Chrom-Ed Book Series

Raymond P. W. Scott

GAS

CHROMATOGRAPHY

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Contents

Introduction

Chromatography, in one of its several forms, is the most commonly used procedure in contemporary chemical analysis and the first configuration of chromatography equipment to be produced in a single composite unit and made commercially available was the gas chromatograph. Gas chromatography was invented by A. J. P. Martin who, with R. L. M. Synge, suggested its possibility in a paper on liquid chromatography published in 1941 (1). Martin and Synge recommended that the liquid mobile phase used in liquid chromatography could be replaced by a suitable gas. The basis for this recommendation was that, due to much higher diffusivities of solutes in gases compared with liquids, the equilibrium processes involved in a chromatographic process (see Book 1) would be much faster and thus, the columns much more efficient and separation times much shorter. So the concept of *gas chromatography* was envisioned more than fifty years ago, but unfortunately, little notice was taken of the suggestion and it was left to Martin himself and his coworker A. T. James to bring the concept to practical reality some years later in 1951, when they published their epic paper describing the first gas chromatograph (2).

The first published gas chromatographic separation was that of a series of fatty acids, a titration procedure being used, in conjunction with a micro burette, as the detector. The micro burette was eventually automated providing a very effective in-line detector with an integral response. After its introduction by James and Martin, the technique of GC developed at a phenomenal rate, growing from a simple research novelty to a highly sophisticated instrument, having a multi-million dollar market, in only 4 years. The gas chromatograph was also one of the first analytical instruments to be associated with a computer which controlled the analysis, processed the data and reported the results.

A more sophisticated form of the gas chromatograph was constructed by James and Martin and described by James in 1955 (3). The instrument was a somewhat bulky device with a straight packed column, 3 ft long, that was held vertically and thermostatted in a vapor jacket. Initially, the detector was situated at the base of the column and consisted of the automatic titrating device, the separation was presented as a chromatogram in the form of a series of steps, the height of each step being proportional to the mass of solute eluted. The apparatus was successfully used to separate some fatty acids, but the limited capability of the device to sense only ionic material motivated Martin to develop a more versatile detector, the Gas Density Balance.

The gas density balance, was the first detector with a truly catholic response that was linearly related to the vapor density of the solute and consequently its molecular weight. The gas density balance had a maximum sensitivity (minimum detectable concentration) of about 10^{-6} g/ml at a signal to noise ratio of two. This detector inspired the invention of a wide range of detectors over the next decade providing both higher sensitivity and selective response.

The modern gas chromatograph is a fairly complex instrument mostly computer controlled. The samples are mechanically injected, the analytical results are automatically calculated and the results printed out, together with the pertinent operating conditions in a standard format. However, the instrument has evolved over many years although the majority of the added devices and techniques were suggested or describe in the first three international symposia on gas chromatography held in 1956, 1958 and 1960.

These symposia, initially organized by the 'British Institute of Petroleum' have been held every two years ever since 1956 and the meetings have remained the major stimulus for developing the technique and extending its capabilities. However, the majority of the techniques and devices that have been incorporated in the modern chromatograph, were described, reported, or discussed in the first triad

of symposia. The layout of the modern gas chromatograph is shown as a block diagram in figure 1.

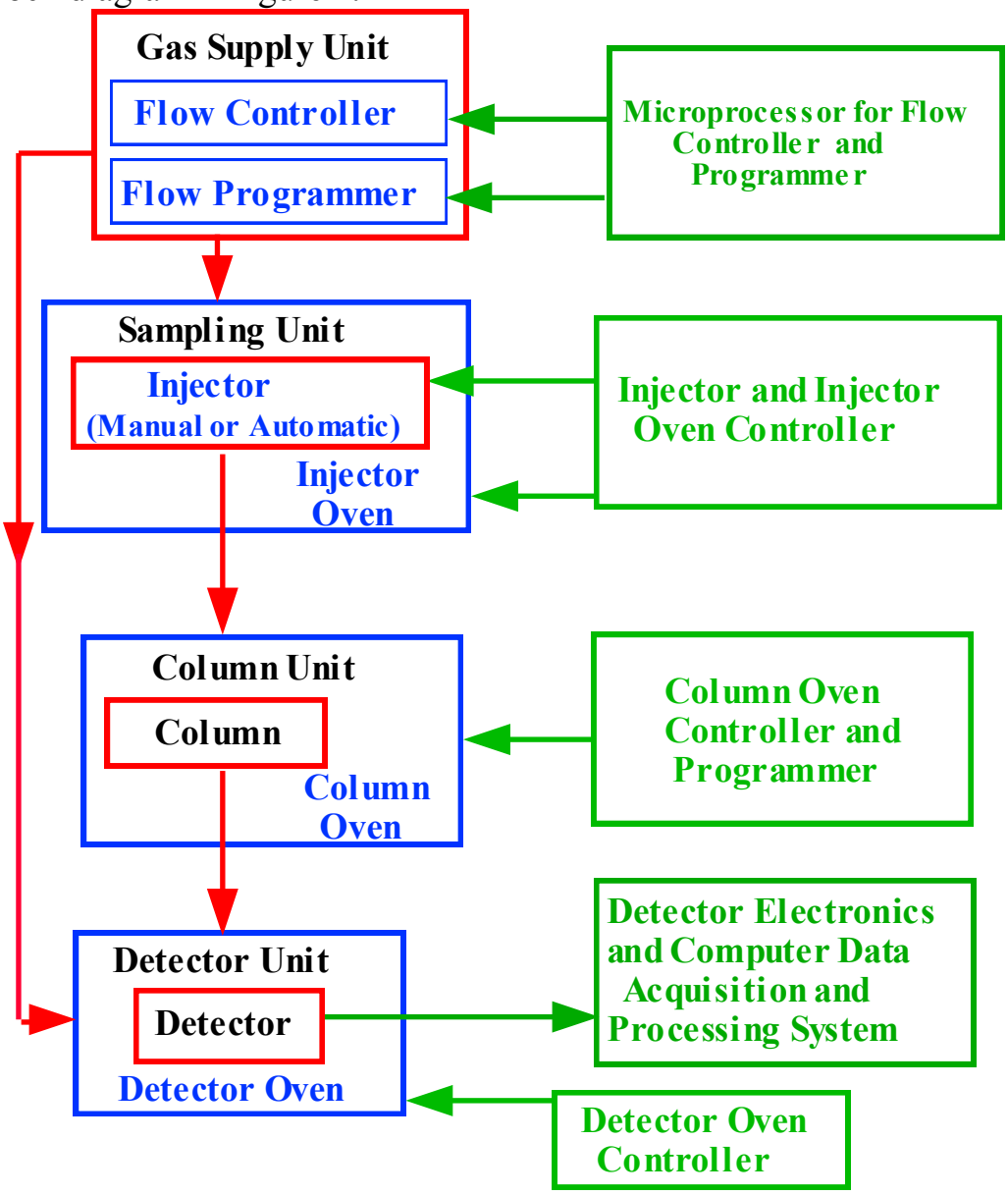


Figure 1 The Design of a Modern Gas Chromatograph

The Modern Gas Chromatograph

Most gas chromatographs consist of four chromatography units, supported by three temperature controllers and 2 micro processors systems. In some instruments, a single microprocessor unit is employed to service the entire chromatograph but this tends to restrict the choice

available for the different parts of the chromatograph. The first unit, the gas supply unit, provides all the necessary gas supplies which may involve a number of different gases, depending on the type of detector that is chosen. For example, a flame ionization detector will require hydrogen or some other combustible gas mixture, air or oxygen to support combustion and a mobile phase supply that could be nitrogen, helium or some other appropriately inert gas. Thus, for the detector postulated, a minimum of three different gases would be required which will also involve the use of three flow controllers, three flow monitors and possibly a flow programmer. In addition the gas supply unit would be serviced by a microprocessor to monitor flow rates, adjust individual gas flows and, when and if necessary, program the mobile phase flow rate.

The second unit is the sampling unit which contains an automatic injector which is situated inside a thermostatically controlled enclosure. The injector usually has its own oven, but sometimes shares the column oven for temperature control. The injector oven, if separate from the column oven, is serviced by its own temperature controller which both monitors and controls the temperature. There is normally a separate controller, usually a microprocessor, that controls the injector itself. The injector can range in complexity from a simple sample valve, or mechanically actuated syringe to an automatic multi sampler that is microprocessor controlled. It can have a complex transport system (such as a carousel) that can take samples, wash containers, prepare derivatives and, if necessary, carry out a very complex series of sample preparation procedures before injecting the sample onto the column. Sample preparation is often carried out using a laboratory robot which then becomes part of the sampling unit. If a robot is used it can be programmed to prepare a wide variety of different samples and so software must be written for each type of sample.

The third unit is the column unit which contains the column, the essential device that actually achieves the necessary separation, and an oven to control the column temperature. It is interesting to note that despite the complexity of the apparatus, and its impressive appearance,

the actual separation is achieved either in a relatively short length of packed tube or a simple wall-coated open tube. The rest of the apparatus is merely there to support this relatively trivial, but critical device. The oven also will contain a temperature sensor and if necessary an appropriate temperature programmer. As the mobile phase is a gas, there are virtually no interactions between the sample components and the mobile phase and thus the elution time can not be controlled by techniques such as solvent programming or gradient elution. The counterpart to gradient elution in gas chromatography is temperature programming. The column temperature is raised continuously during development to elute the more retained peaks in a reasonable time. It is a similar technique to flow programming but decreases the retention exponentially with temperature as opposed to linearly with flow rate. The temperature was originally programmed in a linear manner using electro-mechanical devices but modern temperature programmers contain a dedicated micro processor for the purpose. Sometimes all controls are initiated from a central computer that is also employed for acquiring and processing the chromatographic data.

