CHAPTER TWO

INSTRUMENTATION

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2. Instrumentation

2.1. Introduction

Gas chromatography - specifically gas-liquid chromatography - involves a sample being vaporized and injected onto the head of the chromatographic column. The sample is transported through the column by the flow of inert, gaseous mobile phase. The column itself contains a liquid stationary phase, which is adsorbed onto the surface of an inert solid.

Have a look at this schematic diagram of a gas chromatograph:

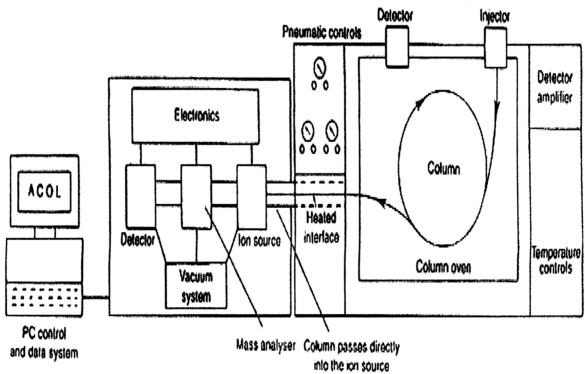

Figure 1: GC-MS combination

2.2. Instrumental components

2.2.1. Carrier gas

The carrier gas must be chemically inert. Commonly used gases include nitrogen, helium, argon, and carbon dioxide.

The choice of carrier gas is often dependant upon the type of detector, which is used. The carrier gas system also contains a molecular sieve to remove water and other impurities.

2.2.2. Sample injection port

For optimum column efficiency, the sample should not be too large, and should be introduced onto the column as a "plug" of vapour - slow injection of large samples causes band broadening and loss of resolution. The most common injection method is where a microsyringe is used to inject sample through a rubber septum into a flash vapouriser port at the head of the column.

The temperature of the sample port is usually about 50°C higher than the boiling point of the least volatile component of the sample.

For packed columns, sample size ranges from tenths of a microliter up to 20 microliters. Capillary columns, on the other hand, need much less sample, typically around 10^{-3} mL.

For capillary GC, split/splitless injection is used. Have a look at this diagram of a split/splitless injector;

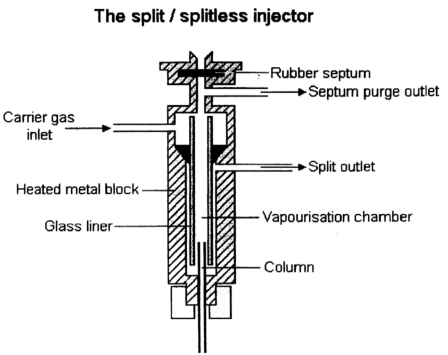

Figure 2: Split/ splitless injector

The injector can be used in one of two modes; split or splitless. The injector contains a heated chamber containing a glass liner into which the sample is injected through the septum.

The carrier gas enters the chamber and can leave by three routes (when the injector is in split mode). The sample vapourises to form a mixture of carrier gas, vapourised solvent and vapourised solutes.

A proportion of this mixture passes onto the column, but most exits through the split outlet. The septum purge outlet prevents septum bleed components from entering the column.

2.2.3. Columns

There are two general types of column, *packed* and *capillary* (also known as *open tubular*). Packed columns contain a finely divided, inert, solid support material (commonly based on *diatomaceous earth*) coated with liquid stationary phase. Most packed columns are 1.5 - 10m in length and have an internal diameter of 2 - 4mm.

Capillary columns have an internal diameter of a few tenths of a millimeter. They can be one of two types; *wall-coated open tubular* (WCOT) or *support-coated open tubular* (SCOT). Wall-coated columns consist of a capillary tube whose walls are coated with liquid stationary phase. In support-coated columns, the inner wall of the capillary is lined with a thin layer of support material such as diatomaceous earth, onto which the stationary phase has been adsorbed. SCOT columns are generally less efficient than WCOT columns. Both types of capillary column are more efficient than packed columns.

In 1979, a new type of WCOT column was devised - the *Fused Silica Open Tubular* (FSOT) column;

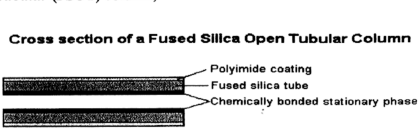

Figure 3:Cross section of Fused Silica Open Tubular Column

These have much thinner walls than the glass capillary columns, and are given strength by the polyimide coating.

These columns are flexible and can be wound into coils. They have the advantages of physical strength, flexibility and low reactivity.

