# CHAPTER II

# THEORY

## 2.1 Types of Chromatography

Chromatography is divided into two main types such are liquid chromatography (LC) and gas chromatography (GC). The former means a case that mobile phase is liquid and the latter means a case that mobile phase is gas. Classification of chromatographic techniques is shown in Figure 2.1.

Liquid-liquid chromatography (LLC) is partition chromatography. The sample is retained by partitioning between the mobile liquid and the stationary liquid. The requirement here is that the mobile liquid should not be a solvent for the stationary liquid. In fact, water is often used as the stationary liquid and organic solvents are used for the mobile liquid. Paper chromatography (PC) is a subgroup of LLC. In paper chromatography, the stationary phase is actually water held on the fibers of cellulose.

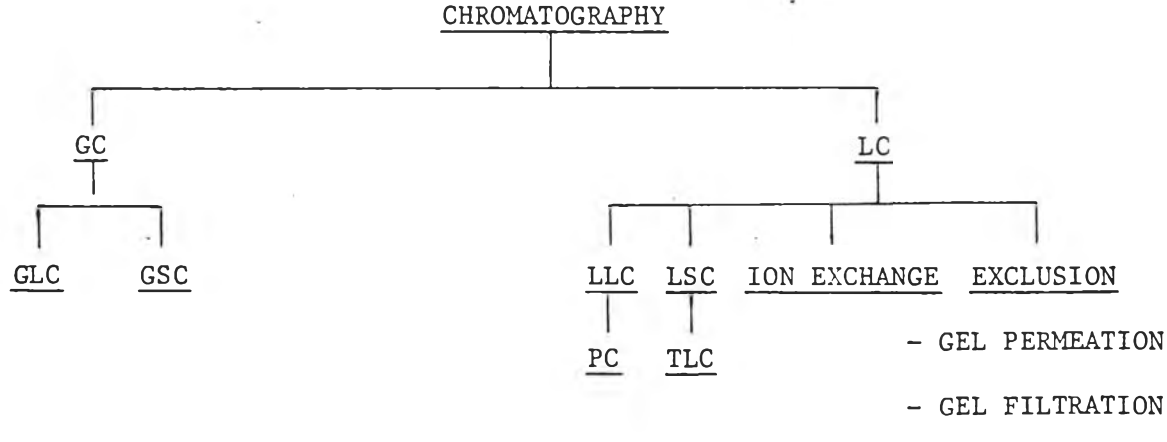



Figure 2.1 Types of chromatography

Liquid-solid chromatography (LSC) is adsorption chromatography. The adsorbents, silica gel, alumina, molecular sieve or porous glass, are packed in a column and the sample components displaced by a mobile phase. Thin-layer chromatography is considered to be LSC, the adsorbent is spread in a thin film on a glass plate instead of having the adsorbent in the column.

Ion-exchange chromatography uses zeolites and synthetic organic and inorganic resins to perform chromatographic separations by an exchange of ions between the sample and the resin.

Exclusion chromatography is another form of liquid chromatography. In this process, a uniform, highly porous, nonionic gel is used to separate materials according to their molecular size. The small molecules can enter into the polymer network and will be retarded, whereas the large molecules cannot enter the polymer network and will be swept out of the column. Exclusion chromatography can perform in two ways such are gel permeation and gel filtration chromatography. For separations performed on polymers which swell in organic solvents the technique is called gel permeation. For separations performed on polymers which swell in water this is called gel filtration.

Gas-liquid chromatography (GLC) is partition chromatography similar to LLC but it differs in that the mobile phase is gas instead of liquid. Therefore, the difficulty about solubility of the stationary phase into the mobile phase is not seen. Mobile phase, carrier gas, for GC is inert gas such as nitrogen, helium and argon. The requirement here is that the sample otherwise its derivatives must be volatile.

Gas-solid chromatography (GSC) is adsorption chromatography. The stationary phase is adsorbent similar to LSC but the mobile phase is gas instead of liquid. The requirement is also the volatile sample likely in GLC.

These chromatographic techniques can be concluded in another two types such are column chromatography and non-column chromatography. Except paper chromatography and thin-layer chromatography, all of them are column chromatography which stationary phase is in a column, i.e., the separation occurs in the column, too. Most of this thesis concerns GLC.

## 2.2 Gas Chromatographic System

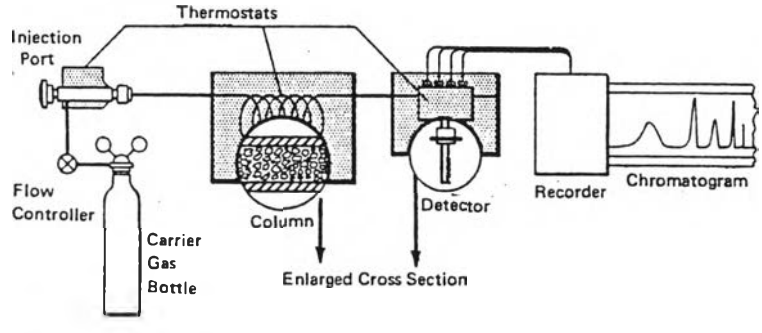



Figure 2.2 Schematic drawing of a gas chromatographic system.

The basic parts of gas chromatograph are:

1. Cylinder of carrier gas
2. Flow controller and pressure regulator
3. Injection port
4. Column
5. Detector (with necessary electronics)
6. Recorder
7. Thermostats for injector, column, and detector

### 2.2.1 Carrier Gas

Common carrier gas is hydrogen, helium, nitrogen and carbon-dioxide, among these gases hydrogen can easily explode. In analysis,

the flow rate of carrier gas must be constant. To obtain this, a pressure regulator and a flow controller are used to control the pressure and speed of carrier gas.

### 2.2.2 Injection Port

Injection port is the inlet of sample to the column, by using a micro-syringe to inject an amount of the sample through a septum which protects the sample to leak back off the column. The temperature of injection port must be high enough to evaporize the sample immediately and should be higher than the temperature of column. For the gaseous sample it is injected on to the column by using a gas-tight syringe or a by-pass sample loop.

### 2.2.3 Column

Generally, the stainless-steel columns are most popular because of their strength and inertness to common substances. For sensitive or corrosive compounds such as pesticides, phenols, steroids and amines, glass columns are necessary. For sulfur gases the teflon columns are required. The length of 3-10 feet is generally used. A long column will provide many numbers of plates and high resolution. However, the too-long column is not good because the flow rate of the carrier gas may be varied along the column and the inlet pressure must be high. The latter causes the difficulty of injecting and leak of the sample through the septum. Another advantage of long column is that more sample can be used.

The standard diameters of columns are 1/8 and 1/4 inch outer diameter. For capillary columns which provide a large number of theoretical plates the diameter of 1/16 inch outer diameter is favored. For separation as preparative scale the column diameter of 3/8, 1/2

inch outer diameter or more is used. However, the large diameter of column causes diffusion and multi-path effect that results in reducing the column efficiency.

Within the column it is the packing material which is adsorbent for gas-solid chromatography and is a liquid phase coating on the solid support for gas-liquid chromatography.

2.2.3.1 Solid Support

There are two factors in choosing a material as a support. One is structure, the other is surface characteristics. The structure means the efficiency of the material as a support and the surface characteristics mean the degree to which it enters into the separation. A good solid support should have these factors as details as the followings:

1. a large specific surface area which is from 1 to 20 $m^2/g$

2. a pore structure with uniform pore diameter in the range of 10 μm or less

3. inertness that is a minimum of chemical and adsorptive interaction with the sample

4. regularity-shape particles, uniform in size for efficient packing

5. mechanical strength, should not crush on handling.

2.2.3.2 Stationary Phase

Stationary or liquid phase is the heart of gas-liquid chromatography. It is the separator of the sample compounds from the carrier gas. The separation depends on the partition coefficient of that compound. The properties of liquid phase are

the followings:

1. non-volatile at the operating temperature
2. Thermally stable, not decomposed at the operating condition
3. inert to sample
4. able to be a good solvent for samples with different distribution coefficient.

