

Chemistry Department

**SUMMER TRAINING**  
**In Research Institute at KFUPM**

**Done by**  
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## **Introduction:**

Last summer, summer 2007, I was a trainee in Research Institute at KFUPM. In addition, my supervisor Dr. Bassam Al-twabini taught me how to use and analyze unknown chemicals. In particular, soils that CAL received by companies and Saudi Custom. However, in this report I will concern and cover the instruments that allowed me to work on and care about the samples, which were analyzed with the help of technician lab, Lewis.

### **I. Soxhlet Extraction:**

A Soxhlet extractor is a piece of laboratory apparatus invented in 1879 by Franz von Soxhlet. It was originally designed for the extraction of a lipid from a solid material. However, a Soxhlet extractor is not limited to the extraction of lipids. Typically, a Soxhlet extraction is only required where the desired compound has only a limited solubility in a solvent, and the impurity is insoluble in that solvent. If the desired compound has a high solubility in a solvent then a simple filtration can be used to separate the compound from the insoluble substance.

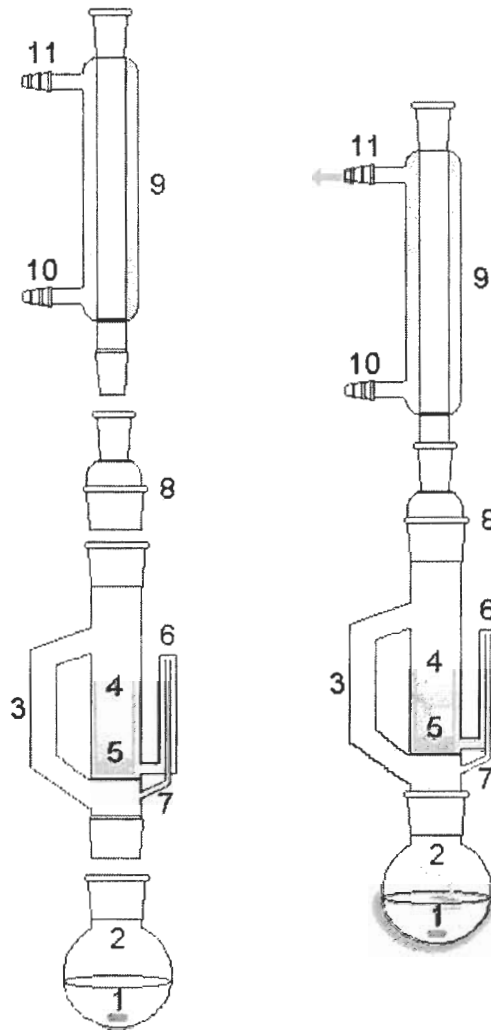
Normally a solid material containing some of the desired compound is placed inside a thimble made from thick filter paper, which is loaded into the main chamber of the Soxhlet extractor. The Soxhlet extractor is placed onto a flask containing the extraction solvent. The Soxhlet is then equipped with a condenser.

The solvent is heated to reflux. The solvent vapour travels up a distillation arm, and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapour cools, and drips back down into the chamber housing the solid material.

The chamber containing the solid material slowly fills with warm solvent. Some of the desired compound will then dissolve in the warm solvent. When the Soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. This cycle may be allowed to repeat many times, over hours or days.

During each cycle, a portion of the non-volatile compound dissolves in the solvent. After many cycles the desired compound is concentrated in the distillation flask. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled.

After extraction the solvent is removed, typically by means of a rotary evaporator, yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble, and is usually discarded.