2.2.4. Column temperature

For precise work, column temperature must be controlled to within tenths of a degree. The optimum column temperature is dependant upon the boiling point of the sample. As a rule of thumb, a temperature slightly above the average boiling point of the sample results in an elution time of 2 - 30 minutes. Minimal temperatures give good resolution, but increase elution times.

If a sample has a wide boiling range, then temperature programming can be useful.

The column temperature is increased (either continuously or in steps) as separation proceeds.

2.2.5. Detectors

There are many detectors, which can be used in gas chromatography. Different detectors will give different types of selectivity. A *non-selective* detector responds to all compounds except the carrier gas, a *selective detector* responds to a range of compounds with a common physical or chemical property and a *specific detector* responds to a single chemical compound. Detectors can also be grouped into *concentration dependant detectors* and *mass flow dependant detectors*.

The signal from a concentration dependant detector is related to the concentration of solute in the detector, and does not usually destroy the sample Dilution of with make-up gas will lower the detectors response. Mass flow dependant detectors usually destroy the sample, and the signal is related to the rate at which solute molecules enter the detector.

The response of a mass flow dependant detector is unaffected by make-up gas. Have a look at this tabular summary of common GC detectors:

Detector	Type	Support gases	Selectivity	Detectability
Flame ionization (FID)	Mass flow	Hydrogen and air	Most organic compounds.	100 pg
Thermal conductivity (TCD)	Concentration	Reference	Universal	1 ng
Electron capture (ECD)	Concentration	Make-up	Halides, nitrates, nitriles etc.	50 ng
Mass flow		Hydrogen and air	Nitrogen, phosphorus	10 pg

Table 1: Summary of common GC detectors

In this study we will use the Flame Ionization Detector and Mass Selective detector. Their description is as below.

2.2.5.1. Flame Ionization Detector

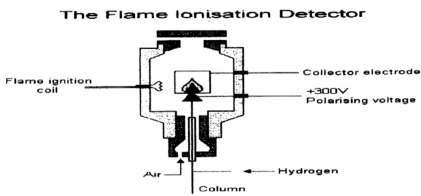

Figure 4: Flame ionization Detector

The effluent from the column is mixed with hydrogen and air, and ignited. Organic compounds burning in the flame produce ions and electrons, which can conduct electricity through the flame.

A large electrical potential is applied at the burner tip, and a collector electrode is located above the flame.

The current resulting from the pyrolysis of any organic compounds is measured. FIDs are mass sensitive rather than concentration sensitive; this gives the advantage that changes in mobile phase flow rate do not affect the detector's response.

The FID is a useful general detector for the analysis of organic compounds; it has high sensitivity, a large linear response range, and low noise. It is also robust and easy to use, but unfortunately, it destroys the sample.

2.2.5.2. Mass Selective Detector

The introduction in recent years of low cost bench-top mass spectrometers, which can readily be combined with high-resolution gas chromatography, justifies the inclusion of the mass spectrometer as a detector.

Mass spectrometry is based upon the ionization of solute molecules in the ion source and the separation of the ions generated on the basis of their **mass/charge ratio** by an analyzer unit.

This may be a magnetic sector analyzer, a quadruple mass filter, or an ion trap. Ions are detected by a dynode electron multiplier.

The detector is maintained under vacuum. Compounds are bombarded with electrons (EI) or gas molecules (CI).

Compounds fragment into characteristic charged ions or fragments. The resulting ions are focused and accelerated into a mass filter. The mass filter selectively allows all ions of a specific mass to pass through to the electron multiplier.

All of the ions of the specific mass are detected. The mass filter then allows the next mass to pass through while excluding all others. The mass filter scans stepwise through the designated range of masses several times per second. The total number of ions is counted for each scan.

The abundance or number of ions per scan is plotted versus time to obtain the chromatogram (called the total ion chromatogram, TIC). A mass spectrum is obtained for each scan, which plots the various ion masses versus their abundance or number.

Operating as a simple detector, in ***acquiring mode***, the mass spectrometer scans the total mass range, typically **30 - 600 atomic mass units (amu), every few seconds**; sums all the ions detected and then produce a trace on the control system PC screen.

This is called a **total ion chromatogram** and is analogous to the trace we might obtain from any other detector.