For GLC the liquid phase is divided into 3 types based on the polarity. These are polar, intermediately polar and non-polar liquid phases. Choosing the liquid phase depends on what the components of the sample are by using the principle "Like dissolves like".

2.2.4 Detectors

The detector indicates the presence and measures the amount of components in the column effluent. The temperature of detector and the connection between the column and the detector must be high enough to prevent the condensation of the sample and liquid phase in the detector.

Requirements for a good detector are the followings:

1. high sensitivity
2. low noise level
3. wide linearity of response
4. response to all types of compounds
5. insensitive to flow and temperature change
6. inexpensive.

There is no detector which has all of these properties. The universal detectors are thermal conductivity and flame ionization detectors. The specific detectors such as electron capture, flame

photometric and thermionic specific detectors have the advantage in that they selectively detect only certain types of compounds. This makes them extremely useful for trace and qualitative analysis.

## 2.3 Chromatographic Theory

Chromatographic separations can be evaluated by the shape of the peaks and depend upon the isotherms that describe the relationship between the solute concentration in the stationary phase to the solute concentration in the carrier gas. The isotherm is a graphical representation of the partition coefficient or distribution constant, K, at a given temperature

$$K = \frac{C_s}{C_g} \qquad 2.1$$

where $C_s$ is the solute concentration in the stationary phase or the solid surface and $C_g$ is the solute concentration in the gas phase. The measurements are done at the equilibrium of concentrations. Three types of isotherms are obtainable: linear, convex and concave isotherms.

The linear isotherm is obtained when the partition coefficient or distribution constant, K, is a constant overall working concentration ranges. Therefore, the bands or the peaks are symmetrical. The convex isotherm demonstrates that the component moves through the column at a faster rate thus it causes the front boundary to be self-sharpening and the rear boundary to be diffuse. In other words, it causes the peak tailing. The concave isotherm results from the opposite effect. In this case the solute is retained in the column for a longer time and the peak leading is obtained.

These effects are depicted in Figure 2.4. Changing the sample concentration or physical condition (temperature, flowrate, pressure,

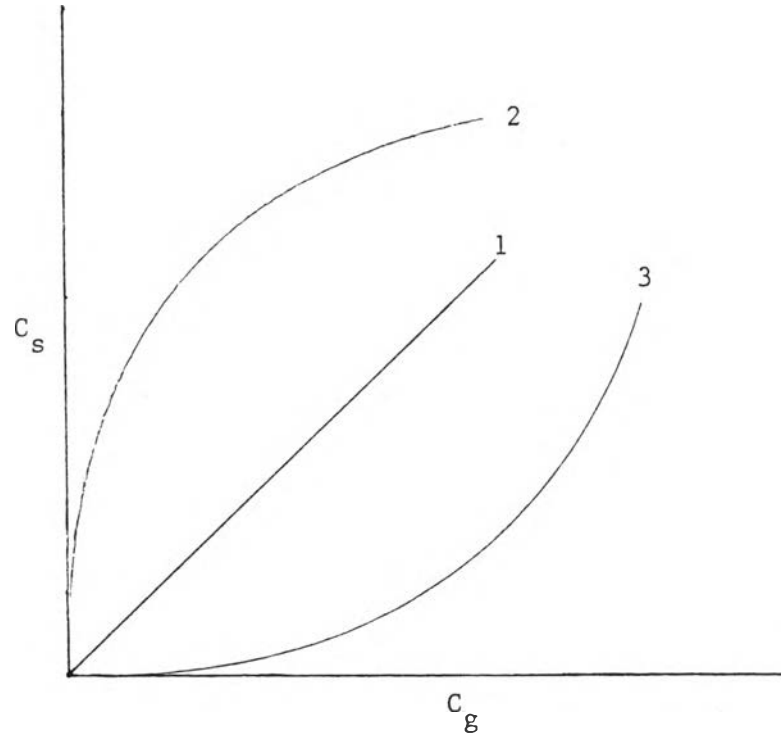



Figure 2.3 Isotherms (1) linear isotherm, (2) convex isotherm and (3) concave isotherm.

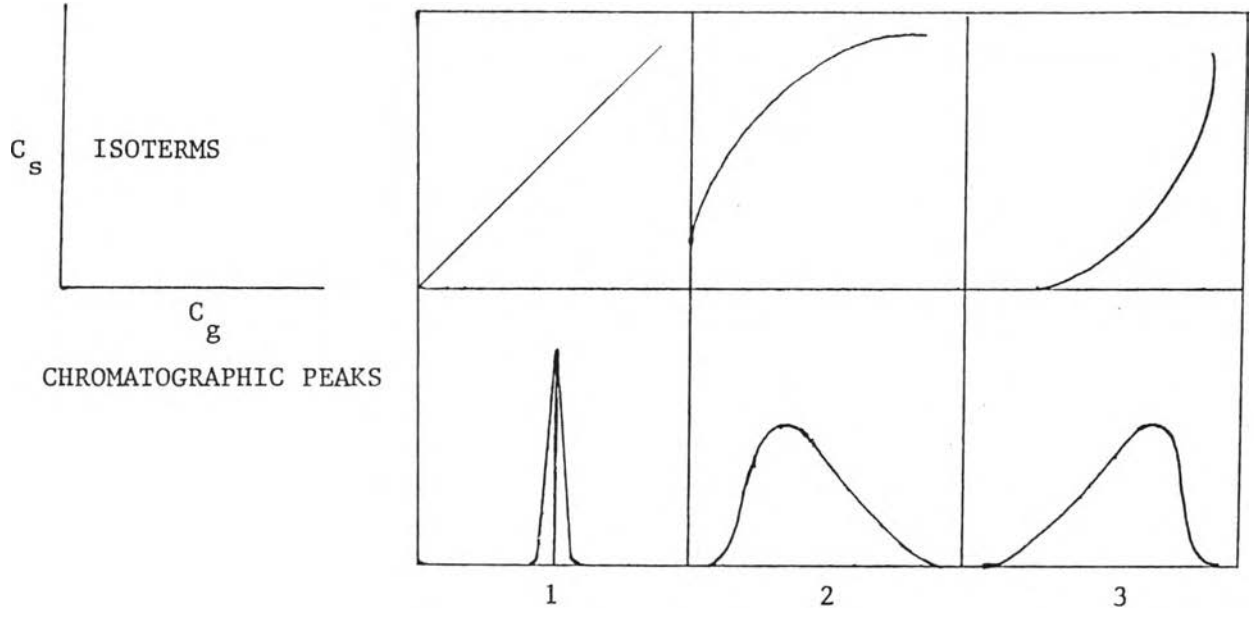



Figure 2.4 The dependence of peak shape on the form of the partition isotherm; (1) symmetrical, (2) tailing, and (3) leading peaks.

etc.) can help in converting the rear and front boundaries to symmetrical shape.

In order to explain chromatographic theory on the basis of a continuous model three assumptions are made:

1. equilibrium between solute concentrations in the two phases is reached instantaneously.

2. diffusion of solute in mobile phase, along the column axis is negligible.

3. column is packed uniformly.

There are two factors such are column efficiency and solvent efficiency which have roles on the resolution of chromatographic peaks.

### 2.3.1 Column Effiency

Column efficiency concerns the peak broadening of an initially compact band as it passes through the column. The broadening results from the column design and operating conditions. Column efficiency can be described by two theories such are plate theory and rate theory.

