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Review Article

REVIEW ON GAS LIQUID CHROMATOGRAPHY

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Abstract: Chromatography is a physical method for separating the components of a mixture in which the components distribute themselves between two phases, one of the phases being mobile and the other stationary. The stationary phase is a solid or a liquid, and the mobile phase is a liquid or a gas (or vapor). The two phases, and the tube that holds them, constitute a chromatographic column. There are, then, f our combinations of phases that can be used in a column, and thus four kinds of chromatography. With the mobile phase given first, they are: gas-liquid (GLC), gas-solid (GSC), liquid-liquid (LLC), and liquid-solid (LSC). This experiment involves the first kind — GLC. (G.L.C) is based upon partition between Gas and immobile liquid coated on solid .so the component of mixture distributes themselves between the gas phase and liquid adsorbent coated on solid and the separation is due to partition properties.
Key**words:** GLC, components, Chromatography, Application

INTRODUCTION

Gas chromatography is a widely used technique for the separation of gaseous and volatile sub which are difficult to separate.The primary limit of s technique is that sample must be capable of being volatilized without undergoing decomposition because of this this technique is replaced by H.P.L.C.It is similar to column chromatography except that the gas is used as the mobile phase instead of the liquid.Here gas as a mobile phase or moving phase is passed through a column a column containing liquid adsorbent coated on inert solid support ,thus the adsorption or partition is possible.Gas solid chromatography(G.S.C) based upon the selective adsorption on solid,so the component of mixture distributes themselves between the gas phase and adsorbent and the separation is due to adsorptive properties.where as Gas liquid chromatography (G.L.C) is based upon partition between Gas and immobile liquid coated on solid,.so the component of mixture distributes them selves between the gas phase and liquid adsorbent coated on solid and the separation is due to partition properties [1].

INSTRUMENTATION

In GLC, an inert solid support is needed to keep the liquid phase stationary in the column. In a packed column, such as is used in this experiment, the support usually consists of porous material of siliceous origin (diatomaceous earth or clay), fairly finely granulated to reduce the turbulence of a gas flowing through it. It should be capable of absorbing the liquid on its surface in a thin, uniform film with a loading of up to 50% by weight. The column packing is prepared by mixing the liquid and solid support prior to filling the tube which holds the packing. The tube material is usually glass or metal (e.g. copper or stainless steel) tubing. Unless the sample to be analyzed reacts with it, metal tubing is preferred because of its infrangibility, and because it can be readily wound into space-saving coils. The operating principle of GLC involves introducing the sample to be studied (the solute) into a heated injector tube where it is vaporized and mixed with an inert gas, usually helium, called the carrier gas. The vapor is then swept onto the column where it distributes itself between the gaseous and the liquid phases. The partial pressure of the solute vapor in the gaseous phase depends upon how soluble it is in the stationary liquid phase (the solvent or substrate). The factors influencing the solubility of the solute in the solvent are discussed later in the section on theory. It should be obvious that the solvent must be a liquid with a very low vapor pressure if it is to remain stationary on the column.

In any event, the amount of solute swept along the column by the carrier gas depends on its partial pressure, and sooner or later some of it will pass out of the column into the detector, which responds to any change in the chemical or physical nature of the gas leaving the column by producing an electrical output, the magnitude of which depends in part on the partial pressure of the solute in the gas. This output actuates the pen of an electrical strip-chart recorder, which makes a trace indicating the amount of solute leaving the column as a function of time.

From what has been written above, it should be obvious that strict control of the carrier gas flow rate and of the column temperature is essential if the detector output is to have any quantitative significance. The gas flow is held constant by suitable controls, and a device can be attached to the gas outlet to measure the flow rate. The column is mounted in a thermostatted oven. In addition, to avoid condensation the injector and detector must be maintained at a constant temperature, higher than that of the column. Hence these components are thermostatted as well. For a detailed description of the chromatograph to be used, and its operation, the instruction manual which is kept with the instrument should be consulted.

