vertical adjustment of the position of the flow cell in the radiation beam. More complicated flow cell design attempt to reduce flow cell design attempt to reduce flow disturbances, which can be caused by changes in the refractive index of the eluent, for instance if the solute has been dissolved in a solvent other than the mobile phase.

PAHs standard mixture can be detected by UV and RI detector. The PAHs standard mixture is injected into the HPLC to verify that the mixture will elute out at almost the same retention time, t_R. This t_R will be used as the reference retention time for PAHs.

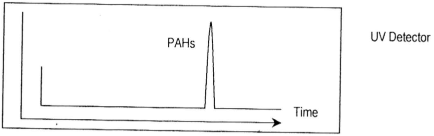


Fig. 2.7 *Chromatogram PAHs standard mixtures*

Mixture of *n*-alkanes and PAHs (standard) can be detected by UV and RI detector. The standard mixture of *n*-alkanes and PAHs is injected into the HPLC to verify that PAHs and *n*-alkanes mixture can be well resolved. And clearly confirms the retention time of each standard.

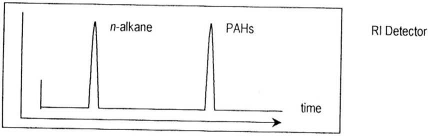


Fig. 2.8 *Chromatogram of n-alkane and PAHs standard mixtures*

2.4 HPLC UTILIZATION

2.4.1 SEMIPREPARATIVE HPLC.

Semipreparative separations are made on a 10 mm x 25 cm column packed with the same 5 or 10 μm packing used in the analytical separation. Simply replace the column and equilibrate with the analytical mobile phase. A 1-5 mg sample can be injected with a flow rate FR2 calculated from the following formula:

$$FR_2 = FR_1 \times (D_2/D_1)^2 \qquad (3.2)$$

Where FR_1 is the analytical flow rate, D_2 is the semipreparative column diameter, is the diameter of the equivalent analytical column, and is the square of the diameter difference. Here we would use a flow rate of about 2-5 ml/min.

By adopting the same method and condition as above, the PAHs and *n*-alkanes fractions are isolated in preparative HPLC.

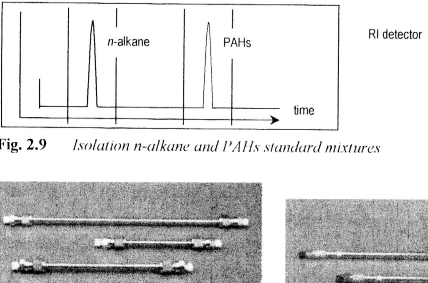
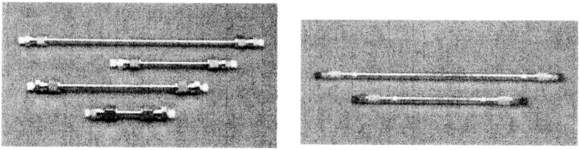


Fig. 2.9 *Isolation n-alkane and PAHs standard mixtures*



(a) (b)

Fig. 2.10 *HPLC analytical columns.* *(a) APEX(R) silica column*
(b) Genesis(R) silica column

2.4.2 COLUMNS FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

Liquid-chromatographic columns are usually constructed from stainless steel (refer Fig. 2.10 (a) and (b)) tubing, although heavy-walled glass tubing is sometimes employed for lower pressure applications (<600psi). Most column range in length from 10 to 30 cm and have inside diameters of 4 to 10 mm. Column packings typically have particle sizes of 5 or 10 µm. Columns of this type often contain 40,000 to 60,000 plates/m. Recently, high-performance microcolumns with inside diameters of 1 to 4.6 mm and lengths of 3 to 7.5 cm have become available. These columns, which are packed with 3 or 5 µm particles, contain as many as 100,000 plates/m and have the advantage of speed and minimal solvent consumption. The latter property is of considerable importance because the high-purity solvent required for liquid chromatography are expensive to purchase and to dispose of after use.