#### 2.3.1.1 Plate Theory

Martin and Synge (32) first applied the plate theory to partition chromatography. The theory assumes that the column is divided into a number of zones called theoretical plates. One determines the zone thickness or height equivalent to a theoretical plate (HETP) by assuming that there is perfect equilibrium between the gas and liquid phases within each plate. The resulting behavior of the plate column is calculated on the assumption that the distribution isotherm is linear and the diffusion of the solute in the mobile phase from one plate to another is neglected. Plates

are useful to compare similar column, or set standards for packing techniques. Theoretical plates can be easily measured from the chromatogram. Tangents are drawn to the peak at the points of inflection (about 2/3 of the height). The number of theoretical plates, N, is given by

$$N = 16\left[\frac{t_r}{W}\right]^2 \qquad 2.2$$

where $t_R$ is the retention time, the distance from injection to peak maximum (including the dead volumn), and W is the base width of the peak, the length of the baseline cut by the two tangents. The number of plates are sometimes measured at the bandwidth at half-height, $W_{0.5}$. From statistics

$$W = \left[\frac{2}{\ln 2}\right]^{\frac{1}{2}} W_{0.5} \qquad 2.3$$

and substituting Equation 2.3 in Equation 2.2, one obtains

$$N = 5.55\left[\frac{t_R}{W_{0.5}}\right]^2 \qquad 2.4$$

Having calculated the number of theoretical plates and knowing the length of the column, L, usually in centimeters, one may determine the height equivalent to a theoretical plate, HETP, by

$$HETP = \frac{L}{N} \qquad 2.5$$

HETP calculation allows comparisons between columns of different lengths and is the preferred measure of column efficiency. Plate theory describes that HETP becomes smaller with decreasing flow rate; however, experimental evidence shows that a plot of HETP versus flowrate always goes through a minimum. For a given column

of constant length, the HETP represents the peak broadening as a function of retention time. In a gas chromatographic column, each component will yield different N and HETP values. Those solutes with high retention (high K values) will result in greater numbers of theoretical plates and thus lower HETP values. It is generally found that a necessary number of the theoretical plates for packed gas chromatographic columns are 10 times greater than in distillation for a similar separation.

2.3.1.2 Rate Theory

Since plate theory does not explain other factors, a more sophisticated approach, the rate theory, is applied. Rate theory is based on such parameters as rate of mass transfer between stationary and mobile phases, diffusion rate of solute along the column, carrier gas flowrate and the hydrodynamics of the mobile phase. The general equation of the rate theory developed by van Deemter (1) is

$$HETP = A + \frac{B}{\mu} + C\mu \qquad 2.6$$

where A is a constant which concerns multipath effect of eddy diffusion, B is a constant which concerns molecular diffusion, C is a constant which concerns resistance to mass transfer and $\mu$ is the linear gas velocity or flow rate through the chromatographic column. A representation of Equation 2.6 is in Figure 2.6 which shows the effect of change in linear gas velocity on HETP. The optimum flowrate, $\mu_{opt}$, is the value which gives the lowest HETP, i.e., the most column efficiency. Equation 2.6 is that of a hyperbola having a minimum at velocity $\mu_{opt} = (B/C)^{\frac{1}{2}}$ and a minimum HETP ($h_{min}$) at $A + 2\,(BC)^{\frac{1}{2}}$. The constants may be graphically calculated

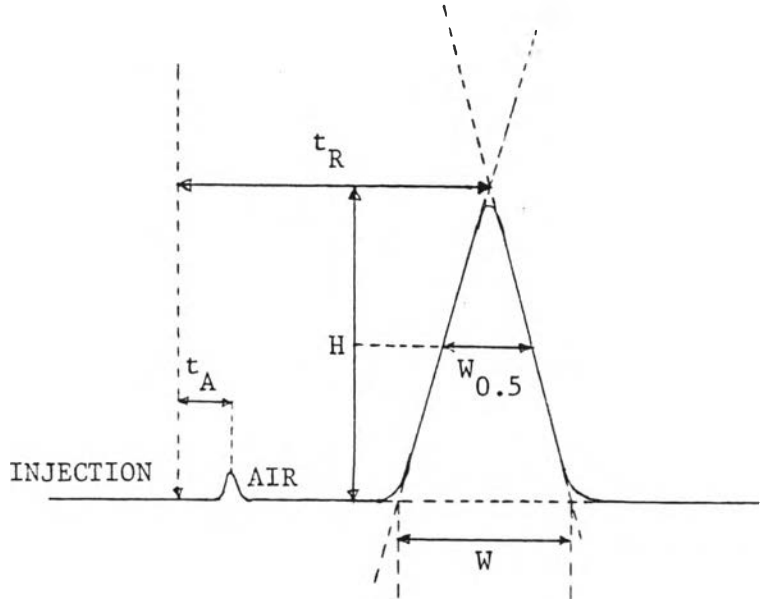



Figure 2.5 Calculation of theoretical plates

$t_R$ is the retention time of solute,

$t_A$ is the retention time of air (dead volume).

W is the base width of the peak,

H is the height of the peak and

$W_{0.5}$ is the peak width at half-height.

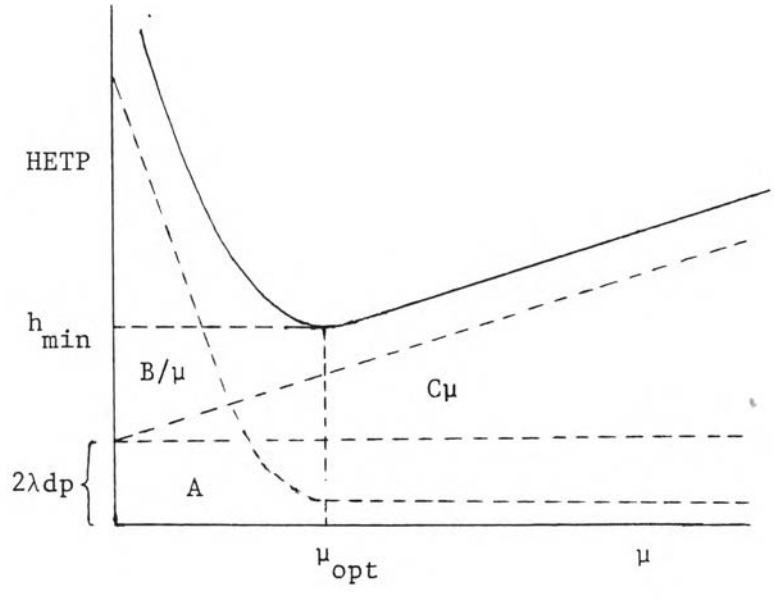



Figure 2.6 Van Deemter plot. Change in HETP versus linear gas velocity, μ. $h_{min} = A + (2\ BC)^{\frac{1}{2}}$. $\mu_{opt} = (BC)^{\frac{1}{2}}$

from an experimental plot of HETP versus linear gas velocity as shown in Figure 2.6. The van Deemter equation is extended as Equation 2.7.

$$HETP = 2\lambda dp + \frac{2\gamma Dgas}{\mu} + \frac{8}{\P^2} \cdot \left[\frac{k'}{(1+k')^2}\right] \cdot \left[\frac{d_f^2}{D_{liq}}\right] \cdot \mu \qquad 2.7$$

where $\lambda$ = a constant which is a measure of packing irregularities.

$dp$ = average particle diameter of the solid support.

$\gamma$ = a correction factor accounting for the tortuosity of the gas channels in the column.

$D_{gas}$ = diffusivity of the solute in the gas phase.

$\mu$ = the linear gas velocity

$k'$ = capacity factor = $K\left[\frac{F_{liq}}{F_{gas}}\right]$

$K$ = partition (distribution) coefficient of the solute, expressed as the amount of solute per unit volume of liquid phase divided by the amount of solute per u unit volume of gas phase.

$F_{liq}$ = fraction of cross section occupied by the liquid phase.

$F_{gas}$ = fraction of cross section occupied by the gas phase.

$d_f$ = effective thickness of the liquid film which is co coated on the particles of the support.

$D_{liq}$ = diffusivity of the solute in the liquid phase.

With the particle size used in analytical gas chromatographic columns, 60/80 mesh (0.25-0.17 mm) it is very difficult to have all the particles the same diameter and some of these particles might fit into void spaces between particles. In any packed column, solute molecules and carrier gas molecules travel

along many paths. These paths have different lengths, therefore the solute molecules have different residence time. These add to peak broadening. The broadening depends upon the size of particles constituting the packing, the shape, and the manner in which they are packed and the column diameter. The pressure drop across the column is another limiting factor on particle size. Small particles increase the pressure drop. To increase column efficiency by minimizing the A term one should use small particles of uniform size constant with low pressure drop, low $\lambda$, and small diameter columns.

