CHAPTER 4: METHODS

Chapter 4

Methods

4.1 Field Work

A field investigation was undertaken in October 2001. About ten outcrops were logged. Previous workers (e.g. Wilson, 1964; Lee, 1977; Mazlan, 1994 &1997; Tongkul, 2001) highlight characteristics and describe the geological column of Labuan Island. Detailed attention was given during field work to describe each lithology. The short litholigical descriptions comprise rock name, color, texture, and primary features. Primary sedimentation structures that originate during or shortly after deposition (i.e. before it is lithified into sedimentary rock) were given attention. More than 40 samples were collected during the field work some of which were selected for analytical study.

4.2 Organic Petrology

4.2.1 Microscopy

Microscopic examination of the polished blocks was performed using a Leitz DMRXP MPV reflected light microscope under oil immersion (Plate 4.1). The fluorescence observations using ultraviolet excitation were carried out using a ploem type filter cube fitted with BP 340 – 380 excitation filters, RKP 400 dichromatic mirror, and, a LP 125 suppression filter. A window-based software package MPV GEOR was used to acquire data.

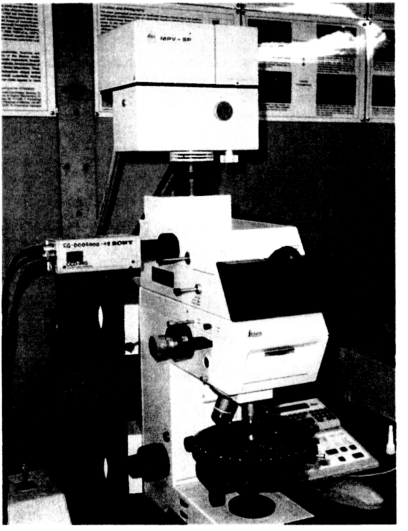

Plate 4.1: Leitz DMRXP photometry microscope

4.2.2 Sample Preparations

A total of thirty five samples were prepared for petrographic study. The samples were crushed to small fragments (about 2 mm) and placed in or mounting molds to about ¼ full. Next, the moulds were half-filled with resin mixed with hardener and stirred, and then more resin, mixed with hardener, was poured to fill up the mold. The molds were then left to harden. Once hardened, the blocks were removed from the molds and engraved with the sample name. The samples were then ground flat on a diamond lap and subsequently polished on a water-lubricated paper coated with different grades of carborundum powders. Finally, the samples were polished to a highly reflecting surface using progressively finer alumina powder (5/20, 3/50 and Gamma.

3.2.3 Maceral Identification

A maceral is an elementary microscopic constituent of coal that can be recognized by its shape, morphology, reflectance and fluorescence (Stopes, 1935). The terminology used to describe organic matter is based on the petrological examination of coals using the Stopes-Heerlen system of nomenclature (Stopes, 1935; Stach et al., 1982), as modified in the Australian Standard for coal-maceral Analysis, 1986 (AS 2856). There are three basic groups of macerals, the vitrinite group derived from coalfield woody tissue; the Liptinitic group derived from resinous and waxy parts of plants; and the inertinite group derived from charred and biochemical altered plant cell wall material. In this study, most attention is given to macerals that considered having a role in oil generation from coal, such as liptinitic macerals and micrinite.

4.2.4 Vitrinite Reflectance

Vitrinite Reflectance (VR) is the most commonly used organic maturation indicator used in petroleum exploration. This is because it is reasonably accurate, quick, non-destructive and inexpensive. Furthermore, vitrinite is the most common constituent of most coals and is also present in shales.

Vitrinite reflectance is a comparative measure of light intensity reflected from a standard of known reflectance, such as sapphire (of mean Ro of 0.589%) to that of the sample. An average of 25 readings per sample was taken, mostly on tellinite and telocollinite.

As coal rank increases, and the chemical composition of the vitrinite correspondingly changes, the vitrinite macerals become increasingly reflective. Therefore, the percentage reflection of a beam of normal incident white light from the surface of polished vitrinite is a function of the rank (maturity) of the maceral. Hunt (1995) has assigned Ro values to the limits of oil generation, the lowest value associated with known generation of conventional oil is about 0.5%, and 0.6% is generally recognised as the minimum reflectance for subsequent commercial accumulations. The peak of oil generation is at a Ro level of around 0.8 to 1%.

However, in this study, the beginning of oil generation is thought to be much lower at Ro of 0.4%, and the peak of oil generation is in range %Ro 0.5 – 0.7. This is based on the maceral composition, reactivity, and proportions.

4.3 Organic Geochemistry

The principal requirement in biomarker analysis is the identification and quantification of individual biomarker compounds or families of compounds. The analytical procedures for biomarker characterisation follow the sequence of sample extraction, extract fractionation, and finally saturate and/or aromatic fraction analysis by GC-MS (Figure 4.1).

4.3.1 Sample Extraction

A known amount of crushed sample (generally 15g) is placed in a pre-weighed, pre-extracted thimble capped with pre-extracted cotton wool and extracted in a Soxhlet apparatus with 200 ml of an azeotropic mixture of dichloromethane and methanol (93:7) (Figure 4.2). Anti-bumping granules and copper are added into the round-bottom flask to prevent rigorous boiling and remove sulfur, respectively.