The fourth unit contains the detector which is situated in its own oven. There is a wide range of detectors available each having unique operating parameters and its own performance characteristics. The detector, and the conduit connecting the column to the detector, must be maintained at a temperature at least 15˚C above that of the maximum temperature the oven will reach during analysis to ensure no sample condenses in the conduits or detector, consequently, separate conduit heaters are necessary. Any condensation introduces serious detector noise into the system and also reduces the detector response thus effecting both the detector sensitivity and the accuracy and precision of the results. The detector oven is set at a user defined temperature and is operated isothermally, controlled by its own detector-oven temperature controller. The output from the detector is usually electronically modified and then acquired by the data

processing computer which processes the data and prints out an appropriate report.

Gas Supplies

Gases for use with the gas chromatograph were originally all obtained from gas tanks or gas cylinders. However, over the past decade the use of gas generators have become more popular as it avoids having gases at high pressure in the laboratory which is perceived by some as potentially dangerous. In addition, the use of a hydrogen generator avoids the use of a cylinder of hydrogen at high pressure which is also perceived by some as a serious fire hazard despite the fact that they have been used in laboratories, quite safely for nearly a century.

Supplies from Gas Tanks

Gasses are stored in large cylindrical tanks fitted with reducing valves that are set to supply the gas to the instrument at the recommended pressure defined by the manufacturers. The cylinders are often situated outside and away from the chromatograph for safety purposes and the gasses are passed to the chromatograph through copper or stainless steel conduits at relatively low pressure. The main disadvantage of gas tanks is their size and weight which makes them difficult to move and replace.

Pure Air Generators

Air generators require an air supply from air tanks or directly from the laboratory compressed air supply. The Packard Zero Air Generator passes the gas through a 0.5 μ filter to remove oil and water and finally over a catalyst to remove hydrocarbons. The hydrocarbon free air is then passed through a 0.01 μ cellulose fiber filter to remove any residual particulate matter that may be present. The manufacturers claim the resulting air supply contains less than 0.1 ppm total hydrocarbons and delivers air at 125 psi at flow rates up to 2,500 cc per min.

Pure Nitrogen Generators

The nitrogen generator can also operate directly from the laboratory compressed air supply. General contaminants are first removed with appropriate filters and adsorbents and the purified air passes over layers of polymeric hollow fiber membranes through which nitrogen selectively permeates. The residual nitrogen-depleted air containing about 30% oxygen is vented to atmosphere. The nitrogen produced by the Air Products nitrogen generator contains less than 0.5 ppm of oxygen, less than 0.5 ppm of water vapor and less than 2.0 ppb of halocarbons or hydrocarbons. It can supply up to 1 l/min. at pressures from 60 to 100 psi.

Hydrogen Generators

In the Packard Hydrogen Generator, hydrogen is generated electrolytically from pure deionized water. Unfortunately, the technology used in hydrogen generators is largely proprietary and technical details are not readily available. The electrolysis unit uses a solid polymer electrolyte and thus does not need to be supplied with electrolytes, only the deionized water. The manufacturers claim the device generates 99.999% pure hydrogen with a reservoir capacity of 4 liter, and an output pressure that ranges from 2 to 100 psi. Other units can produce hydrogen flows that range from 0 to 125 ml/min. to 0 to 1200 ml/min. The oxygen, produced simultaneously with hydrogen at half the flow rate, is vented to air.

Pressure Controllers

The first control on any gas line is afforded by a simple pressure controller. There are a number of pressure controllers associated with a gas chromatograph. The reducing valves on the gas tanks are examples of simple pressure controllers and the flow controllers that are used for detector and column flow control often involve devices based on the same principles. A diagram of a pressure controller is shown in figure 2.

The pressure controller consists essentially of two chambers separated by a diaphragm, in the center of which is a needle valve that is actuated by the diaphragm. The diaphragm is held down by a spring that is adjustable so that the pressure in the second chamber, and thus the outlet flow, can be set at any chosen value. When gas enters the lower chamber, the pressure on the lower part of the diaphragm acts against the spring setting, and opens the valve. Gas then passes into the upper chamber and pressure is built up in the upper chamber to the value that has been set at which time the diaphragm moves downward closing the valve. If the pressure falls in the upper cylinder, the diaphragm again moves upward due to the pressure in the lower chamber, which opens the valve and the pressure in the upper chamber is brought back to its set value.

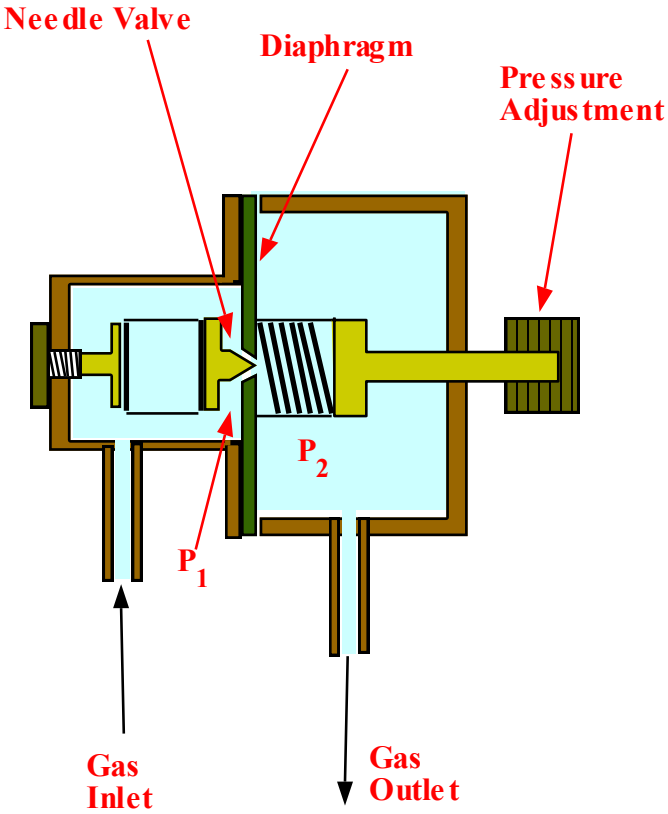
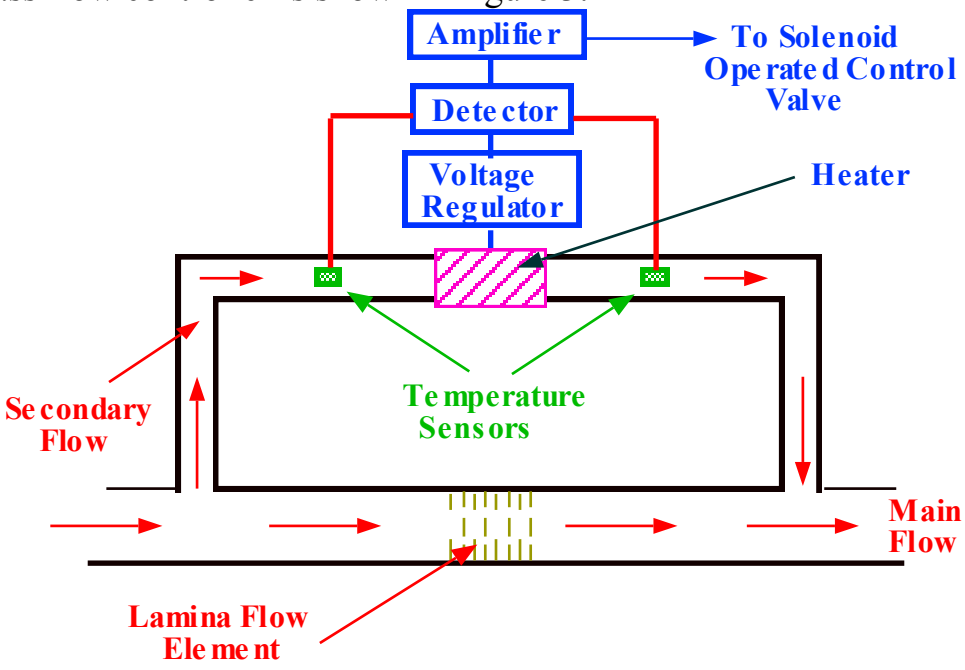


Figure 2 The Pressure Controller

Flow Controllers

A constant pressure applied to a column does not ensure a constant flow of mobile phase though the chromatographic system, particularly if the column is being temperature programmed. Raising the temperature of a gas causes the viscosity to increase, and at a constant inlet pressure, the flow rate will fall. The reduction in flow rate will be related to the temperature program limits and to a certain extent on the temperature gradient. To obviate the flow rate change, mass controllers are used which ensure a constant mass of mobile passes through the column in unit time irrespective of the system temperature. A diagram of a mass flow controller is shown in figure 3.



Courtesy of Porter Instrumentation Company Inc.

Figure 3 The Mass Flow Controller

The sensing system consists of a bypass tube with a heater situated at the center. Precision temperature sensors are placed equidistant up stream and down stream of the heater. A proprietary set of baffles situated in the main conduit creates a pressure drop that causes a fixed proportion of the flow to be diverted through the sensor tube. At zero

flow rate both sensors are at the same temperature. At a finite flow rate, the down stream sensor is heated, producing a differential temperature across the sensors. The temperature of the gas will be proportional to the product of mass flowing and its specific heat and so the differential temperature that will be proportional to the mass flow rate. The differential voltage from the two sensors is compared to a set voltage and the difference used to generate a signal that actuates a valve controlling the flow. Thus, a closed loop control system is formed that maintains the mass flow rate set by the reference voltage. The device can be made extremely compact, is highly reliable and affords accurate control of the carrier gas flow rate irrespective of gas viscosity changes due to temperature programming.