The molecular diffusion, B term is proportional to $D_{gas}$, the solute diffusivity in the carrier gas. High solute diffusivity leads to band broadening and a consequence loss in the efficiency. Diffusivity is a property of both solute and carrier gas. The solute diffusivity in the liquid phase, $D_{liq}$, is extremely small compared to that in the gas phase and can be neglected. To reduce the B term one should increase the linear velocity and use a high molecular weight carrier gas.

Obviously keeping $d_f$, the thickness of the film, small will reduce the C term. This causes a reduction of capacity factor, $k'$ and an increase in the term $k'/(1+k')^2$. However, using thinly coated column packings increases the probability of adsorption of solute molecules on the surface of support material, which might result in peak tailing.

The $k'$ term is temperature dependent, so we increase $k'$ and decrease $k'/(1+k')^2$ by lowering temperature. Lowering of temperature increases viscosity and thus decreases $D_{liq}$. Therefore,the effects of the factors $k'/(1+k')^2$ and $1/D_{liq}$ counteract each other.

A liquid phase which exhibits high diffusivity, $D_{liq}$, tends to reduce this C term. For this reason liquid phase of low viscosity produce more efficient columns.

To minimize the C term, a thin uniform film of a low viscosity liquid should be used. The flow rate must be low enough and the distribution coefficient must be high enough to favor equilibrium between the liquid and gas phases.

### 2.3.2 Solvent Efficiency

Substances having the same vapor pressure can be easily separated by appropiate selection of the liquid phase. There are numerous selective liquid phases available and the following comments should be aid in choosing the proper one.

#### 2.3.2.1 Interaction forces and partition coefficient

There are four interaction forces which can aid in the gas chromatographic separation:

1. Orientation or Keesom forces: forces resulting from the interaction between two permanent dipoles. The hydrogen-bond is a particularly important type of orientation force encountered in gas chromatography.

2. Induced dipole, or Debye forces: forces resulting from the interaction between a permanent dipole in one molecule and the induced dipole in a neighboring molecule. These forces are usually very small.

3. Dispersion, London or non-polar forces: forces arising from synchronized variations in the instantaneous dipoles of the two interaction species. These forces are present in all cases, and are the only source of attraction energy between two non-polar substances. They are weak compared to the two former

forces.

4. Specific interaction forces: forces resulting from chemical bonding, complex formation between solute and solvents. These forces of interaction determine the solubility and thereby the separation achieved. Their combined effects are expressed by the partition coefficient, $K = \frac{C_s}{C_g}$ The value of K is high when most of a substance is retained in the liquid phase. This means that the substance moves slowly along the column because only a small fraction will be in the carrier gas at any given time. Transport is negligible in the liquid phase, and only that fraction in the gas phase is carried through the column.

Thus separation between two compounds is possible if their partition coefficients are dissimilar. The greater the difference in their K values is, the fewer the plates or the shorter the column length is required to achieve a separation.

2.3.2.2 Solvent efficiency and temperature

Solvent efficiency is measured by α, the relative retention. It is the ratio of adjusted retention times or partition coefficients as in Equation 2.8.

$$\alpha = \frac{t'_{R_2}}{t'_{R_1}} = \frac{K_2}{K_1} \qquad 2.8$$

where α is the relative retention time, $t'_R$ is adjusted retention time of each peak and K is partition coefficient of solute. The measuring of data is demonstrated in Figure 2.7. Relative retention differs from the separation factor, S.F., where S.F. is the ratio of unadjusted retention times as in Equation 2.9.

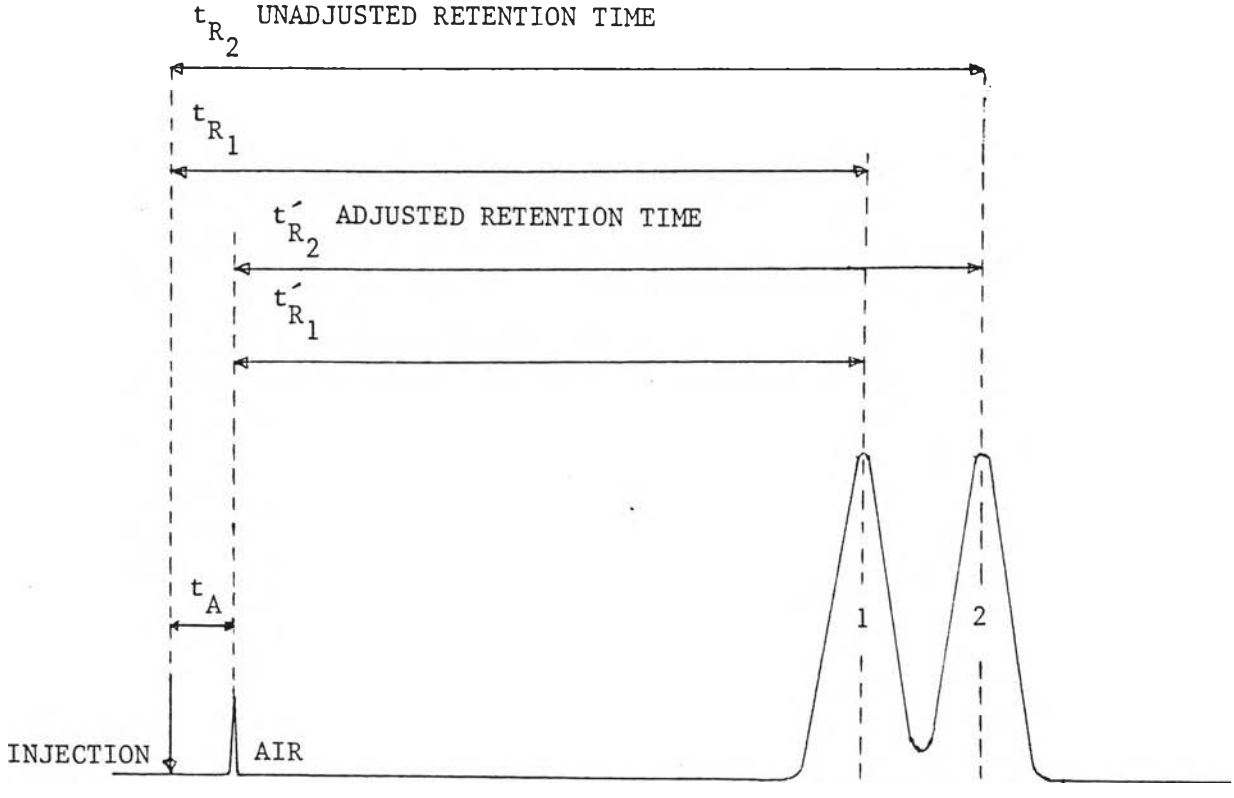



Figure 2.7 Characteristic data of the peaks for calculation of solvent efficiency.

$$S.F. = \frac{t_{R_2}}{t_{R_1}} \qquad 2.9$$

where $t_R$ is unadjusted retention time of each peaks. The measuring of these data is also shown in Figure 2.7. Both $\alpha$ and K are temperature dependent. However, over a limited temperature range $\alpha$ will be constant. The distribution coefficient, K, decreases with increasing temperature, i.e., the fraction of the solute in the gas phase will increase and hence the elution time will decrease. This results in decreasing separation. To improve separations, lower temperature should be used. Lower temperature means more liquid phase interaction, more separation, longer analysis time. As a minimum, the solute should spend 50% of the time in the liquid phase, so that the retention time exceeds twice the retention time of air.