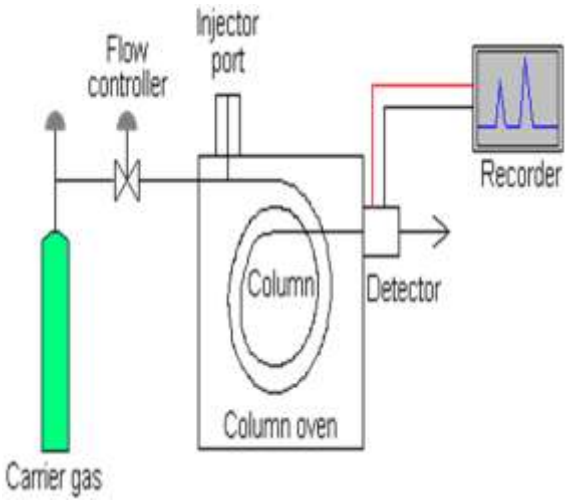

Fig-Instrumentation

PRACTICAL REQUIREMENTS [2]:

1. carrier gas.
2. Flow regulator and flow meter.
3. Injection devices.
4. Columns.
5. Temperature control device.
6. Detector.
7. Recorders and integrators

(1). CARRIER GAS:

The main purpose of carrier gas is to transport the sample compoments through the column. For selection of the carrier gas, following factor must be conider.

1. It should be chemically inert and should not interact with the sample or stationary phase.
2. It should be suitable for the detector to be utilized and the type of the sample or stationary phase.
3. It should give best column performance consistent with the desired speed of analysed.
4. It should be readily available cheap and of high purity.
5. It should not cause the risk of fire or explosion hazard.

Most widely used are Hydrogen, Helium, Nitrogen, and Argon.

Hydrogen: It has better thermal conductivity ,low density.it is useful in case of thermal conductivity detector and flame ionization detector. The disadvantages is that it reacts with unsaturated compounds and it is inflammable.

Helium:it also has excellent thermal conductivity, but it is expensive.it is good carrier gas when used with thermal conductivity detector.

Nitrogen: it is in expensive but has reduce sensitivity. By considering the requirements ,and a compromising among the inertness efficiency and operating cost .Make the nitrogen and helium as the most common carrier gas. As carrier gas are compressible it must be stored under high pressure in cylinder and used when required.

(2)FLOW REGULATOR AND FLOW METER:

As the carrier gas are stored at high pressure flow regulator are used to deliver the gas with uniform pressure or flow rate.Flow meter are used to measure the flow rate of carrier gas. They are Rotameter and soap bubble flow meter.

Rota meter: it is placed conveniently before the column inlet . it has an ordinary glass tube (like burette) with a float held on to a spring .the level of the float is determine by the flow rate of carrier gas and is precalibrated.

(**a) soap bubble meter** **(b) Rotameter**

Soap bubble meter: it is similar to rotameter and instead of float , soap bubble formed indicates the flow rate.it has a glass tube with a inlet tube at the bottom through which gas comes in .A rubber bulb is used to store the soap solution when the bulb is gently pressed , a drop of soap solution is converted in to a bubble by the pressure of carrier gas and travel up. The distance travelled upwards is measure of flow rate of carrier gas.

(3) SAMPLE INJESCTION DEVICE:

The sample injection system is very important because one of the feature of gas chromatography is the use of very small amount of the sample. this system must introduce the sample in a reproducible manner and must vaporized it instantaneously so that the sample will enter the column as a single slug.

Liquid samples are generally introduced by hypodermic syringe through a self sealing rubber septum in to a small inlet chamber, which may be heated to cause flash evaporation .

Solid sample must be dissolved in volatile liquids for introduction or may be introduced directly if they can be liquefied.

Gas samples require special gas sampling valves for introduction in to the carrier gas stream.

(4)COLUMNS:

The column can be constructed of glass or metal tubing and for analytical work it has 4.8 meter diameter . It can be of any length from few centimeter to over a hundred meter and can be of coiled ,bent or straight.