Flow Programmers

Flow programming is a procedure where the mobile phase flow-rate is increased during chromatographic development. If the mobile phase is compressible the relationship between retention volume, flow rate and inlet pressure is given by,

$$V_r = \frac{V_{r(0)} 3\left(\gamma^2 - 1\right)}{2\left(\gamma^3 - 1\right)} \qquad (1)$$

Where (V_r) is the true retention volume of the solute,
($V_{r(0)}$) is the retention volume measured at the outlet.
and (γ) is the inlet/outlet pressure ratio

(for the derivation of this equation see Book 8, The Thermodynamics of Chromatography)

Thus, $$V_{r(0)} = \frac{2V_r\left(\gamma^3 - 1\right)}{3\left(\gamma^2 - 1\right)} = t_{r(0)}Q_0$$

Now, from Book $Q_0 = \varepsilon P_o\left(\gamma - 1\right)$ where (ε) is a constant

Thus, $$t_0 = \frac{2V_r(\gamma^3 - 1)}{\varepsilon P_o 3(\gamma - 1)(\gamma^2 - 1)} = \frac{2V_r(\gamma^2 + \gamma + 1)}{\varepsilon P_o 3(\gamma^2 - 1)} \quad (2)$$

If (γ) is large compared with unity, Then

$$t_0 = \frac{2V_r(\gamma^2 + \gamma + 1)}{\varepsilon P_o 3(\gamma^2)} = \frac{2V_r}{\varepsilon P_o 3}\left(1 + \frac{1}{\gamma} + \frac{1}{\gamma^2}\right) \rightarrow \frac{2V_r}{\varepsilon P_o 3} \quad (3)$$

It is seen that at high values of (γ), the retention time approaches a constant value.

The relationship between $\left(1 + \frac{1}{\gamma} + \frac{1}{\gamma^2}\right)$ and (γ) is depicted in figure 4.

Figure 4 shows that there is little advantage in employing inlet/outlet pressure ratios much above 5 as values in excess of this do not reduce elution time significantly. If the column is very long, and consequently has a high flow impedance, higher inlet pressures may be necessary to obtain the optimum flow rate but this may not significantly reduce the elution time.

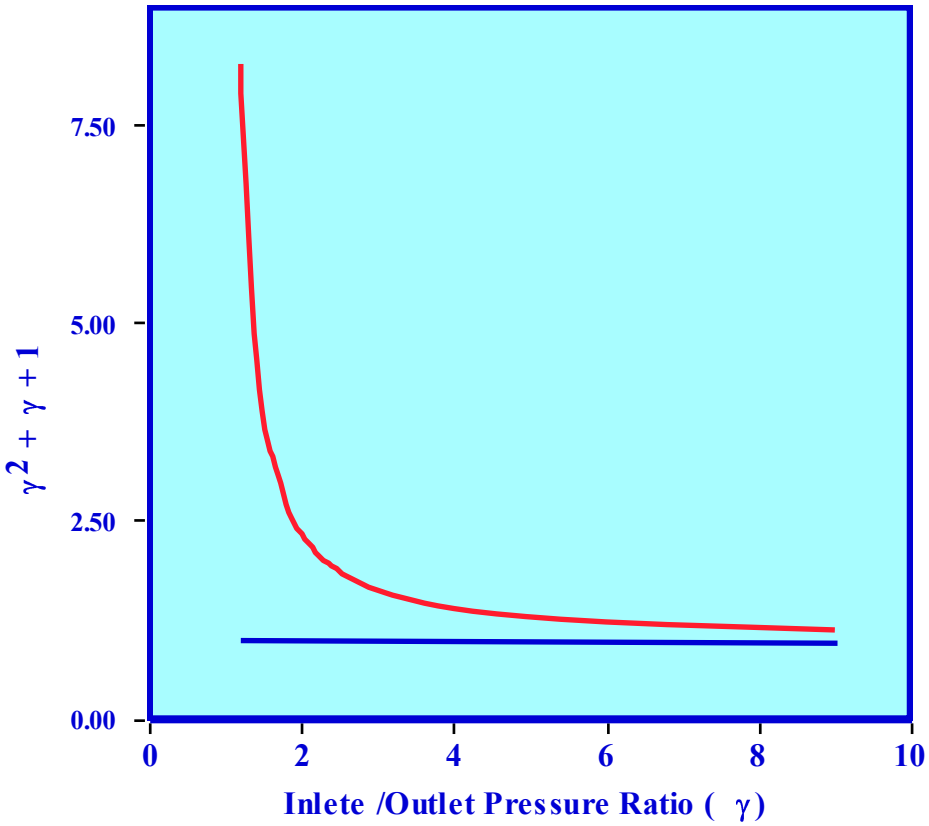


Figure 4 Graph of $\left(1+\frac{1}{\gamma}+\frac{1}{\gamma^2}\right)$ **against (γ)**

In figure 5, the log of the retention time is plotted against (γ) for both compressible and incompressible mobile phases. It is seen that for a compressible mobile phase the retention time falls to a constant level when (γ) is about 5 or 6. In contrast, for an incompressible mobile phase (*i.e.* in liquid chromatography), the retention time is continuously reduced as (γ) is increased. The advantages of flow programming with a compressible mobile phases are much less than for incompressible mobile phases. It should be noted, however, that the effect of increasing the flow rate above the optimum will progressively denigrate the column efficiency, whether the mobile phase is a liquid or a gas.

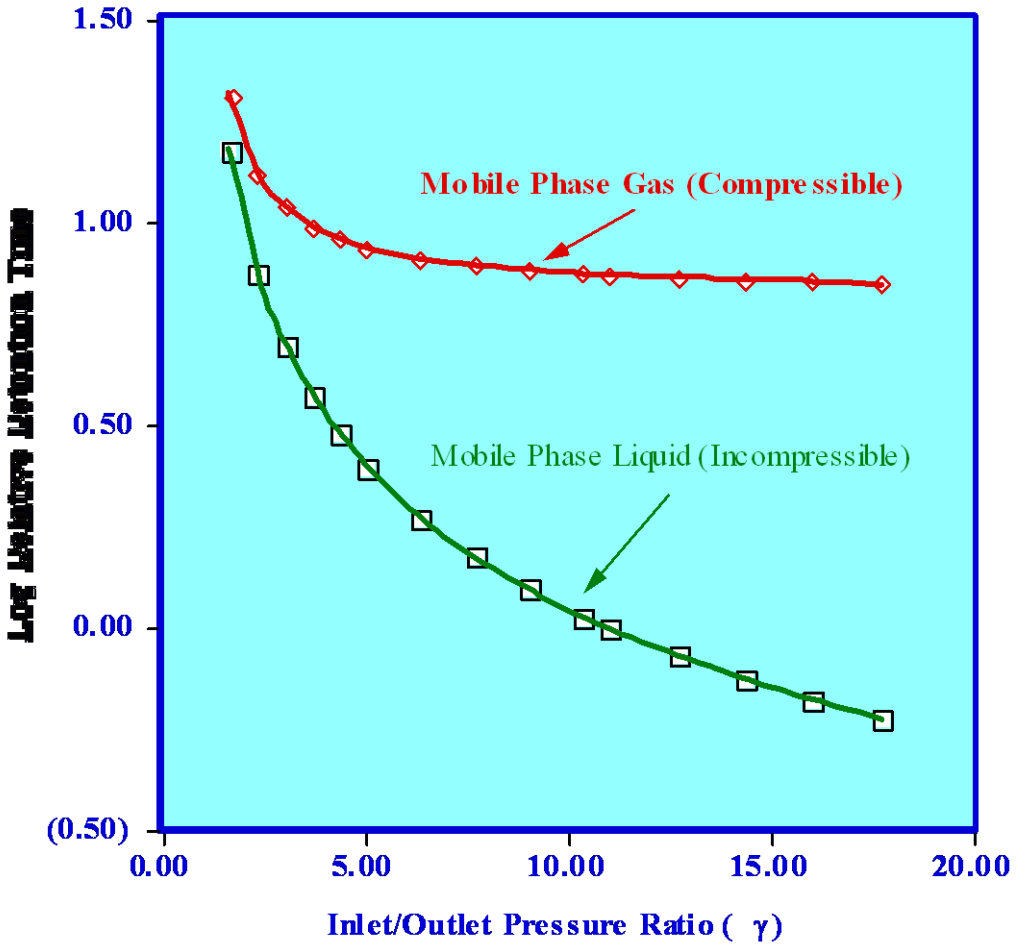


Figure 5 Graphs of Log Retention Time against Inlet/Outlet Pressure Ratio for Compressible and Incompressible Mobile Phases

The compressibility of the mobile phase in GC has interesting implications for the use of pressure or flow programming. The net effect of pressure programming on elution time can be evaluated as follows.

Rearranging equation (2), $V_r = \frac{\varepsilon P_o 3\left(\gamma^2 - 1\right)t_0}{2\left(\gamma^2 + \gamma + 1\right)}$ (4)

Therefore, during a range of values for (γ) that occur during a pressure or flow program, over a time interval (Δt), the contribution of the column flow to the retention volume ($\Delta V_{r(0)}$) will be given by,

$$\Delta V_{r(p)} = \frac{\varepsilon P_o 3\left(\gamma_p^2 - 1\right)\Delta t}{2\left(\gamma_p^2 + \gamma_p + 1\right)} \qquad (5)$$

Taking (Δt) as unit time (1 second)

Then $$V_r = \sum_{p=1}^{p=n} \Delta V_{r(p)} = \sum_{p=1}^{p=n} \frac{\varepsilon P_o 3\left(\gamma_p^2 - 1\right)}{2\left(\gamma_p^2 + \gamma_p + 1\right)} \qquad (6)$$

and n = $T_{t(0)}$, where ($T_{t(0)}$) will be the retention time of the solute under the defined pressure programming conditions.