2.3.2.3 Resolution

The true separation of two consecutive peaks is measured by the resolution, R. Resolution is a measure of both the column and solvent efficiencies. It accounts for both the narrowness of peaks and the separation between maxima. It is calculated from Equation 2.10.

$$R = \frac{2d}{W_1 + W_2} \qquad 2.10$$

where d is the difference of retention time and W is the peak width at the baseline. From statistics $W = (2/\ln 2)^{\frac{1}{2}} W_{0.5}$, so Equation 2.10 can be expressed as

$$R = \frac{1.18d}{(W_{0.5})_1 + (W_{0.5})_2} \qquad 2.11$$

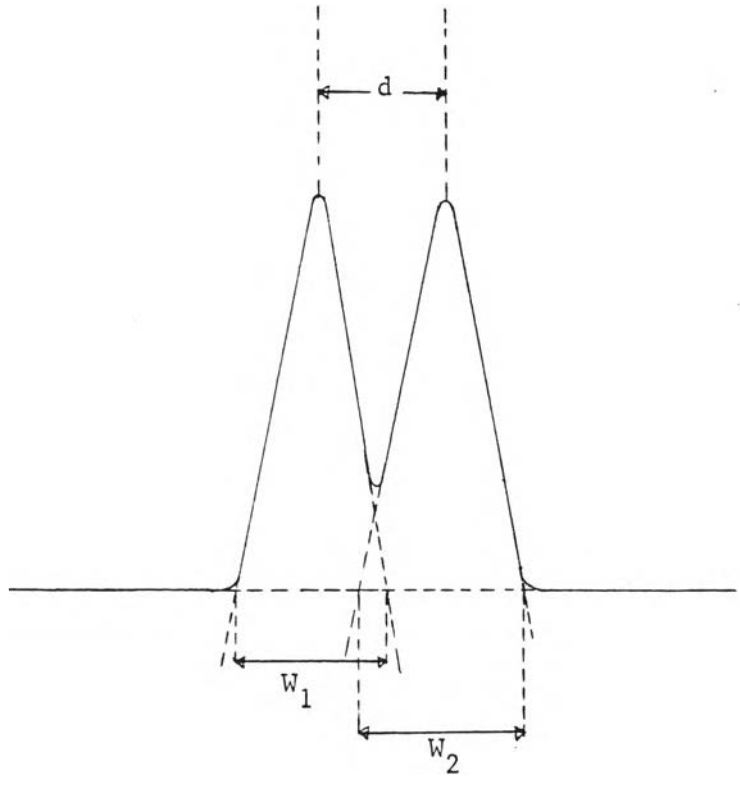



Figure 2.8 Characteristic data of the peaks for calculation of resolution.

if R = 1, the resolution of two equal-area peaks is approximately 98% complete,

if R = 1.5, baseline separation (99.7% resolution) is achieved.

2.3.2.4 Number of plates for required separation

An equation which is useful in determining the number of plates and the length of column required is

$$N_{req} = 16R^2\left|\frac{\alpha}{\alpha-1}\right|^2\left|\frac{k'_2+1}{k'_2}\right|^2 \qquad 2.12$$

where $N_{req}$ = the number of required plates

$R$ = the resolution required

$\alpha$ = the solvent efficiency = $\frac{t_{R_2}-1}{t_{R_1}-1}\frac{t'_{R_2}}{t_A}$

$k'$ = capacity factor for peak 2 = $\frac{t'_{R_2}}{t_A}$

## 2.4 Deactivation of the Support Surface

Usually, the support should not enter the separation. The significant feature to indicate that the support is not sufficient inert is the peak tail. Tailing increases proportionally as the ability of the compound to hydrogen bond increases. Except hydrocarbons, most classes of compounds exhibit some degree of tailing. Basic and acidic compounds also show little tail. The degree of tailing of compounds are tabulated in Table 2.1. Generally, for homologous series of compounds, the lowest member of them will tail the most severely.

An important consideration in tailing is that of sample size. As the sample size or concentration is reduced, the tailing becomes more severe. In practice one finds as the sample size is reduced, the retention time of the peak increases as shown in Figure 2.9. Note that the tailing edge of the peak is related to the adsorption isotherm of the support.

Table 2.1 Showing degree of tailing of compounds with non-inert support.

| Degree of Tailing | Class |
|---|---|
| Fair | Ketones, aldehydes, esters |
| Bad | Alcohols, acids, amines, phenols |
| Very bad | Glycol, polyamines |
| Terrible | Water |

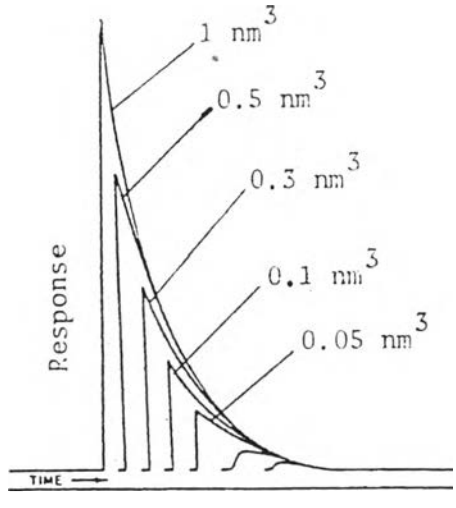



Figure 2.9 Effect of sample size on retention time in the case of adsorption on the solid support.

To reduce adsorption of the sample or tailing of the peak the active sites on the support surface must be deactivated. There are several methods to deactivate the support surface. Those methods are acid washing, base washing, silanization, saturation of the sites, and priming.

### 2.4.1 Acid Washing

It is believed that acid washing can deactivate some active sites of the support surface by removing mineral impurities from its surface. Generally, the support is washed with hydrochloric acid. It is found that the non-acid washed support may cause the decomposition of liquid phase and samples. After acid washing, this event disappears. Undoubtedly, acid washing is beneficial in certain cases, but possibly not in all cases.

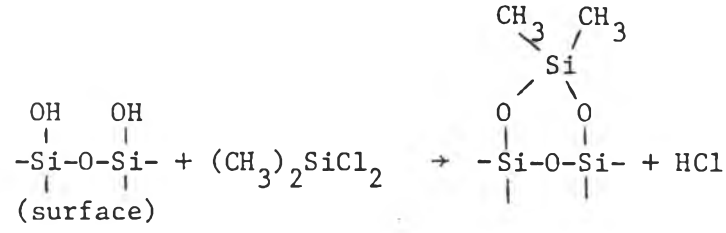
### 2.4.2 Base Washing

As prescribed above acid washing is not sufficient in some cases such as nitrogen compounds. The tail reducer used for nitrogen compounds must be alkaline. It has been the practice to wash the support with sodium or potassium hydroxide to neutralize the acid sites.

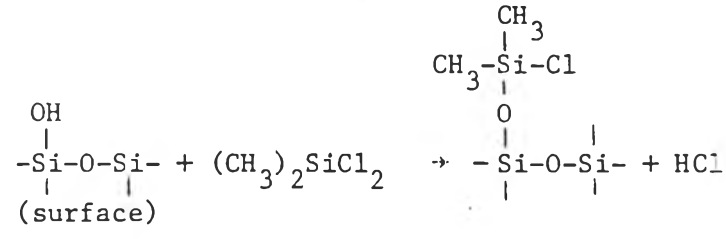
### 2.4.3 Silanization

In addition to acid and base sites, the support surface has the silanol group (Si-OH) which must be removed also. There are several compounds which produce silyl ethers which are inert. Such are dimethyl dichlorosilane (DMCS), hexamethyl disilizane (HMDS) or Siliclad and trimethyl chlorosilane (TMCS). The reaction of the silanization occurs like these:

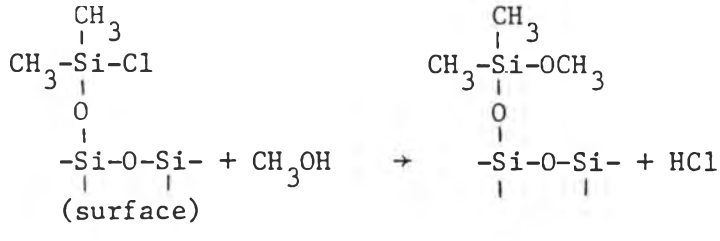
1. Dimethyl dichlorosilane (DMCS)