- 1: Stirrer bar/anti-bumping granules
- 2: Still pot (extraction pot)
- 3: Distillation path
- 4: Soxhlet Thimble
- 5: Extraction solid (residue solid)
- 6: Syphon arm inlet
- 7: Syphon arm outlet
- 8: Expansion adapter
- 9: Condenser
- 10: Cooling water in
- 11: Cooling water out

## II. Experimental (Example of what I did)

Fourteen soil samples marked as SS4-01 to 06 (six samples) and SS2-02 to 09 (eight samples) were analyzed for its PAH contents. The method was modified EPA Methods 3540C (soxhlet extraction). About 10 grams of sample was soxhlet extracted

with 150ml methylene dichloride (MDC) for 6 hours. The MDC extract was concentrated to about 2ml and solvent exchange with hexane by adding 10ml hexane and further concentrated to 1ml by blowing nitrogen. This 1 ml extract is passed through column for the separation of aliphatics from aromatics fraction.

The column for separation was prepared containing 4.5gm silica with 1.5gm alumina on the top. Anhydrous sodium sulfate of about 1cm high was placed above the alumina layer. The column was prewashed by eluting 20ml hexane. When hexane was about 1- 2mm above sodium sulfate layer, the 1ml extract was placed on top and eluted with 15ml of hexane. The 15ml hexane elute was set aside (this contain the aliphatic fraction). Before column gets dry, 40ml of hexane-methylene dicloride mixture (7:3) was passed through. The 40ml elute about 10ml hexane was added for solvent exchange. The final volume was made up to 1ml. this is ready for GCMS-SIM analysis. One (1) microliter of the final concentrate was injected to the GCMS instrument.

## II. Instruments:

In this section a general scheme of some devices were discussed.

### Infrared Spectroscopy

Infrared spectroscopy (IR spectroscopy, see Fig 2) is the subset of spectroscopy that deals with the infrared region of the electromagnetic spectrum. It covers a range of techniques, the most common being a form of absorption spectroscopy. As with all spectroscopic techniques, it can be used to identify compounds or investigate sample composition. Infrared spectroscopy correlation tables are tabulated in the literature.

#### Theory

The infrared portion of the electromagnetic spectrum is divided into three regions; the near-, mid- and far- infrared, named for their relation to the visible spectrum. The far-infrared, approximately  $400\text{-}10\text{ cm}^{-1}$  ( $1000\text{-}30\text{ }\mu\text{m}$ ), lying adjacent to the microwave region, has low energy and may be used for rotational spectroscopy. The mid-infrared, approximately  $4000\text{-}400\text{ cm}^{-1}$  ( $30\text{-}1.4\text{ }\mu\text{m}$ ) may be used to study the fundamental vibrations and associated rotational-vibrational structure. The higher energy near-IR, approximately  $14000\text{-}4000\text{ cm}^{-1}$  ( $1.4\text{-}0.8\text{ }\mu\text{m}$ ) can excite overtone or harmonic vibrations. The names and classifications of these subregions are merely conventions. They are neither strict divisions nor based on exact molecular or electromagnetic properties.

Infrared spectroscopy exploits the fact that molecules have specific frequencies at which they rotate or vibrate corresponding to discrete energy levels. These resonant frequencies are determined by the shape of the molecular potential energy surfaces, the masses of the atoms and, by the associated vibronic coupling. In order for a vibrational mode in a molecule to be IR active, it must be associated with changes in the permanent dipole. In particular, in the Born-Oppenheimer and harmonic approximations, i.e. when the molecular Hamiltonian corresponding to the electronic ground state can be approximated by a harmonic oscillator in the neighborhood of the equilibrium molecular geometry, the resonant frequencies are determined by the normal modes corresponding to the molecular electronic ground state potential energy

surface. Nevertheless, the resonant frequencies can be in a first approach related to the strength of the bond, and the mass of the atoms at either end of it. Thus, the frequency of the vibrations can be associated with a particular bond type.

Simple diatomic molecules have only one bond, which may stretch. More complex molecules have many bonds, and vibrations can be conjugated, leading to infrared absorptions at characteristic frequencies that may be related to chemical groups. For example, the atoms in a CH<sub>2</sub> group, commonly found in organic compounds can vibrate in six different ways: symmetrical and antisymmetrical stretching, scissoring, rocking, wagging and twisting.