Taking a simple practical situation where the retention volume is 1000 ml on a given column operating at a (γ) value of 2 and the retention time of the solute is 10 minutes (600 seconds). The flow properties of the column *i.e.* $\frac{3\varepsilon P_o}{2}$ can be defined in the following manner,

From equation (3) $$t_0 = \frac{2V_r\left(\gamma^2 + \gamma + 1\right)}{\varepsilon P_o 3\left(\gamma^2 - 1\right)}$$

or $$600 = \frac{2x1000\left(2^2 + 2 + 1\right)}{\varepsilon P_o 3\left(2^2 - 1\right)} = \frac{2}{3\varepsilon P_o}\frac{7000}{3}.$$ Thus, $$\frac{3\varepsilon P_o}{2} = 3.89$$

Substituting for $\frac{3\varepsilon P_o}{2}$ in (6), $$V_r = \sum_{p=1}^{p=n} \Delta V_{r(p)} = \sum_{p=1}^{p=n} \frac{3.89\left(\gamma_p^2 - 1\right)}{\left(\gamma_p^2 + \gamma_p + 1\right)} \qquad (7)$$

Equation (7) can now be employed to calculate the change in retention time for a series of solutes separated under pressure programming conditions in the defined column

Consider five solutes having actual retention volumes of 150, 300, 600, 900 and 1200 ml eluted under pressure programming conditions where, (γ_1) is 1.2 and at (p) seconds after the start, $\gamma_p = \gamma_1 + pa$, where (a)

takes values that range from 0.0025/s (0.0375 psi/s) and 0.025/s (0.15 psi/s) . Employing equation (7) the retention time of the solutes can be calculated for the series of different programming rates.

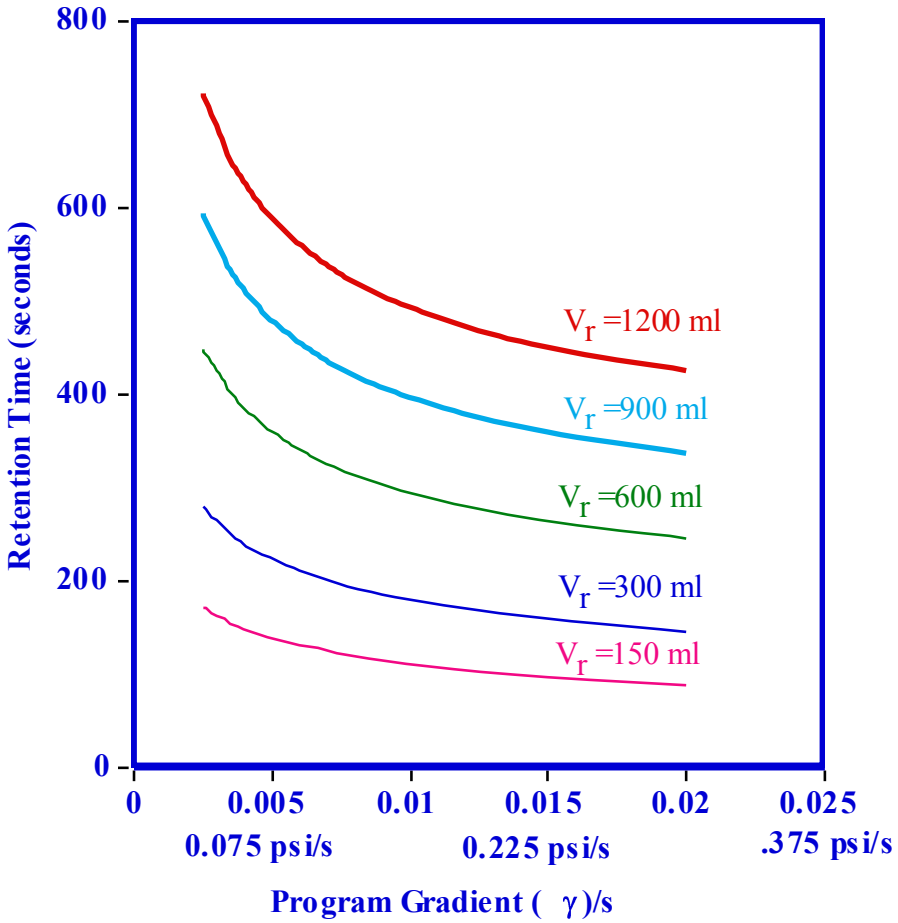


Figure 6 Graph of Retention Time against Pressure Program Rate for a Series of Solutes.

The results are shown in figure 6. It is seen that. although the use of pressure programming does indeed reduce the retention time of all solutes, program rates much above 0.2255 psi/s (13.5 psi/min.) provides very little advantage as far as reduction of analysis time is concerned.

Injection Devices

The basic injection devices that are used in chromatography, such as the external loop valve, have been discussed in book 1. In gas chromatography two basic types of sampling system are used, those suitable for packed columns and those designed for open tubular columns. In addition, different sample injectors are necessary that will be appropriate for alternative column configurations. It must be stressed, however, that irrespective of the design of the associated equipment, the precision and accuracy of a GC analysis will only be as good as that provided by the sample injector. The sample injector is a very critical part of the chromatographic equipment and needs to be well designed and well maintained.

Packed Column Injectors

In general, the sample injected onto a packed GC column ranges in volume from 0.5 μl to 5 μl and usually contains the materials of interest at concentrations ranging from 5%v/v to 10%w/v. The sample is injected by a hypodermic syringe, through a silicone rubber septum directly into the column packing or into a flash heater. Although the latter tends to produce broader peaks it also disperses the sample radially across the column.

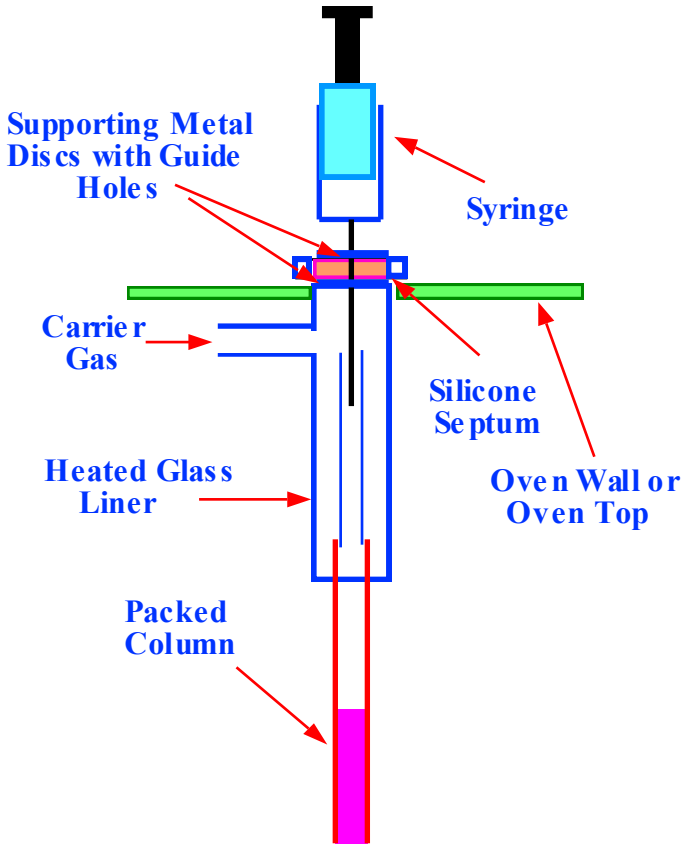


Figure 7 A Packed Column Injector

Direct injection into the packing constrains the sample into a small volume, but can cool the front of the packing. An example of a septum injection system used for packed columns is shown in figure 7. The silicone septum is compressed between metal surfaces in such a manner that a hypodermic needle can pierce it, but when it is withdrawn the hole is closed as a result of the septum compression and there is no gas leak. The glass liner prevents the sample coming in contact with the heated metal wall and thus, reduces the chance of thermal decomposition.The glass liner can be fitted with a separate heater and the volatalization temperature can, thus, be controlled. This "flash heater" system is available in most chromatographs. By using a

syringe with a long needle, the tip can be made to penetrate past the liner and discharge its contents directly into the column packing. This procedure is called 'on-column injection' and, as it reduces peak dispersion on injection and thus, provides higher column efficiencies, is often the preferred procedure.

Open Tubular Column Injection Systems

Due to the very small sample size that must be placed on narrow bore capillary columns, a split injection system is necessary, a diagram of which is shown in figure 8.

The basic difference between the two types of injection systems is that the capillary column now projects into the glass liner and a portion of the carrier gas sweeps past the column inlet to waste. As the sample passes the column opening, a small fraction is split off and flows directly into the capillary column, *ipso facto* this device is called a split injector. The split ratio is changed by regulating the portion of the carrier gas that flows to waste which is achieved by an adjustable flow resistance in the waste flow line. This device is only used for small diameter capillary columns where the charge size is critical.

The device has certain disadvantages due to component differentiation and the sample placed on the column may not be truly representative. The solutes with the higher diffusivities (low molecular weight) are lost preferentially to those with lower diffusivities (higher molecular weights). Consequently, quantitative analyses carried out using the high efficiency small diameter capillary columns may have limited accuracy and precision, depending on the nature of the sample.