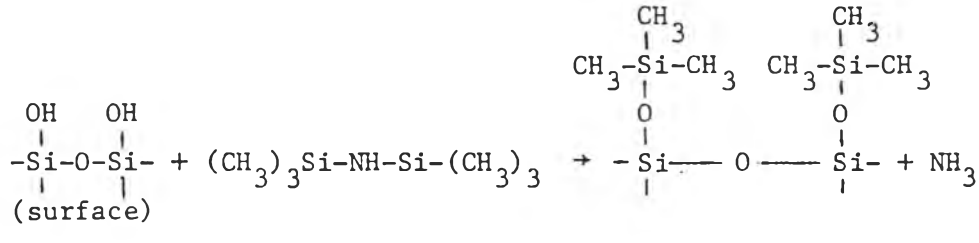
and/or



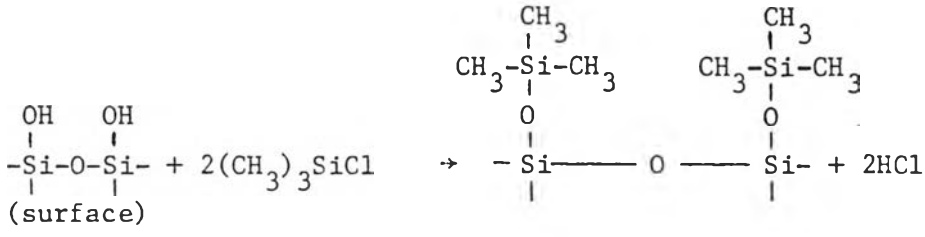
For the treatment to be successful it is necessary to thoroughly wash the support with methanol to convert the chlorosilyl ether in the latter reaction to a methoxy derivative:



2. Hexamethyldisilizane (HMDS)



3. Trimethylchlorosilane (TMCS)



Considering all above reactions it shows that DMCS can react both single silanol group or adjacent silanol group while another reagents cannot. This may be a reason why the silanization with DMCS provides the most inert support comparing to another silane reagents. However, the support treated with DMCS after acid washing is much more inert than those without acid washing before.

When working with non-silicone stationary phases, such as polyesters, polyglycols, polyamides, etc., one obtains a great deal of deactivation by the stationary phase, and it is less critical in terms of tailing to have a silanized support. However, columns made with silanized supports appear to last longer than those made with non-silanized supports. The surface silanol appears to react with the stationary phase causing them to deteriorate rapidly.

The silanized support is hydrophobic rather than hydrophilic as it is normal. A highly hydrophilic stationary phase such as glycerol or diglycerol will not readily cover a silanized surface. A high concentration of these phases will show poor efficiency on a silanized support because of the wetting problem. Therefore, the amount of stationary phase loading on the silanized support is usually 1-10% by weight.

### 2.4.4 Saturation of the Active Sites

Adsorption or tailing can be reduced by saturating the active sites with an active agent. The active agent which can be the

sample itself, the stationary phase, an additive such as KOH to the stationary phase or an agent such as steam ammonia or formic acid is introduced into the column with the carrier gas. The active agents except the sample are called tail reducers. The saturation with the sample itself is called priming. The procedure of deactivation is a temporary one, but one that may allow a worker to get some results if no alternative is available. Several large injections of the sample are made, and after the retention time or peak area has stabilized, the actual sample size is then introduced. If the column has not been used for a matter of hours, then used again for an analysis, severely tailing may be encountered. It is again necessary to saturate or prime the column with sample before it can be effectively used.

### 2.4.5 Coating the Support with a Solid

With this technique the diatomite becomes a carrier for a solid imparting to support its structure not its surface. The surface is shielded from the solute by the solid such as silver and teflon. The effectiveness of this technique will depend on the ability to obtain complete coverage. However, it was found that the silvered support had the disadvantage of reacting with sulfur compounds and amines (16).

## 2.5 Column Proparation

### 2.5.1 Support Coating

A few simple rules of the support coating must be observed:

1. The stationary phase must be coated uniformly over the surface of the support.

2. The support particles must not be damage

3. The stationary phase must not be oxidized, hydrolyzed, or evaporated during the preparation.

There are several methods using to coat the liquid phase on the surface of the support to obtain a good uniform coating. There are:

2.5.1.1 Rotating Evaporator Method

The correct amount of liquid phase dissolved in a suitable solvent is placed in a round bottom flask. The weighed amount of solid support is added. The flask is connected to the rotating evaporator. A water aspirator is used to reduce pressure in the flask. The flask is rotated until all of the solvent is evaporated. Use of a heat lamp helps evaporation. This method may cause the support crushing. So the soft support such as Chromosorp T, Teflon support, should not be coated by using this method.

2.5.1.2 Pan Coating Method

The weighed amount of liquid phase dissolved in the correct amount of solvent is added to the weighed solid support in a pan. The amount of solvent used is just enough to wet the solid support with no excess. The solvent is allowed to evaporate spontaneously or with judicious application of heat. The mixture is gently agitated during drying by shaking the pan. Do not stir, as this may crush the diatomite particles. The amount of solvent to use may be calculated from Table 2.2. The volume of solvent required is obtained by multiplying the grams of support by the Z factor.

2.5.1.3 Funnel Coating or Solution Coating Method

The amount of 20 g. of support is added to 100 $cm^3$ of the proper solution of the liquid phase in a filter flask.

Table 2.2 Amount of solvent to use with supports (1).

| Support | $cm^3$ of solvent/g of support Z | Example for 20 g use |
|---|---|---|
| Chromosorp P | 1.5 | 30 $cm^3$ |
| Chromosorp W | 2.0 | 40 $cm^3$ |
| Chromosorp G | 0.5 | 10 $cm^3$ |
| Chromosorp T | 1.0 | 20 $cm^3$ |
| Firebrick | 1.5 | 30 $cm^3$ |
| Fluoropack 80 | 0.8 | 16 $cm^3$ |

The pressure in the flask is reduced for a few minutes with a water aspirator. Then the pressure is released and the flask is let to stand for 15 minutes. Otherwise, the support is added slowly to the solution of liquid phase while the solution is stirred until all of support is in suspension. The slurry is poured into a sintered glass and let to drain freely until the support settles. Vacuum is applied for approximately 5 minutes, then the support is spread on filter paper to dry. After air drying, the coated support is dried in an oven at 80-100°C. Low temperature liquid phases should be dried at the room temperature. The coated support must not be resieved before use. The correct solution to obtain a given weight coating on the support may be calculated from Table 2.3 and the following equation:

$$\% \text{ solution} = (\% \text{ coating}) \times (\phi \text{ factor}) \qquad 2.13$$

where $\phi$ factor is the ratio of interparticle porosity by the total porosity of the support.

The solution coating technique provides a more uniform coating of the liquid phase on the support. It is suitable for the case of low concentration of liquid phase loading. The evaporating technique and the pan coating technique are more convenient for preparing high concentration packings of the viscous phase such as carbowax, SE-30 and OV-1, and for preparing packings with two or more stationary phases which are not soluble in a common solvent.