Three type of column are generally used.
(1)packed column.
(2) open tubular column.
(3) support coated open tubular column.(SCOT)

Packed column.: packed column are prepared by packing metal or glass tubing with granular stationary phase. For G.S.C the column are packed with size graded adsorbent or porous polymer,where as for G.L.C the packing is prepared by coating the liquid phase over a size graded inert solid support. A wide variety of stationary phase like poly ethyleneglycols, high molecular weight ester, amides, hydrocarbons, polysiloxanes, microporous cross-linked poly aromatic beds.

The advantages of using porous material are

- **(a)** There is no column –bleed.Most of the porous polymer are stable up to the 250^0c and cause no base line drift. It there for ,allows the use of highly sensitive detector.
- **(b)** There is no adsorption of porous compound such as water alcohols, or acids and they are eluted rapidly as sharp symmetrical peaks.
- **(c)** Retention data are highly reproducible.
- **(d)** Some of the separation provided are unique.
- **(e)** Porous chemical band are mechanically strong and can be packed on column.

Open tubular column: These column are also called as capillary column OR golay column. They are made up of long capillary tubing (30-90meters) having uniform and narrow inernal diameter of(0.025-0.075c.m).they are made up of stainless steel ,copper, nylon ,or glass etc. the stainless steel being the most popular. The inside wall of the capillary tubing is coated with liquid phase in the form of a thin (0.5-1micron)and uniform film.

These column offers the least restance to the flow of carrier gas and hence they are most efficient than the packed column. But here disadvantages is that more sample can not be loaded.

(c) Support coated open tubular column (S.C.O.T): This is an improved version of golay column or capillary columns. As golay or capillary column have small sample capacity ,they can be modified in to S.C.O.T column.These columns are made by depositing a micron size porous layer of support material on the inner wall of of the capillary column and then coated with a thin film of liquid phase. These column also have reistance to the flow of carrier gas but offer the advantages of more sample load.

(5) TEMPERATURE AND CONTROL DEVICE.

Pre heater: pre heater are used in Gas chromatography to convert the sample in to its vapour form and mix them with the mobile phase or carrier gas. The preheater are present along with the injection devices.As soon as liquid sample are injected ,they are converted in to vapour form.

Thermostatically controlled oven: The principle of separation in gas chromatography is partition. Partition co-efficient is the ratio of concentration of of a solute distributed between two immiscible liquids. Since partition co-efficient as well as solubility of a solute is depend upon temperature, so the temperature maintenances in column in highly essential for efficient temperature. Hence column as well as injection device should be maintained at a particular temperature.

For this two type of operation are available:

- **(a) Isothermal programming :** (iso means same) In which the same temperature is maintained throught the process.
- **(b) Linear programming:** in which the oven is heated lineary over a **period** of time Eg 150^0C initially to 200^0Cat the end of separation.with increase the temperature rate of 5^0c/minutes. this required when the sample contain mixture of low nd high boiling point temperature.

(6)DETECTORS:-

Detectors are the most important part of the gas chromatographic instruments.they are consider as a heart of the apparatus. A detector uses some property by which it can detect the difference between a pure carrier gas and elutd components.

The requirements of an ideal detector are-

I. Applicability to wide range of samples.
II. .High sensitivity to even small concentration.
III. Rapidity of responces.
IV. Linerity i.e less response to low concentration and proportional response to high concentration.
V. Responance should be un affected by the temperature, flow rate, and character of carrier gas
VI. simple and easy to maintain.
VII. in expensive.

(1)Thermalconductivity detector Katharometer:

The Principle is based upon thermal conductivity difference between carrier gas and that of the components. Kathrometer has two platinum wire of uniform dimension which form a part of a wheatstone bridge.Through one of them pure carrier gas always flows through and through the other ,the effulents of the column passes. The two platinum wires are heated electrically and hence assume equilibrium condition of temperature and electrical resistance. When pure gas passes through both of them , there is no difference in temperature or resistance of the wire.Hence this produce a difference in resistanceand so conductivity between two wires,which ic amplified and recorded as a signal.