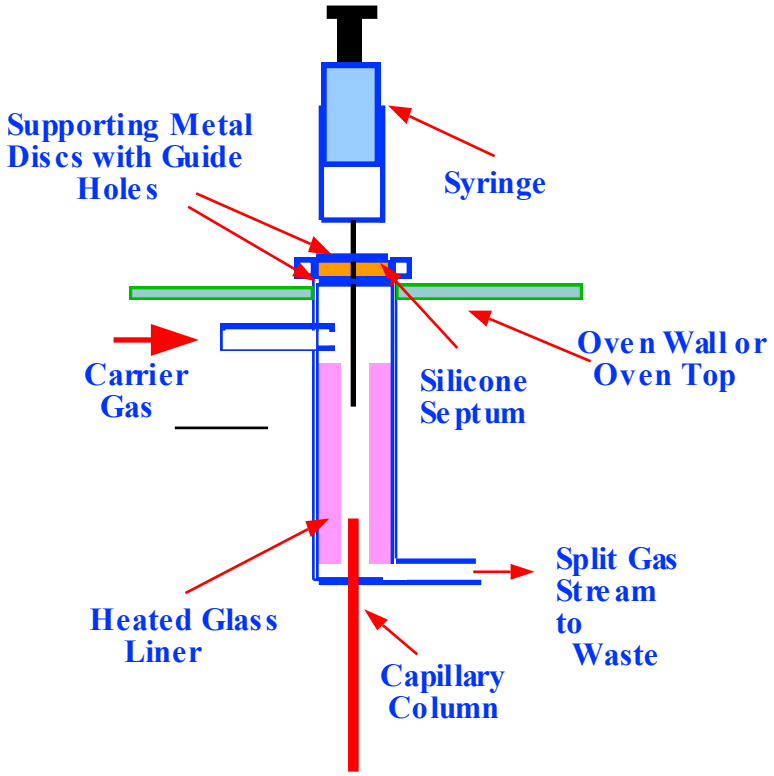


Figure 8 The Split Injection System

This problem was partially solved by using larger diameter columns that would permit *on-column* injection. The columns are constructed to have an I.D. of about 0.056 in; which is slightly greater than the diameter of a certain hypodermic needles. This injection system is depicted in figure 9.

However, there are also difficulties associated with this type of injector. On injection, the sample breaks up into separate portions, and bubbles form at the beginning of the column causing the sample to be deposited at different positions along the open tube as the solvent evaporates. On starting to develop the separation, each local concentration of sample acts as a separate injection. As a consequence, a chromatogram containing very wide or multiple peaks is produced.

Procedures have been introduced in an attempt to eliminate sample splitting in this manner.

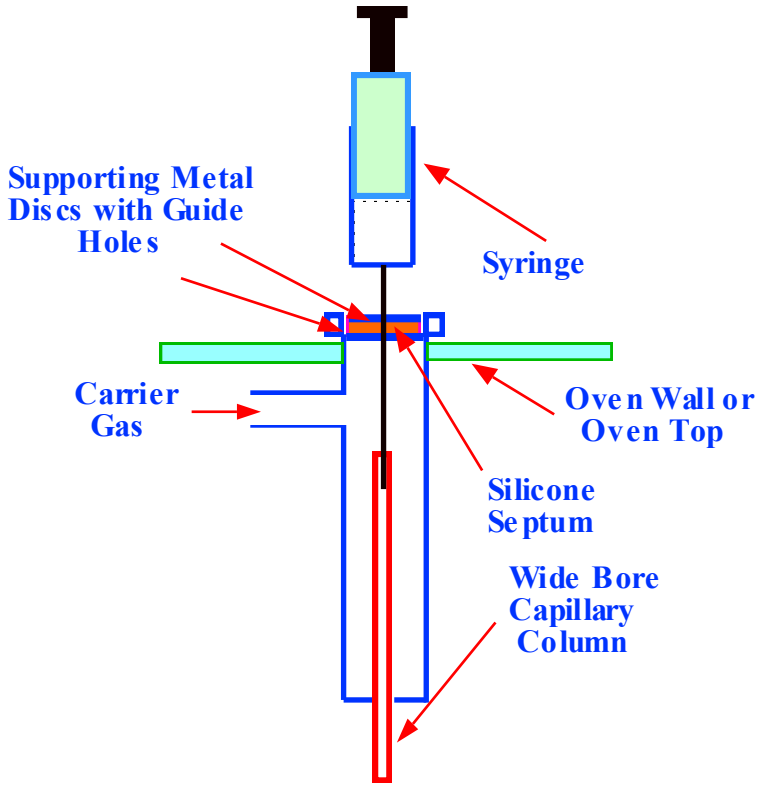


Figure 9 On-Column Injector for Large Bore Open Tubular Columns

Retention Gap Sampling

The first solution to the problem of sample splitting was the 'retention gap method' which is depicted in figure 10.

In this procedure stationary phase is removed from the first few centimeters of column. The sample is injected into this section and, if the sample becomes split, on commencing development, each split portion will still vaporize in the normal way. However, as there is no stationary phase present, the solutes will all travel at the velocity of the mobile phase until they reach the beginning of the coated section of the column. On reaching the start of the coating, the sample will be absorbed into the stationary phase and be concentrated at that point. As a result the sample is again at one point in the column. The retention

gap procedure is normally used in conjunction with temperature programming, the program being initiated at a fairly low temperature.

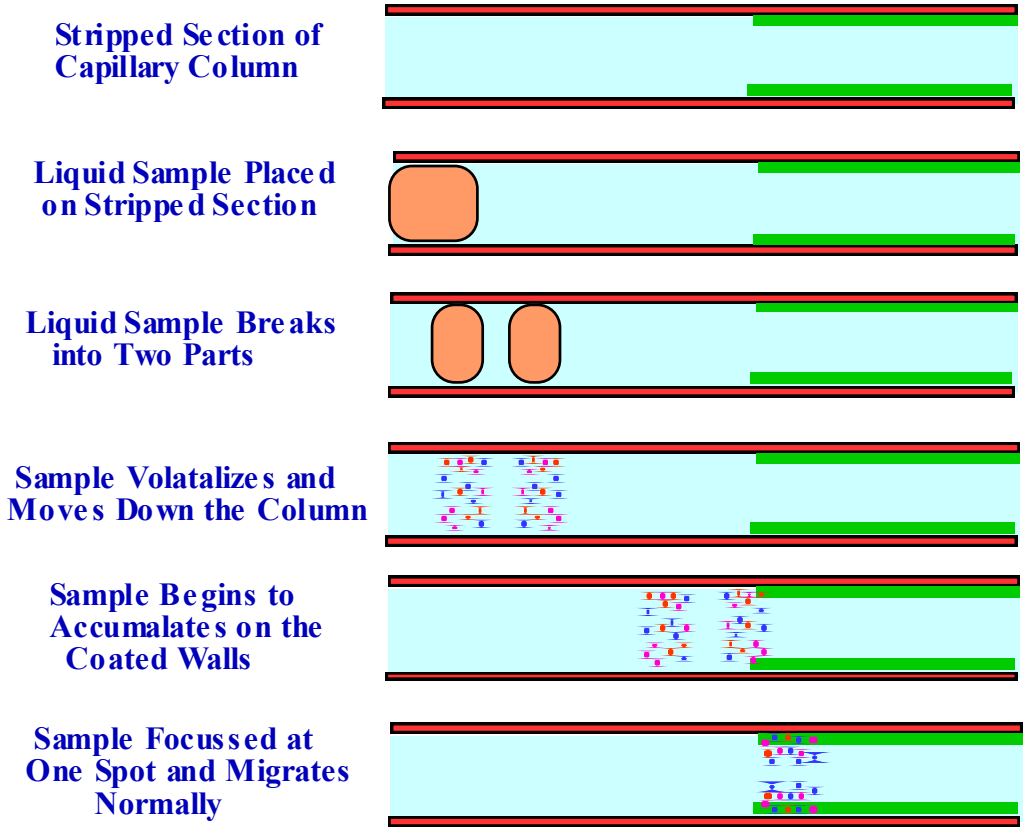


Figure 10. The Retention Gap Method of Sampling

The lower temperature aids in the accumulation of all the solutes where the stationary phase coating begins. In order for this method of sampling to be successful there must be a significant difference between the boiling points of the sample solvent and those of the components of the sample.

Sampling by Solute Focusing

Another method of sampling that avoids sample splitting is the 'solute focusing method' which is more effective, but requires more complicated and expensive equipment. The injector is designed so that there are two consecutive, independently heated and cooled zones

located at the beginning of the column. A diagram of the solute focusing system is shown in figure 11.

Figure 11 The Solute Focusing Method sampling

Initially the two zones are cooled and the sample is injected onto the first zone. The sample usually splits, but the carrier gas is allowed to remove the solvent, which is eluted through and out of the column. This leaves the sample spread along the first zone in dispersed fragments. The first zone is then heated while the second zone kept cool.

The solutes in the first zone are eluted through the zone at the higher temperature and the sample accumulate at the beginning of the cooled second zone. The sample has now been focused as a compact band at the beginning of the column. The second zone is now heated and the separation developed normally. This technique is more flexible than the

'retention gap method' but the apparatus is more expensive and the procedure more complex.

GC Columns

There are two types of columns in common use in GC and they are the conventional packed column and the open tubular column. The former are usually 2 to 4 mm I.D. and 1 to 4 meters long and, packed with a suitable adsorbent, are mostly used for gas analysis. As a result of the simpler injection procedure and the more precise sampling method, the packed column tends to give greater quantitative accuracy and precision. However, despite its problems with sample injection, the open tubular column is seen as the 'state of the art' column and is by far the most popular column system in general use. The length of open tubular columns range from about 10 m to 100 m and can have internal diameters from 100 μm to 500 μm. The stationary phase is coated on the internal wall of the column as a film 0.2 μm to 1 μm thick.