In Soxhlet extraction, solvent is vaporised from a flask, which passes upward through the side arm of the apparatus and subsequently condenses and drips down into the thimble containing the sample. The hot solvent extracts soluble organic matter (including biomarkers) from the sample and is periodically recycled into the reservoir flask via a siphon. Any cycles of this process are required to ensure exhaustive extraction. The extraction is typically completed after 72 hours when the solvent surrounding the thimble becomes clear. The solvent in the reservoir flask is then reduced using a Butchi rotary evaporator (Plate 4.2).

The organic extract recovered is divided into two aliquots. One of the aliquots is blown dry and weighed; the total amount of extract is then calculated. The undried fraction is used for further analysis such as fractionation.

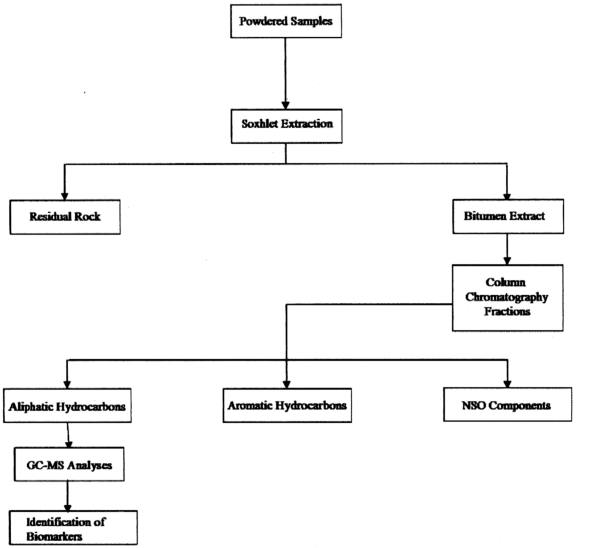

Figure 4.1: Scheme for Extraction and analysis geochemical fossils.

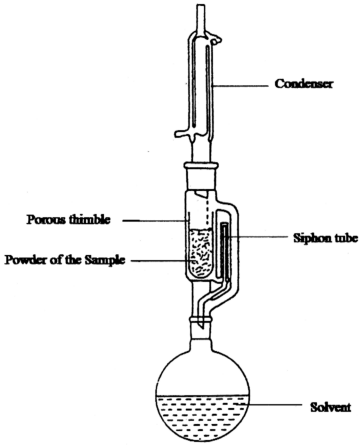

Figure 4.2: Soxhlet continuous extractor for the extraction of powder of the samples

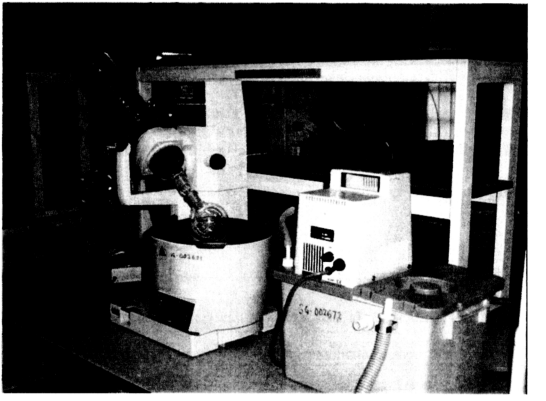

Plate 4.2: Buchi evaporation

4.3.2 Sample Fractionation

Extracts obtained require fractionation in order to separate the extract into fractions according to chemical functionality (aliphatic, aromatic, polar or NSO). Column chromatography and thin layer chromatography were used. These involve chromatographic separation, which is governed by the principal of partitioning of compounds between a stationary phase, typically composed of silica gel or alumina, and a mobile phase (solvent), resulting in different compounds eluting at different rates and time. Overall, the fractionation methods permit separation of whole rock extracts into less complex and distinct fractions containing compounds of specific polarity, unsaturation and structure; these are generally saturate, aromatic, and NSO fractions.

4.3.2.1 Column Chromatography

The extracted organic matter was separated into aliphatic, aromatic and NSO (nitrogen, sulphur and oxygen) fractions by column chromatography.

The fractionation was preformed using a column (30 cm x 0.72 cm i.d.) packed with approximately 20 cm of silica gel (BDH 60-120 mesh), supporting a 2-3 cm layer of alumina. The silica was slurry-packed using light petroleum (Bp 40-60° C), whilst the alumina was gravity-packed. Careful tapping of the column, while packing, avoided air bubbles from being trapped in the packed column.

A known quantity (approximately 30-100 mg) of extract was adsorbed onto alumina and dried using a stream of nitrogen. The adsorbed extractable organic matter was then added to the top of the column.

The column was then developed with solvents of increasing polarity, i.e. light petroleum (100 ml), dichloromethane (100 ml) and methanol (50 ml), respectively.

The eluates were collected in separate 250 ml round-bottom flask. The solvents were later reduced by Buchi evaporation.