The most common packing for liquid chromatography is from silica, prepared by agglomerating submicron silica particles under conditions that lead to larger particles with highly uniform diameters. The resulting particles are often coated with thin organic films, which are chemically or physically bonded to the surface. Other packing materials include particles, porous polymer particles, and ion-exchange resins

2.4.3 NORMAL- AND REVERSED-PHASE PACKINGS

Two types of partition chromatography are distinguishable based upon the relative polarities of the mobile and stationary phases. Early work in liquid chromatography was based upon highly polar stationary phases such as triethylene glycol or water; a relatively nonpolar solvent such as hexane or *i*-propyl ether then served as the mobile phase. For historic reasons, this type of chromatography is called *normal-phase chromatography*. In *reversed-phased chromatography* the stationary phase is nonpolar, often a hydrocarbon, and the mobile phase is a relatively polar solvent. [42]. In normal-phase silica chromatography, the *least* polar component is eluted first (in this study it is the *n*-alkane group); increasing the polarity of the mobile phase then *decreases* the elution time. In contrast, in the reversed-phase method, the *most* polar component elutes first, and increasing the mobile phase polarity increases the elution time.

It has been estimated that more than three quarters of all HPLC separations are currently performed with reversed-phase, bonded, octadecyl siloxane packings. With such preparations, the long-chain hydrocarbon groups are aligned parallel to one another and perpendicular to the surface of the particle, giving a brushlike, nonpolar, hydrocarbon surfaces. The mobile phase used with these packings is often an aqueous solution containing various concentrations of such solvents as methanol, acetonitrile, or tetrahydrofuran.

2.4.4 CHOICE OF MOBILE AND STATIONARY PHASES

Successful partition chromatography requires a proper balance of intermolecular forces among the three participants in the separation process - the analyte, the mobile phase, and the stationary phase. These intermolecular forces are described qualitatively in terms of the relative polarity possessed by each of the three reactants. In general, the polarities of common organic functional groups in increasing order are aliphatic hydrocarbons < olefins < aromatic hydrocarbons < halides < sulfides < ethers < nitro compounds < esters ≈ aldehydes ≈ ketones < alcohols ≈ amines < sulfones < sulfoxides < amides < carboxilic acids < water [43].

As a rule, most chromatographic separations are achieved by matching the polarity of the analyte to that of the stationary phase; a mobile phase of considerably different polarity is then used. This procedure is generally more successful than one in which the polarities of the analyte and the mobile phase are matched but are different from that of the stationary phase. Here, the stationary phase often cannot compete successfully for the sample components; retention times then become too short for practical application. At the other extreme is the situation where the polarities of the analyte and stationary phase are too much alike; here, retention times become inordinately long.

2.5 ROCK (COAL) EXTRACTION USING SOXHLET APPARATUS.

Typically sediments are extracted using either Soxhlet apparatus or ultrasonication [27]. Wet sediments may be lyophilized (freeze-dried) prior to either of these methods to remove the pore water and increase the effectiveness of penetration of organic solvents into the pore spaces. The extraction efficiency of all of the methods tends to be enhanced by the use of a combination of solvents of differing polarity. In part, this approach reflects the recognition that the components in the less polar solvent (e.g. dichloromethane, trichloromethane or hexane) but that the more polar solvent (e.g. methanol or isopropanol) is more effective at swelling or expanding clays and thereby providing better access to absorbed organic compounds.

In Soxhlet extraction solvent is vaporized from the flask, passes upward through from a flask, passes upward through the side arm of the apparatus, condenses and drips down into a cellulose thimble containing the powdered sample within the Soxhlet. The hot solvent extracts the component from the sample and is periodically recycled into the solvent reservoir flask via a siphon. Several cycles of this process are required to ensure exhaustive extraction; hence the apparatus is typically operated for 24 hour, dependent on the sample size and richness. Extraction is typically complete when the solvent surrounding the thimble becomes clear, rather than some variant shade of black, brown, red or yellow.

One important concern with extraction and all methods employed on the investigation of coal extract are the need to use high-grade solvents that are effectively free of contamination hydrocarbons, plasticizers or other components that can interfere with the analysis or invalidate the results. Procedural blanks provide a direct and convenient means of ensuring that potential contaminants can be screened and evaluated.

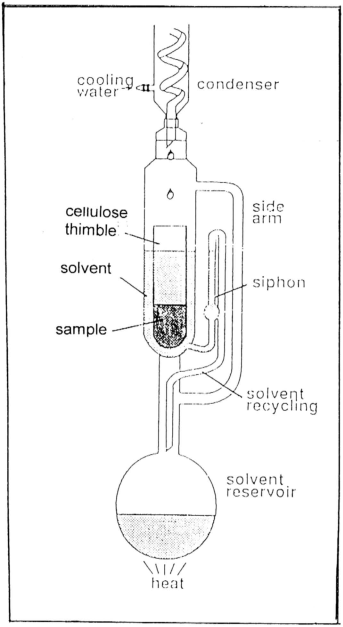


Fig. 2.11 *Illustration of Soxhlet extraction in progress*