The Packed GC Column

Packed columns are usually constructed from stainless steel or Pyrex glass. Pyrex glass is favored when thermally labile materials are being separated such as essential oils and flavor components. However, glass has pressure limitations and for long packed columns, stainless steel columns are used as they can easily tolerate the necessary elevated pressures. The sample must, of course, be amenable to contact with hot metal surfaces. Short columns can be straight, and installed vertically in the chromatograph. Longer columns can be U-shaped but columns more than a meter long are usually coiled. Such columns can be constructed of any practical length and relatively easily installed. Pyrex glass columns are formed to the desired shape by coiling at about 700˚C and metal columns by bending at room temperature. Glass columns are sometimes treated with an appropriate silanizing reagent to eliminate the surface hydroxyl groups which can be catalytically active or produce asymmetric peaks. Stainless steel columns are usually washed with dilute hydrochloric acid, then extensively with water

followed by methanol, acetone, methylene dichloride and n-hexane. This washing procedure removes any corrosion products and traces of lubricating agents used in the tube drawing process. The columns are then ready for packing.

Adsorbents

There are two types of packing employed in GC, the adsorbents and the supports, on which the stationary phase is coated. There are both inorganic and organic types of GSC adsorbents, each of which have specific areas of application. All are ground and screened to provide a range of particle sizes that extend from about 30/40 mesh to 100/120 mesh. In general, the smaller the particle size the higher the column efficiency, but the packing procedure is more difficult. It is also essential that the particle size *range* should be as narrow as possible. Packing materials that have a wide size range not only produce columns with poor efficiencies, but again, are also far more difficult to pack.

Alumina, in an activated form, is used to separate the permanent gases and hydrocarbons up to about pentane. Alumina is usually activated by heating to 200˚C for about an hour. A common particle size is about 100/120 mesh and the pore size range from about 1 Å to 100,000Å. Silica gel in spherical form (prepared by spraying a neutralized silicate solution (a colloidal silica sol) into fine droplets, allowing the silica gel to be formed, and subsequently drying the droplets in a stream of hot air). Silica is produced with a wide choice of surface areas and porosity's, which can range from about 750 m^2/g and a mean pore size of 22 Å, to a material having a surface area of only 100m^2/g and a mean pore diameter of 300 Å. It is used for the separation of the lower molecular weight gases and some of the smaller hydrocarbons. In a specially prepared form, silica can be used for the separation of the sulfur gases, hydrogen sulfide, sulfur dioxide and carbon disulfide.

Molecular sieves are used for the separation of small molecular weight gases largely by exclusion. The naturally occurring aluminosilicates are called zeolites, the *synthetic* zeolites are the Linde Molecular Sieves of

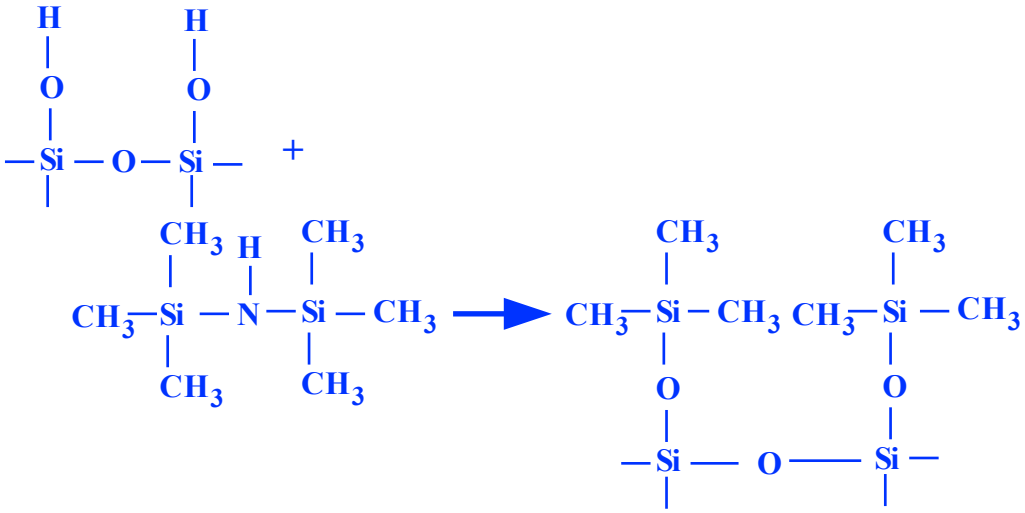
which there are a number of different types available for specific applications. The zeolites have a crystalline structure which does not collapse when dehydrated. When water is removed from the crystals, channels of uniform dimensions are left within the structure which becomes very porous and the size of the channels changes only slightly with temperature. Molecular sieves are used to separate substances of different molecular size and shape, *e.g.* straight chain hydrocarbons can be separated from their branched chain isomers. The molecular sieves designated 5A and 13X are commonly used for the separation of hydrogen, oxygen, nitrogen, methane and carbon monoxide and also argon, neon and the other rare gasses.

Carbon is also used as an adsorbent of which there are two types. The high surface area active carbon and the graphitized carbon (surface areas ranging from 5 m^2/g to about 100 m^2/g). The high surface area carbon, (*ca* 1000 m^2/g) is used for the separation of the permanent gases and may need special treatment to modify its activity. The graphitized carbon adsorbents are much less active and separations appear to be based largely on exclusion. Macroporous Polymers such as the packings founded on the co-polymerization of polystyrene and divinylbenzene are also popular GC adsorbents. The extent of cross-linking determines its rigidity and the greater the cross-linking the harder the resin becomes until, at the extreme, the resin formed is very brittle. The macro-porous resin consists of resin particles a few microns in diameter, which in turn are composed of a fused mass of polymer micro-spheres, a few Angstroms in diameter. Consequently, the resin polymer has a relatively high surface area as well as high porosity. They exhibit strong dispersive type interaction with solvents and solutes with some polarizability arising from the aromatic nuclei in the polymer.

Supports for GLC

There have been a number of materials used as supports for packed GC columns including, Celite (a proprietary form of a diatomaceous earth), fire-brick (calcined Celite), fire-brick coated with metallic silver or

gold, glass beads, Teflon chips and polymer beads. Today however, the vast majority of contemporary packed GLC columns are filled with materials that are either based on of Celtic or polystyrene beads as a support. Diatomaceous supports comprise the silica skeletons of microscopic animals that lived many millions of years ago in ancient seas and lakes. As food transfer through the cells could only occur by diffusion, the supporting structure had to contain many apertures through which the cell nutrients could diffuse. This type of structure is ideal for a gas chromatography support, as rapid transfer by diffusion through the mobile and stationary phases is an essential requisite for the efficient operation of the column. The original Celite material is too friable and the brickdust too active, and thus a series of modified Celites had to be introduced. There are two processes used to modify Celite. One was to crush, blend and press the Celite into the form of a brick and then calcine it at a temperature of about 900˚C. Under these conditions some of the silica is changed into cristobalite and traces of iron and other heavy metals interact with the silica causing the material to become pink in color. This material is sold under the trade name of Chromosorb P. The second process involves mixing the Celite with sodium carbonate and fluxing the material at 900˚C. This causes the structure of the Celite to be disrupted and the fragments adhere to one another by means of glass formed from the silica and the sodium carbonate. As the original Celite structure is disrupted, the material exhibits a wide range of pore sizes which differs significantly from the material that was calcined in the absence of sodium carbonate. This materials is sold under the name of Chromosorb W together with two similar materials called Chromosorb G and Chromosorb S. The residual deleterious adsorptive properties of the support are due to silanol groups on the surface and these can be removed by silanization. The support is treated with hexamethyldisilazane which replaces the hydrogen of the silanol group with a trimethylsilyl radical. The reaction proceeds as follows,



.In this way the strongly polar silanol groups are methylated and assume dispersive characteristics that do not produce peak tailing. Although the major contributors to adsorption by the support are the silanol groups, a residual adsorption results from the presence of trace quantities of heavy metals such as iron. which can be largely removed by acid washing priorto silanization. All three types of support are commercially available. None of these supports, however, are completely devoid of adsorptive properties and in may cases the effect of the residual adsorption must be further reduced by suitable stationary phase additives.

To try to completely eliminate adsorption effects from the support, Teflon was explored as a possible alternative to a diatomaceous earth. Teflon powder proved to have little adsorption, but also proved to be extremely difficult to pack into a column. So difficult, that it is very rarely used in general GLC analyses. Its inert character makes it useful for the separation of certain highly corrosive materials. It has a temperature limit of about 250˚C.

Glass beads have also been used as supports for packed GC columns and, if silanized, have little adsorption properties. Being non-porous, all the stationery phase must reside on the surface of the beads which gives them limited loading capacity. If the loading is increased, the stationary phase collects at the contact points of the spheres and form relatively thick accumulations, producing a high resistance to mass

transfer and consequently low column efficiency. Glass beads appears to be the worst compromise between a column packed with modified Celite and a wall coated glass ,or fused silica, capillary column. The macroporous polymer beads are used as supports as well as adsorbents. They exhibit significant adsorption as the support itself acts as a stationary phase and makes a substantial contribution to retention. However, with normal sample loads, the adsorption isotherm is linear and so the eluted peaks are symmetrical. Only stationary phases that do not affect the polymer in any way can be used with such beads, which is a distinct disadvantage. They also have relatively poor temperature stability.

Coating the Supports

It is important to have an accurate measure of the amount of stationary phase that has been placed on a support to ensure retention time reproducibility and qualitative accuracy. The reproducibility of the coating procedure may have particular significance when the analytical results are to be used for forensic purposes. The material can be coated by the direct addition of the stationary phase to the support, by the filtration method or by the slurry method. The slurry method of coating is the one that is recommended.

Coating by direct addition would appear to be the ideal quantitative method of preparing the column packings. A weighed amount of stationary phase is added directly to a known mass of support contained in a glass flask. The material is well mixed by rotating the flask for several hours, but even with extensive mixing, the stationary phase being is still irregularly distributed throughout the packing. As a result, the efficiency of the column slowly increases with use, as the stationary phase distributes itself more evenly throughout the packing. It may take several weeks of use for the column to give a constant maximum efficiency.