Table 2.3 Factors for calculating solution percentages (1).

| Support | $\phi$ factor for 1% | Example (for 10% liquid phase) solution by weight |
|---|---|---|
| Chromosorb P | 0.75 | 7.5% |
| Chromosorb W | 0.55 | 5.5% |
| Chromosorb G | 1.15 | 11.5% |
| Chromosorb T | 1.50 | 15.0% |
| Firebrick | 0.75 | 7.5% |
| Fluoropack 80 | 2.00 | 20.0% |

### 2.5.2 Packing the Column

Before packing the column, it is imperative that the inner surface of the column be properly cleaned and treated. For metal tubing, which contains residual oils and greases, must be cleaned by thorough washing with polar and nonpolar solvents. Glass columns may contain residual alkali materials and should be thoroughly cleaned with diluted acid. They should then be silanized since glass columns are usually used for analysis of sensitive compounds. The object in packing the column is to fill it as tightly as possible without fracturing or deforming the particles. Many techniques have been described but their effectiveness depends primarily on the skill and experience of the person filling the column. For metal columns 6 feet and shorter it is advisable to fill the column after it has been coiled in the same manner as a glass column (30). First, a large wad of glass wool is placed partially into the end of the column which will connect to the detector. The excess glass wool is bent back around the outside of the tubing and a piece of rubber tubing is connected to the column. This method assures that the glass wool will not be pulled out of the column during filling. The other end of the rubber tubing is then connected to a vacuum source such as an aspirator or pump. A funnel is then connected to the other end of the column and the packing added slowly while vacuum is applied at the other end. During the entire filling operation it is advisable to provide a slight agitation to the packing by vibrating or tapping the column at approximately the point at which the packing is settling. When the column is filled, a small piece of silane-treated glass wool or fritted disk is placed in the inlet end; the vaccuum source is then disconnected from the outlet end. The large glass wool plug is then removed from the outlet and replaced with a small piece of silane-treated glass wool or a fritted disk.

Long metal columns up to 20 feet are packed in straight sections and then carefully coiled with a minimum of flexing of the tubing. For still longer columns it is advisable to pack shorter lengths and to connect these with a Swagelok union which has been bored out to permit a butt to butt seal of the column ends.

Glass U-tube columns are usually filled by gravity without the need for vacuum. It is best to add packing to both arms simultaneously, or in small segments to each arm. Silane-treated glasswool is recommended in the inlet side.

### 2.5.3 Conditioning the Column

Most columns must be conditioned prior to connection to the detector in order to purge volatile components that would foul the detector and cause unsteady baselines. The conditioning procedure depends upon the column material used; excessive conditioning temperatures result in short column life. Normal carrier gas flow should be maintained during conditioning in most cases.

Columns prepared with polymeric stationary phases such as Carbowax, polyesters, or polyphenyl ethers, should be conditioned at a temperature at which the column will be used. While conditioning overnight will usually suffice, the level of column bleed will gradually decrease and will be indicated drifting baseline. Silicones require special conditioning. Technical grade material such as UC-W98, DC-200, SF-96, 2F-1 and JXR should be treated as any other polymeric phases, and conditioned overnight at slightly above the planned operating temperature. These materials are not recommended for high temperature use even though the literature contains many references to use at temperature up to 300$^{o}$C.

Chromatographic grade silicones such as OV-1, SE-30, and Silar-5CP required a special conditioning procedure. Columns should be heated slowly to slightly above the operating temperature for an hour or two hours. Then the column is ready for use. Conditioning such columns at 300°C if the columns are to be used at 230°C will only shorten the column life.

Columns should never be removed from the chromatograph while the packing material is still hot. The recommended procedure is to turn off oven and injector heaters, open the oven and allow to cool for approximately 30 minutes. The carrier gas is then turned off, the exit end is uncoupled and when no flow can be measured at the exit of the column, the inlet end is disconnected. The two ends of the column should then be sealed with a suitable cap to prevent contamination of the packing material.

## 2.6 Application of Gas Chromatography

Gas chromatography can be used for both qualitative and quantitative analysis. Qualitative identification is easily achieved by using the retention time of peak compared with the standard peak at the same condition. However, the identification of compounds by GC is generally used to confirm the results from other methods such as mass spectroscopy, infrared spectroscopy, UV-visible spectroscopy and NMR spectroscopy. Most of chromatographers prefer to use GC for the quantitation of components. However, the accuracy depends on several factors such are sample injection, sample adsorption or decomposition, performance of detector and recorder, integration techniques and calculation.

## 2.7 Other Techniques Used in This Research

### 2.7.1 Flame Emission Spectroscopy

Flame emission spectroscopy (33) is based on the principle that atoms in the ground state are excited by thermal energy of the flame to higher energy levels and those excited atoms then radiate energy as emission spectrum to return the ground state. With an appropiate filter or a monochromator the characteristic energy of emission spectrum is detected by using a photoelectric detector. The detector response, E is expressed by

$$E = k\alpha c \qquad 2.14$$

where k is a constant governed by the efficiency of atomization and of self absorption, $\alpha$ is the efficiency of the atomic excitation, and C is the concentration of the test solution.

The schematic diagram of the apparatus required for flame emission spectroscopy is shown in Figure 2.10. Combustion flame produces temperatures in excess of 2000K. In most cases, the flame is formed by burning the fuel gas in an oxidant gas which is usually air, nitrous oxide, or oxygen diluted with either nitrogen or argon.

The nebulizer-burner system is used to convert the test solution to gaseous atoms. The function of the nebulizer is to produce a mist or aerosol of the test solution. The solution to be nebulized is drawn up a capillary tube by the Venturi action of a jet of air blowing across the top of the capillary; a gas flow at high pressure is necessary in order to produce a fine aerosol. There are two main types of burner system such are the pre-mix or larminar-flow burner and the total consumption or turbulent-flow burner. In the pre-mix type of burner, the aerosol is produced in a vaporizing chamber where the larger

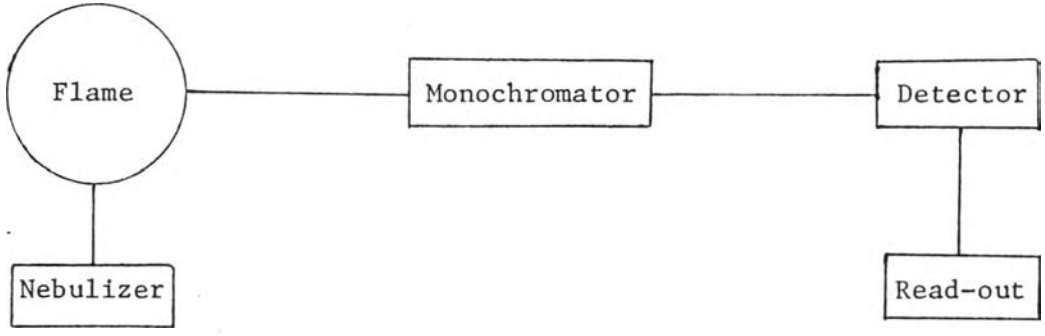



Figure 2.10 A schematic diagram of apparatus for flame emission spectroscopy.

droplets of liquid fall out from the gas stream and are discharged to waste. The resulting fine mist is mixed with the fuel gas and the carrier (oxidant) gas, and the mixed gases then flow to the burner head. A typical burner of this type is shown in Figure 2.11. Its advantages are a long light path, being quiet in action and with little danger of incrustation around the burner head since large droplets of solution have been eliminated from the stream of gas reaching the burner. Its disadvantage is that with solutions made up in mixed solvents, the more volatile solvents are evaporated preferentially.

The total consumption type of burner consists of three concentric tubes as shown in Figure 2.12. The sample solution is carried by a fine capillary tube directly to the flame. The fuel gas and the oxidant gas are carried along separate tubes so that they only mix at the tip of the burner. Since all liquid sample which is aspirated up the capillary tube reaches the flame, it would appear that this type of burner should be more efficient than the pre-mix type of burner. However, the total consumption burner gives a flame of relatively short path length, and hence such burners are predominantly used for flame emission studies. This type of burner has the advantages that it is simple to manufacture, it allows a totally representative sample to reach the flame, and it is free from explosion hazards arising from unburnt gas mixtures. Its disadvantages are that the aspiration rate varies with different solvents and there is a tendency for incrustations to form at the tip of the burner which can lead to variations in the signal recorded.