In ***selected ion monitoring mode*** **(SIM),** during the acquisition, the appearance of a specific compound can be traced by selecting an ion, which is characteristic of that compound; either the molecular ion, or the characteristic ion of a group of compounds for example. **[21]**

Sensitivity: 1-10 ng (full scan)

Linear range: 10^5-10^6

Gases: None

Temperature: 250- 300^{0}C

2.2.6. Current Study description

The parameters used in this experiment are as follows:

2.2.6.1.GC system

GC System Name – Shimadzu GC – 17AA

GC Type – Shimadzu GC – 17A Ver.2

2.2.6.2.Detector

Flame Ionization Detector

Mass Selective Detector

2.2.6.3.Temperature

Column Oven – Begin at 150^{0}C (holding for 5 minute) then elevating to 200^{0}C at a rate of 5^{0}C per minute then elevating to 250^{0}C at a rate of 10^{0}C per minute the final temperature was held for 22minutes.

Injector Port– 250^{0}C

Detector – 250^{0}C

2.2.6.4.Column

BP-5 (5% phenyldimethylsiloxane) non-polar fused silica capillary column)

Length – 30m

Internal diameter – 0.32mm

Pressure – 48 kPa

Flow – 1.0ml/min

2.2.6.5.Carrier gas

Oxygen Free Nitrogen Gas (OFN)

Pressure: N_2 – 75kPA, H_2 – 60kPa, compressed air - 60kPA

Control mode – split (split ratio - 1: 50)

2.2.7. Preparation of standards

Individual analytical standards of PE's were purchased from Fluka Chemika. The purities of all standards were stated to be > 98%. They were prepared in hexane at a concentration of 100 000 ppm in 5ml stock solution. Further dilution of the stock solution to various concentrations was carried out for the calibration studies.

NOTE: Due to some unforeseen reasons the standards available were of DOP, DEHP and DIBP

	Molecular weight / $gmol^{-1}$	Density / gml^{-1}	Volume to prepare 5ml of 100 000 ppm standard (ml)
1	DEHP	0.985	0.51
2	DIBP	1.039	0.48
3	DOP	0.980	0.51

Table 2: Preparation of standard solution

The appropriate standards were prepared using the serial dilution method.

2.3. Sampling

2.3.1. Location

4 sampling stations were chosen in the Klang Valley. They were Jambatan Kota, Bridge at LDP, river next to Guinness and Bridge Next to Citibank.

2.3.2. Sampling Technique

Each water sample was collected in a clean dark glass bottle. All sampling glass bottles used were thoroughly rinsed with dilute nitric acid, washed with soap water and finally with distilled water to avoid contamination. The bottles were rinsed with the respective river water before being filled up until the neck.

The time of collection, the color and the flow pattern of the river, the weather and the pH of the water sample were recorded for further justification of the results.

2.4. Water sample extraction

The liquid-liquid extraction technique was applied in this study to separate out the PE's in the river water. 2.5L of water sample was collected from each station.

The water sample was separated into two sections; one spiked with a known amount of standard PE's and another one is not spiked. 500ml water sample was extracted with 3 X 50ml dichloromethane in a 1-liter separator funnel and the mixture was then shaken vigorously for about 2 minutes.

The organic layer was allowed to drain through a funnel containing anhydrous sodium sulfate to adsorb any water moisture before being drained into a clean round bottom flask. The filter paper and anhydrous sodium sulfate were washed with additional dichloromethane to ensure qualitative transfer. The extract was then evaporated to dryness by using a rotary evaporator. 3ml of petroleum ether was then used to transfer the extract into a sample vial before further clean – up.

2.5. Extract clean – up method

Extracts were then subjected to clean – up procedure by passage through a column containing deactivated florisil. This procedure is important in trace analysis because it helps to reduce background levels caused by other compounds, so that a satisfactory chromatogram can be obtained. This will therefore simplify the task of identification and qualification.

PE's were eluted from the column with two different eluents, first with 10ml of petroleum ether and then with 50ml of 20% diethyl ether in petroleum ether. Three fractions were collected where the first fraction is from 0 –20ml, second fraction from 20 – 40ml and the third fraction 40 – 60ml. It was discovered that the second fraction contained PE's.

This fraction was then evaporated to dryness and then transferred quantitatively to a sample vial with hexane. This fraction was then injected into the GC for analysis of the PE's content.

2.6. Recovery study

The percentage of recovery study would indicate the efficiency of the method employed. For the determination of the recovery rate, a known amount of the PE was spiked in a portion of the sample and mixed well. The percentage of recovery can be determined from the concentration values of compound in spiked and not spiked sample using the following equation.

% Of recovery = (A – B) / C X 100%

A = amount of compound in spiked sample

B = amount of compound in un-spiked sample

C = amount of standard compound spiked in