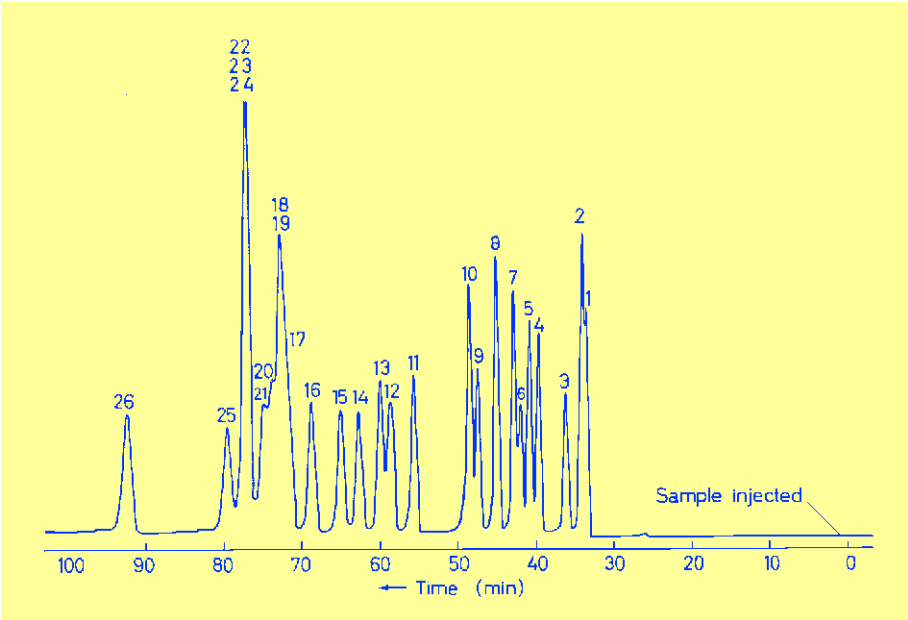
The filtration method gives a packing with the stationary well distributed over the support but the loading can not be accurately calculated. A known mass of stationary phase is dissolved in sufficient

solvent to provide excess liquid when mixed with a weighed amount of the support. The mixture is filtered under vacuum and the volume of the filtrate measured. From the volume of filtrate, the amount of solvent remaining on the support can be calculated and hence this stationary phase loading can be accessed. The bed is then sucked dry, the solvent evaporated and the coated support packed into the column. The amount of stationary phase on the support is not determined accurately by this method due to solvent losses by evaporation.

In the slurry method of coating, a weighed amount of the support is placed in the flask of a rotary evaporator and the required mass of stationary phase added. An appropriate volatile solvent is then added in sufficient quantity to produce a free flowing slurry. The flask is then rotated at room temperature for ten minutes to ensure complete mixing. The rotating flask is then heated and the solvent removed by evaporation. When the packing appears dry, the material is then heated to about 150˚C in and oven to remove the final traces of solvent. This method of coating gives an extremely homogeneous surface distribution of stationary phase throughout the support and an accurate value for the stationary phase loading.

Column Packing

Short columns are usually straight and can be packed vertically.



1. 2,2-Dimethylpentane
2..2,4-Dimethylpentane
3. 2,3,3-Trimethylbutane
4. 3,3-Dimethylpentane
5.2-Methylhexane
6. 2,3-Dimethylpentane
7.3-Methylhexane
8.3-Ethylpentane
9.2,3,4-Trimethylpentane
10.*n*-Heptane
11.2,2-Dimethylhexane
12.2,5-Dimethylhexane
13.2,4-Dimethylhexane
14.2,2,3-Trimethylpentane
15.3,3-Dimethylhexane
16.2,3,4-Trimethypentane
17.2,3-Dimethylhexane
18. 2-Methylheptane
19.2-Methyl-3-ethyl-pentane
20.2,3,3-Trimethylpentane
21.4-Methylheptane
22.3-Methylheptane
23.3-Ethylheptane
24. 2,4-Dimethylhexane
25.#-Methyl-2-ethylpentane
26.*n*-Octane

The column temperature was 78.6˚C, and the column packing 2.5w/w Apiezon Oil on C22 firebrick 100-120 mesh. The column diameter was 2 mm, the inlet pressure 200 p.s.i., and the column efficiency 30,000 theoretical plates. The argon detector was used and the sample weight was 20 µg.

Figure 12. Chromatogram from a 50 ft Column Showing the Separation of the Isomeric Heptanes and Octanes

The packing is added, about 0.5 ml at a time, and the column tapped until the packing had settled. Another portion of packing is then added and the process repeated until the column is full. U-shaped columns are packed in the same manner. Columns up to 50 ft long can be packed in a series of U's and then each U column joined with a low dead volume connection. If the columns were glass they were usually filled through

an opening at the top of each U which was terminated in a plug of quartz wool and sealed-off in a blow-pipe flame. These long packed columns could be operated at a maximum of 200 psi. and could provide efficiencies of up to 50,00 theoretical plates. Such columns could tolerate charges of several microliters. A chromatogram of the isomeric heptanes and octanes obtained from a 50 ft column is shown in figure 12.However, straight columns are clumsy to use and occupy a large amount of space which is often difficult to thermostat.

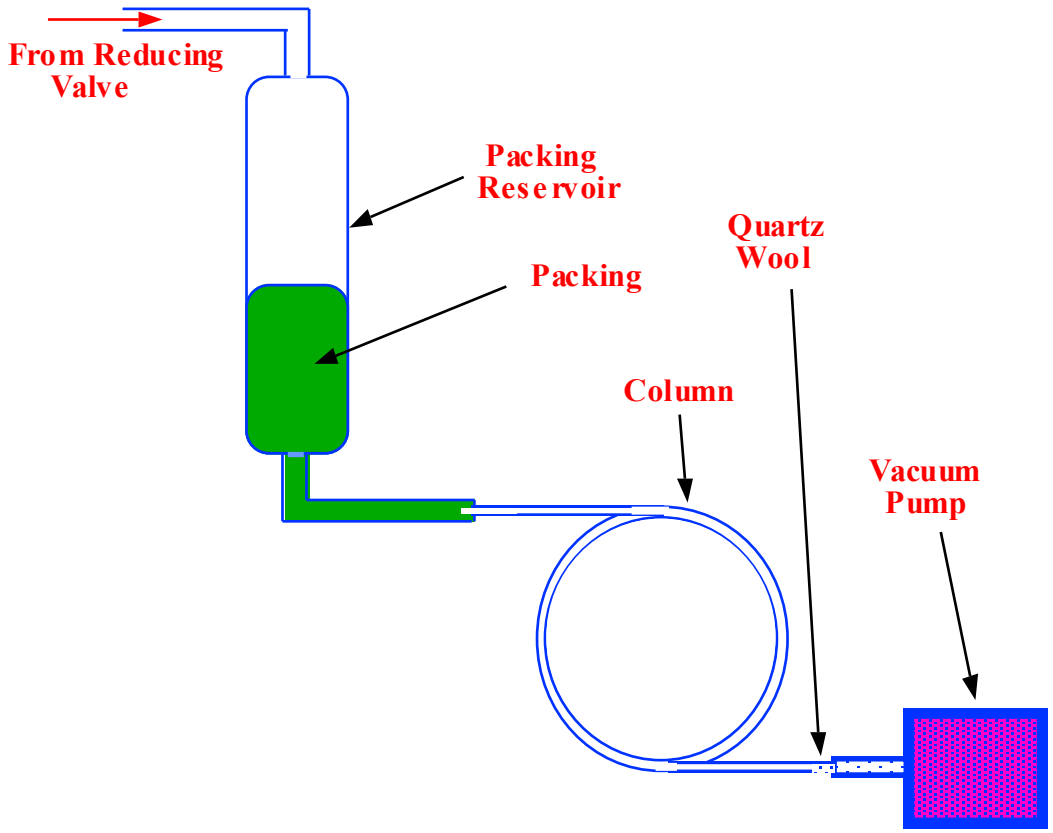


Figure 13 An Example of a Column Packing Apparatus

The coiled column although more difficult to pack has been readily accepted due to the compact nature of their design. To obtain adequate efficiencies, however, a special packing procedure had to be developed. The apparatus used is shown in figure 13. The packing is placed in a reservoir attached to a gas supply that forces the packing through the column. The column exit is connected to a vacuum pump. A wad of quartz wool is placed at the end of the column, constrained

by a small restriction, that prevents the wad from being sucked into the pump. The vacuum and gas flow are turned on simultaneously and the packing is swept rapidly through the column. This causes the material to be slightly compacted along the total length of the column and has been shown to produce well-packed columns. The procedure is a little tedious and the success rate is sometimes less than 90%. In addition, the process does not lend itself to automation. The difficulties involved preparing packed columns have also contributed to the preferential popularity of the open tubular columns.