The infrared spectra of a sample is collected by passing a beam of infrared light through the sample. Examination of the transmitted light reveals how much energy was absorbed at each wavelength. This can be done with a monochromatic beam, which changes in wavelength over time, or by using a Fourier transform instrument to measure all wavelengths at once. From this, a transmittance or absorbance spectrum can be produced, showing at which IR wavelengths the sample absorbs. Analysis of these absorption characteristics reveals details about the molecular structure of the sample.

This technique works almost exclusively on samples with covalent bonds. Simple spectra are obtained from samples with few IR active bonds and high levels of purity. More complex molecular structures lead to more absorption bands and more complex spectra. The technique has been used for the characterization of very complex mixtures.

### **Sample preparation**

Gaseous samples require little preparation beyond purification, but a sample cell with a long pathlength (typically 5-10 cm) is normally needed, as gases show relatively weak absorbances.

Liquid samples can be sandwiched between two plates of a high purity salt (commonly sodium chloride, or common salt, although a number of other salts such as potassium bromide or calcium fluoride are also used). The plates are transparent to the infrared light and will not introduce any lines onto the spectra. Some salt plates are highly soluble in water, so the sample and washing reagents must be anhydrous (without water).

It is important to note that spectra obtained from different sample preparation methods will look slightly different from each other due to differences in the samples' physical states.

### **Typical method**

A beam of infrared light is produced and split into two separate beams. One is passed through the sample, the other passed through a reference which is often the substance the sample is dissolved in. The beams are both reflected back towards a detector, however first they pass through a splitter which quickly alternates which of the two

beams enters the detector. The two signals are then compared and a printout is obtained.

A reference is used for two reasons:

-This prevents fluctuations in the output of the source affecting the data

-This allows the effects of the solvent to be cancelled out (the reference is usually a pure form of the solvent the sample is in)

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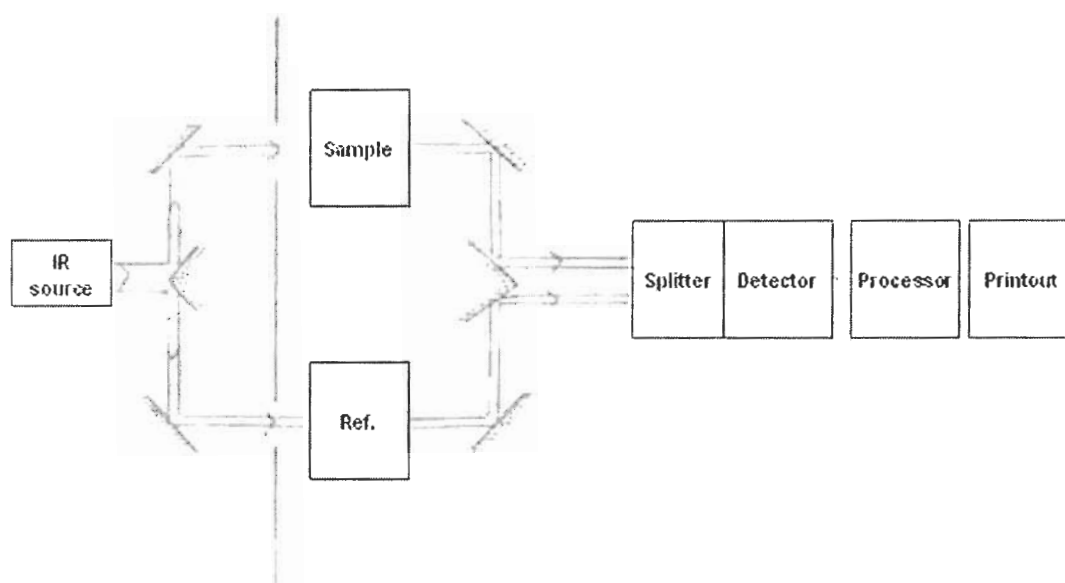


Fig. 2 IR Device Scheme.