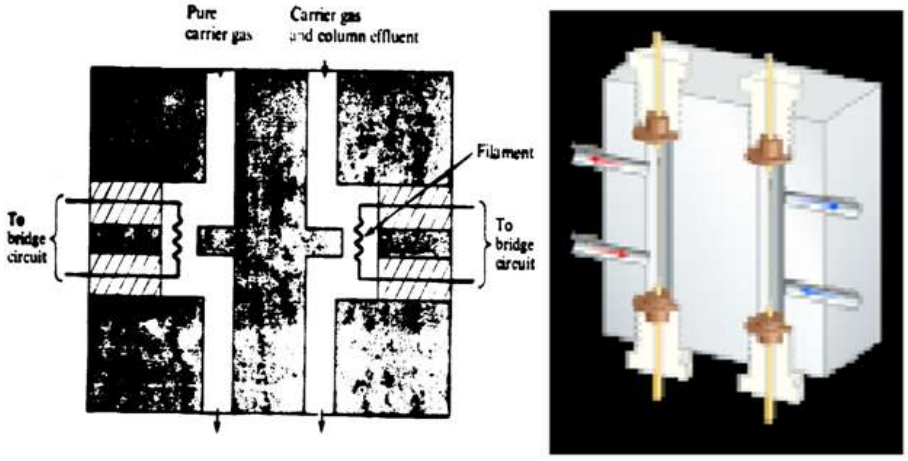

Fig: thermal conductivity detector

The thermal conductivities of some carrier gases are given as follows.

H_2	He	N_2	Methane	Hexane
32.7	33.9	6.5	5.2	3

APPLICATIONS [3,4]**:**

1 **Quality analysis :** it is nothing but the identification of a compound .this is done by comparising the retention **time of sample as well as** standard. Under identical condition .the retention time of the standard and sample are same . if there is deviation than they are not same compound.Retention time meansit the difference between the point of injection and the peak maxima . it is the time required for 50% of component to be eluted from the column . it measured in time in second or minutes.

2 .**checking the purity of the compound:** By comparing the chromatogram of the standard and that of the sample, the purity of the compound can be reported. If the additional peak are present and hence the compound is not purified from the percentage area of the peaks obtained. The percentage purity can also be reported.

3. **presence of impurity:** This can be seen by the presence of additional peaks when compared to the reference or standard material. The percentage impurities may also be calculated from peak areas.

4.Quantitative analysis: The quantity of the compound can be determine by the following method.

A.Direct comparing method:

By injecting a sample and standard separately and comparing their peak areas , the quantity of the sample can be determine.

$A_1/A_2=W_1/W_2$

Where $A_1$and $A_2$are peak of the sample and standard W_1 and W_2 are the concentration or weight of sample and standard.

B. Calibrationcurvemethod.

In this method the standard of various concentration are used to determine their peak areas. A graph of peak area v/s concentration is plotted .from the peak area of the unknown sample the concentration of the unknown is calculated by interpolation method.

C.Internalstandardmethod.

In this method , a compound with similar retention characteristics is used. A known concentration of the internal standard is added separately to the standard solution and sample solution whose concentration is not known. The chromatogram is recorded and the peak area ratio of the sample and internal standard is determine. By using the peak area ratio of standard and internal standard , the concentration of the unknown solution is determined. By using the peak ratio of sample and internal standard , the concentration of the unknown solution is determined .this method is useful when more extraction step are involved in sample preparation and the sample preparation and the sample matrix is complex.

5 Multi components analysis or Determination of mixture of drugs: Similar to the quantification of a single drug , multi component, analysis is determined by using any one of the above methods. Marketed formulation are available which contain several drugs and each component can be determined quantitatively .

6 Isolation and identification of drug or metabolites in urine , plasma, serum etc can be carried out.

7 Isolation and identification of mixture of components like amino acids ,plant extracts, volatile oiletc.

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