Flame emission spectroscopy is used for quantitative analysis preferable to qualitative analysis. The intensity of emission spectrum at the maximum wavelength which corresponds to the pattern or type of element is proportional directly to the concentration as in

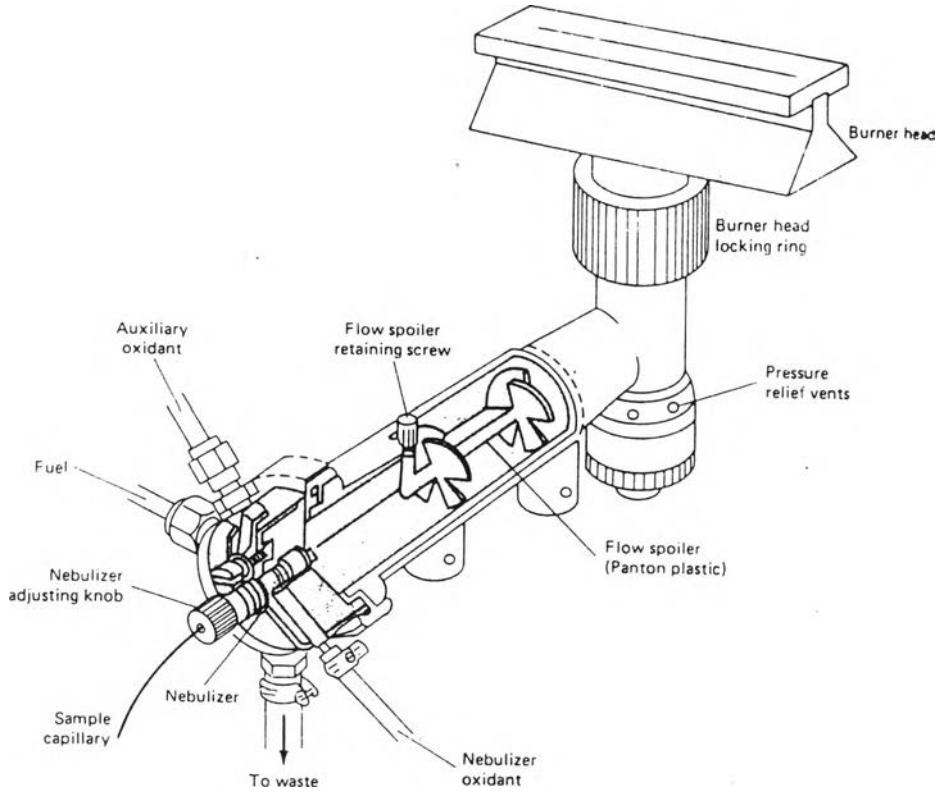



Figure 2.11 A typical pre-mix type of burner (33).

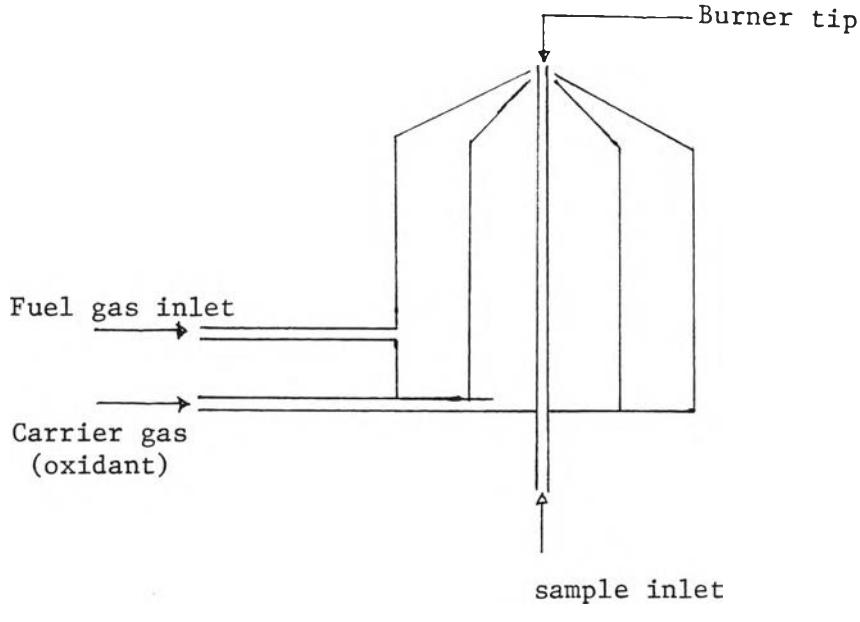



Figure 2.12 A typical total consumption type of burner (33).

Equation 2.12 above.

### 2.7.2 Inductively Coupled Plasma (ICP) Spectroscopy

The ICP spectroscopy (34) is an atomic emission spectroscopy. Unlike the flame emission spectroscopy, the sample is heated in an inductively heated argon plasma instead of being atomized in a flame. The ICP source is shown in Figure 2.13. The sample is aerosol and carried into the plasma by the flowing argon. Ionization of the flowing argon is initiated by a spark from a Tesla coil. The resulting ions, and their associated electrons, then interact with the fluctuating magnetic field produced by the induction coil. This interaction causes the ions and electrons within the coil to flow in the closed annular paths depicted in the figure; ohmic heating is the consequence of their resistance to this movement. The temperature of the plasma formed in this way is high enough (8000 to 10,000$^{o}$K) to require thermal isolation from the outer quartz cylinder. This isolation is achieved by flowing argon tangentially around the wall of the tube, as indicated by the arrows in Figure 2.13.

After the atoms at the ground states are excited, relaxation of the atomized particles to their ground states produces emission spectra. The emission spectra of the sample in an inductively coupled argon plasma is generally rich in lines from excited ions rather than neutral ions as in a flame. This is because the ICP source is more energetic than the other. Similar to a common emission spectroscopy, the emission radiation has a maximum wavelength corresponding to the pattern of element and an intensity corresponding to the concentration of element.

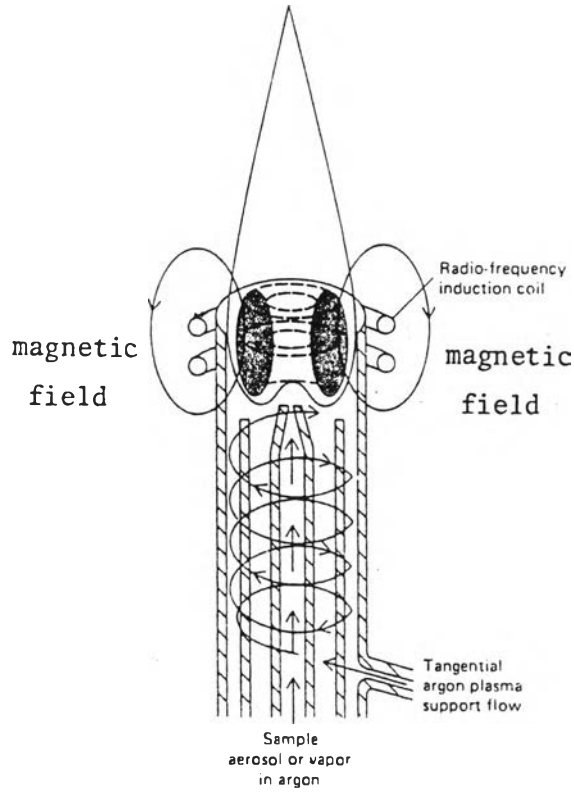



Figure 2.13 A typical inductively coupled plasma source (34).

### 2.7.3 Scanning Electron Microscope (SEM)

The scanning electron microscopy is a technique of x-ray analysis. The beam of electrons from a source, a tungsten filament in a high voltage field, is swept on to the sample surface by a scanning coil similar to the television scene. After the striking of electron on the surface of material, the electron may pass through the material without colliding to any atom, this is called a scattered electron. If the electron collides to an atom or an electron within atom, and no change of energy or all of energy is transferred to the momentum, this is called an elastic electron. If the energy and direction of electron is changed, this is called an inelastic electron. If the electron bounces back without collision to any atom, this is called a back scattered electron. If another or the same electron bounces off after colliding to the atom, this is called a secondary electron. Occurring the secondary electron always gives the radiation of x-ray wavelength. With scanning a detector corresponding to this energy, the image of material can be obtained. The magnification power of a SEM depends on the design, one can magnify as high as 20 thousandfold, and one can magnify very high to 180 thousandfold.