### GC analysis:

A gas chromatograph is a chemical analysis instrument for separating chemicals in a complex sample. A gas chromatograph uses a flow-through narrow tube known as the *column*, through which different chemical constituents of a sample pass in a gas stream (carrier gas, *mobile phase*) at different rates depending on their various chemical and physical properties and their interaction with a specific column filling, called the *stationary phase*. As the chemicals exit the end of the column, they are detected and identified electronically. The function of the stationary phase in the column is to separate different components, causing each one to exit the column at a different time (*retention time*). Other parameters that can be used to alter the order or time of retention are the carrier gas flow rate, and the temperature.

In a GC analysis, a known volume of gaseous or liquid analyte is injected into the "entrance" (head) of the column, usually using a microsyringe (or, solid phase

microextraction fibers, or a gas source switching system). As the carrier gas sweeps the analyte molecules through the column, this motion is inhibited by the adsorption of the analyte molecules either onto the column walls or onto packing materials in the column. The rate at which the molecules progress along the column depends on the strength of adsorption, which in turn depends on the type of molecule and on the stationary phase materials. Since each type of molecule has a different rate of progression, the various components of the analyte mixture are separated as they progress along the column and reach the end of the column at different times (retention time). A detector is used to monitor the outlet stream from the column; thus, the time at which each component reaches the outlet and the amount of that component can be determined. Generally, substances are identified (qualitatively) by the order in which they emerge (elute) from the column and by the retention time of the analyte in the column.

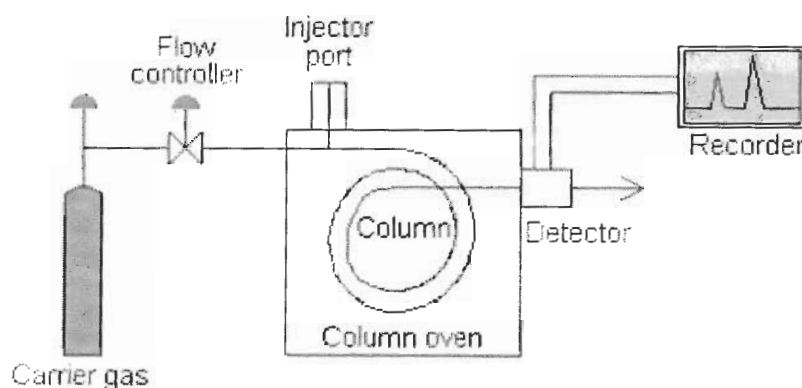


Fig 3. Gas chromatography scheme.

### Instrumental components

#### Carrier gas

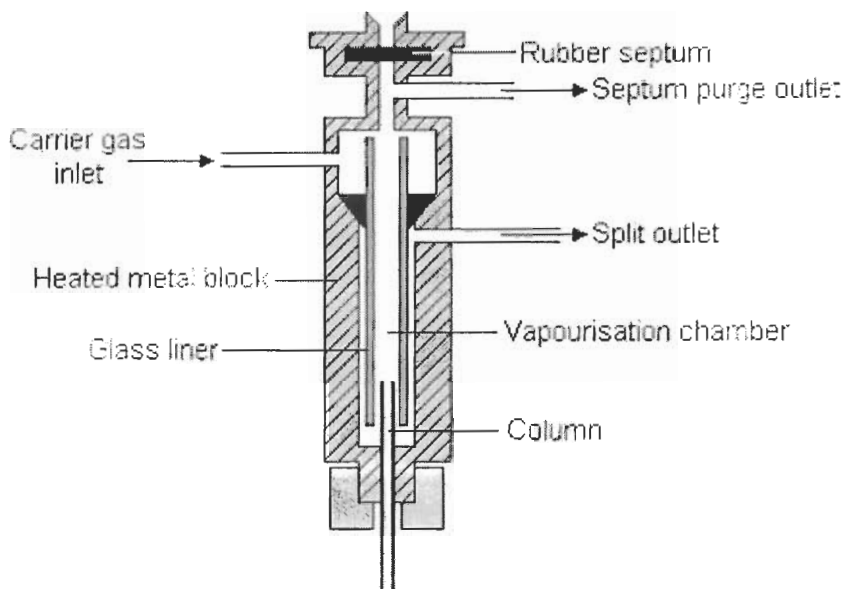
The carrier gas must be chemically inert. Commonly used gases include nitrogen, helium, argon, and carbon dioxide. The choice of carrier gas is often dependant upon the type of detector which is used. The carrier gas system also contains a molecular sieve to remove water and other impurities.