4.3.2.2 Thin Layer Chromatography (TLC)

The TLC plates were prepared using glass plates (20cm x 20cm) that were detergent cleaned, rinsed with distilled water and oven dried. Slurry was prepared with 50 to 60 g of silica (Merk Kiesel gel G nach stahl type 60) in approximately 90ml of distilled water. It was noticed that the best slurry was obtained by just getting the silica wet and then adding a small volume of water resulting in slurry that is not too fluid but still easy to spread.

The glass plates are placed on a plate spreader; the slurry is quickly spread across the plates with a 1mm coating and allowed to set. The plates are dried and activated in an oven at 110 °C for several hours. Approximately 0.05g of extract diluted in 1ml of hexane is spotted in a straight line approximately 2cm above the plate bottom with a fine point pipette.

The plate is then developed with light petroleum. It is important to avoid overshooting the top of the plate. Therefore, once the solvent front is 1cm from the top of the plate it is removed from the tank. The developed plate is left to dry before it is viewed under the light. The band containing the aromatics is noted and the plate is subdivided into three bands, from top to bottom these are the aliphatic, aromatic and polar or NSO bands.

The three bands are scraped off and, respectively, diluted in 20ml of light petroleum, DCM and methanol. The fractions are then recovered by filtering through short columns of 1cm silica and 0.5cm alumina. Each fraction is divided into two parts, one is blown to dryness under a stream of nitrogen and weighed and the total weight of the fraction is calculated. The other fraction is used for GC-MS analysis.

4.3.4 Sample Analysis

The identification of organic compounds can be achieved using a variety of analytical methods. However, biomarker analyses are best met by the techniques of gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS)

4.3.4.1 Gas Chromatography-Mass Spectrometry (GS-MS)

A typical GC-MS system used for biomarker analysis consists of a capillary Gas Chromatograph connected to a mass spectrometer and a computer to receive the data (Figure 4.3). The mixture to be analyzed is injected into the Gas Chromatograph, where the various compounds are separated according to the speed at which they move through the gas-chromatographic column.

Separation of the saturated hydrocarbon is primarily according to molecular weight and volatility, although molecular shape may also play a role. The separated compounds leave the gas chromatograph in sequence and enter the mass spectrometer, where they are analyzed in the same sequence.

Each compound entering the mass spectrometer is bombarded in the ion chamber with a high-energy electron beam that ionizes the molecules by knocking off one electron. The molecular ions formed in this manner are unstable and most break apart to give a variety of smaller fragment ions. The molecular and fragment ions produced in this manner differ in mass, but all bear a +1 charge. Because of the difference in their mass/charge (m/z) ratios, they can be separated by a magnetic field or a quadrupole. The separated ions move to the detector where the relative abundance of each mass is recorded.

The mass spectrometer is repeatedly scanned over the chosen mass range (50-550) at a time interval (scan time) that ideally is shorter than the elution width of a single

component peak. The complete record of quantities and masses of all ions produced from a compound is called its "mass spectrum". The various classes of biomarkers all fragment in characteristic ways in the mass spectrometer, depending upon their molecular structures.

In this study the GC-MS system used for the analysis of the aliphatic fractions of all the samples was a Shimadzu 17 A coupled with a QO-5000 mass spectrometer.

The separation was performed on a 30m x 0.25mm x 0.25um fused silica column. The GC temperature was programmed from 60 °C to 300 °C at 6 °C/min. The final temperature is held for 15 min. Hydrogen was used as a carrier gas.

4.3.5 Biomarker Identification

Identification of biomarkers was performed by comparison of TIC retention time data and mass spectra with published data (Philp, 1985; Peters and Moldowan, 1993 and references therein). Relative peak intensities in gas chromatograms and mass fragmentograms were determined from their heights.

The reconstructed ion chromatograph (RIC) or (TIC) typically resembles the GC trace for the same sample. Most steranes molecules yield a large amount of the m/z 217 fragment ion, whereas triterpanes generally yield large quantities of the m/z 191 fragment ion.

However, steranes were excluded in the current study due to low concentration of these compounds.

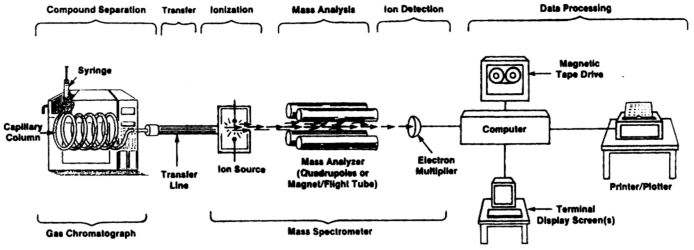

Figure 4.3 A typical Gas Chromatograph/Mass Spectrometer performed six functions (from left to right): (1) Compound separation by gas chromatography, (2) transfer of separation compounds to the ionizing chamber of the mass spectrometer, (3) ionization and acceleration of the compounds down the flight tube, (4) mass analysis of the ions, (5) detection of the focused ions by the electron multiplier, and (6)