#### 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  $10^{-3}$   $\mu\text{L}$ . For capillary GC, split/splitless injection is used. Have a look at this diagram of a split/splitless injector;

### The 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.

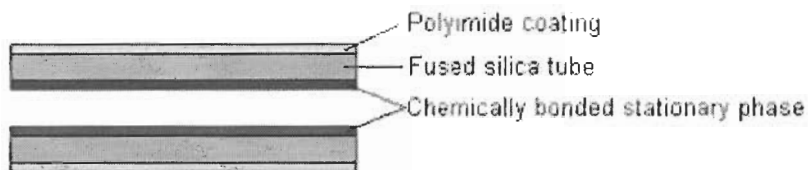
### Columns

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;

### Cross section of a 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.

### 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.

## 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	Dynamic range
Flame ionization (FID)	Mass flow	Hydrogen and air	Most organic cpds.	100 pg	10 <sup>7</sup>
Thermal conductivity (TCD)	Concentration	Reference	Universal	1 ng	10 <sup>7</sup>
Electron capture (ECD)	Concentration	Make-up	Halides, nitrates, nitriles, peroxides, anhydrides, organometallics	50 fg	10 <sup>5</sup>
Nitrogen-phosphorus	Mass flow	Hydrogen and air	Nitrogen, phosphorus	10 pg	10 <sup>6</sup>
Flame photometric (FPD)	Mass flow	Hydrogen and air possibly oxygen	Sulphur, phosphorus, tin, boron, arsenic, germanium, selenium, chromium	100 pg	10 <sup>3</sup>
Photo-ionization (PID)	Concentration	Make-up	Aliphatics, aromatics, ketones, esters, aldehydes, amines, heterocyclics, organosulphurs, some organometallics	2 pg	10 <sup>7</sup>
Hall electrolytic conductivity	Mass flow	Hydrogen, oxygen	Halide, nitrogen, nitrosamine, sulphur		

## The Flame Ionisation Detector

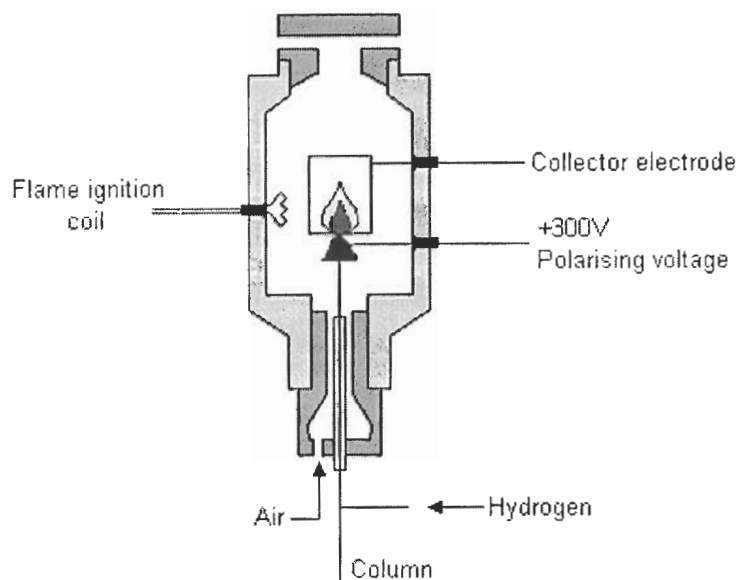


Fig 4 Flame Ionization Detector

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.

### Conclusion:

It was really grate summer. I knew new techniques and used devices that I just read about them in chemistry books. I concluded working with company tatally different from academic life. Moreover, we should take a precaution whenever we are inside laboratory "safety first".