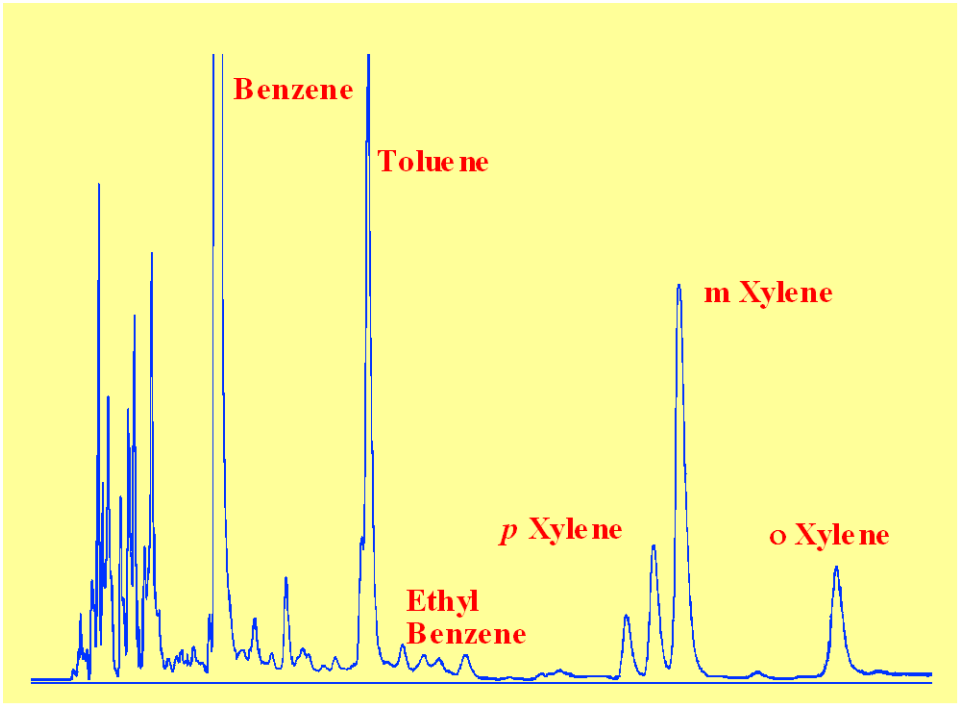


Figure 14. The Separation of a "Benzole" Mixture on a Packed Column, 40 ft Long

The production of capillary columns can be largely automated and several columns can be prepared simultaneously. Another example of a chromatogram from a 40 ft packed column 2 mm I.D., this time of a "benzole" mixture is shown in figure 14.

The column was packed with 5% w/w polyethylene glycol adipate coated on deactivated fire brick and operated isothermally at 130˚C with an inlet gas pressure of 140 psi. The analysis time was about 3.5 hours. The column efficiency was about 40,000 theoretical plates and

all the xylene isomers are separated. The two previous off-scale peaks are benzene and toluene. This separation could be achieved equally well on a open tubular column and probably in less than half the time. The advantage of the packed column would be that much higher sample loads can be placed on the column and thus the dynamic range of the analysis can be made much greater. Components present at a level of 0.001% can be easily separated and determined quantitatively without any preliminary fractionation or concentration.

The Capillary or Open Tubular Column

Capillary columns are fabricated from stainless steel or quartz. Metal capillary columns must be carefully cleaned to remove traces of extrusion lubricants before they can be coated, usually by washing with methylene dichloride, methanol and then water. After removing oil and grease, the columns are washed with dilute acid to remove metal oxides or other corrosion products that may remain adhering to the walls, washed with water and the again washed with methanol and methylene dichloride. Finally the column is dried in a stream of hot nitrogen. Metal columns provide the high efficiencies expected from open tubular columns and were used for the analysis of petroleum and fuel oils, etc. Metal columns, however, have some disadvantages as although easily coated with dispersive stationary phases (*e.g.*, squalane, Apiezon grease etc.) they are not so easily coated with the more polar stationary phases such as CARBOWAX®. In addition, hot metal surfaces can cause decomposition or molecular rearrangement of many thermally labile materials such as the terpenes contained in essential oils. Metal can also react directly with some materials by chelation and adsorb polar material which results in asymmetric and tailing peaks. Nevertheless, metal columns are rugged, easy to handle and easy to remove and replace in the chromatograph consequently, their use has persisted in many application areas despite the introduction of fused silica columns.

Desty *et al.* (4), tried to eliminate the activity of the open tubular column surface by developing the first silica-based columns and invented an extremely clever device for drawing soft glass capillaries. Desty produced both circular rigid soft glass and circular rigid Pyrex capillary columns, but their permanent circular shape, made them difficult to fit to unions connecting columns to injector and column to detector. By careful surface treatment the rigid glass tubes could be coated with polar stationary phases such as CARBOWAX®. Dandenau (5) introduced flexible fused silica capillary columns using the quartz fiber drawing technique. The solid quartz rod used in quartz fiber drawing was replaced by a quartz tube and the drawing rates adjusted appropriately. The quartz tubes had to be coated on the outside with polyimide to prevent moisture attacking the surface and producing stress corrosion. Coating the capillary tube with a polyimide polymer immediately after drawing prevents moisture coming in contact with the surface and thus stabilizes the tube. Soft glass capillaries can be produced by the same technique at much lower temperatures (6) but the tubes are not as mechanically strong or as inert as quartz capillaries. Surface treatment is still necessary with fused quartz columns to reduce adsorption and catalytic activity and also make the surface sufficiently wettable to coat with the selected stationary phase. The treatment may involve washing with acid, silanization and other types of chemical treatment, including the use of surfactants.

Deactivation procedures used for commercial columns are kept highly proprietary. However, a deactivation program for silica and soft glass columns that is suitable for most applications would first entail an acid wash. The column is filled with 10% w/w hydrochloric acid, the ends sealed and the column is then heated to 100°C for 1 hour. It is then washed free of acid with distilled water and dried. This procedure is believed to remove traces of heavy metal ions that can cause adsorption effects. The column is then filled with a solution of hexamethyldisilazane contained in a suitable solvent, sealed, and again heated to the boiling point of the solvent for 1 hour. This procedure blocks any hydroxyl groups that were formed on the surface during the

acid wash. If the column is to be coated with a polar stationary phase, it may be advantageous to employ a polar or semipolar reagent as opposed to the dispersive silicone to facilitate coating. The column is then washed with the pure solvent, dried at an elevated temperature in a stream of pure nitrogen and is then ready for coating.

Open tubular columns can be coated internally with a liquid stationary phase or with polymeric materials that can be polymerized to form a relatively rigid, internal polymer coating. There are two methods for coating a capillary column the *dynamic method* and the *static method.*

Dynamic Coating

A plug of solvent containing the stationary phase is placed at the beginning of the column. The strength of the solution, among other factors, determines the thickness of the stationary phase film. In general the film thickness of an open tubular column ranges from 0.25 μm to about 1.5 μm.

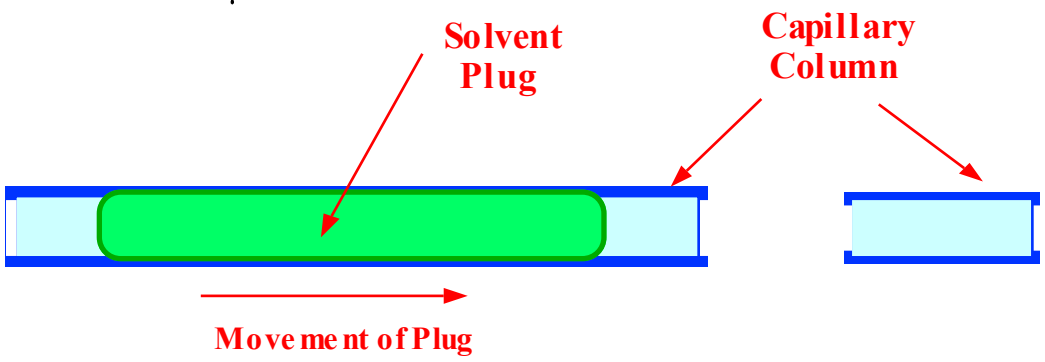


Figure 15. The Dynamic Coating Procedure for an Open Tubular Column

In practice, a 5% w/w of stationary phase in the solvent will produce a film thickness of about 0.5 μm. However, this is only approximate, as the film thickness is also determined by the physical properties of the surface, the solvent and the stationary phase. The coating procedure is depicted in figure 15. After the plug has been run into the front of the column (sufficient to fill about 10% of the column length), pressure is applied to the front of the column to force the plug through the column at 2-4 mm per second (it will take about 5.5 hours for the plug to pass

through a 60 m column). When the plug has passed through the column, the gas flow is continued for about an hour. The gas flow must not be increased too soon, or the stationary phase solution on the walls of the tube is displaced forward in the form of ripples, which produces a very uneven film. After an hour the flow rate can be increased and the column stripped of solvent. The last traces of the solvent are removed by heating the column above the boiling point of the solvent at an increased gas flow rate. Complete solvent removal can be identified by connecting the column to a detector and observing the baseline drift of the detector.

Static Coating

The entire column is filled with a solution of the stationary phase and one end is connected to a vacuum pump. As the solvent evaporates, the front retreats back down the tube leaving a coating on the walls. A diagram of the static coating procedure is shown in figure 16. The column is filled with a solution of stationary phase having a concentration appropriate for the deposition of a film of the desired thickness. Again the required concentration will depend on the stationary phase, the solvent, the temperature and the condition of the wall surface. Unfortunately, the optimum solvent concentration is not theoretically predictable and requires some preliminary experiments to be carried out to determine the best coating conditions.

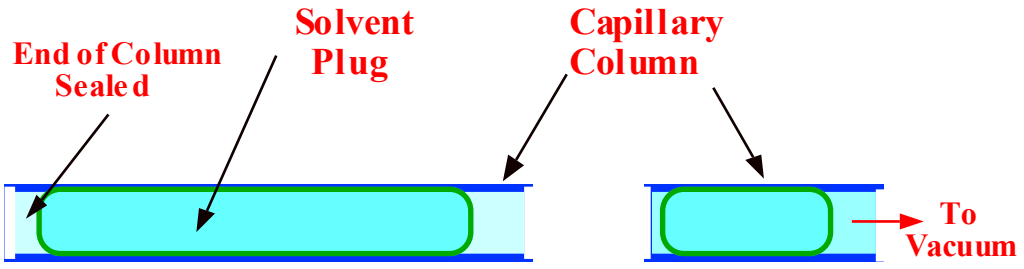
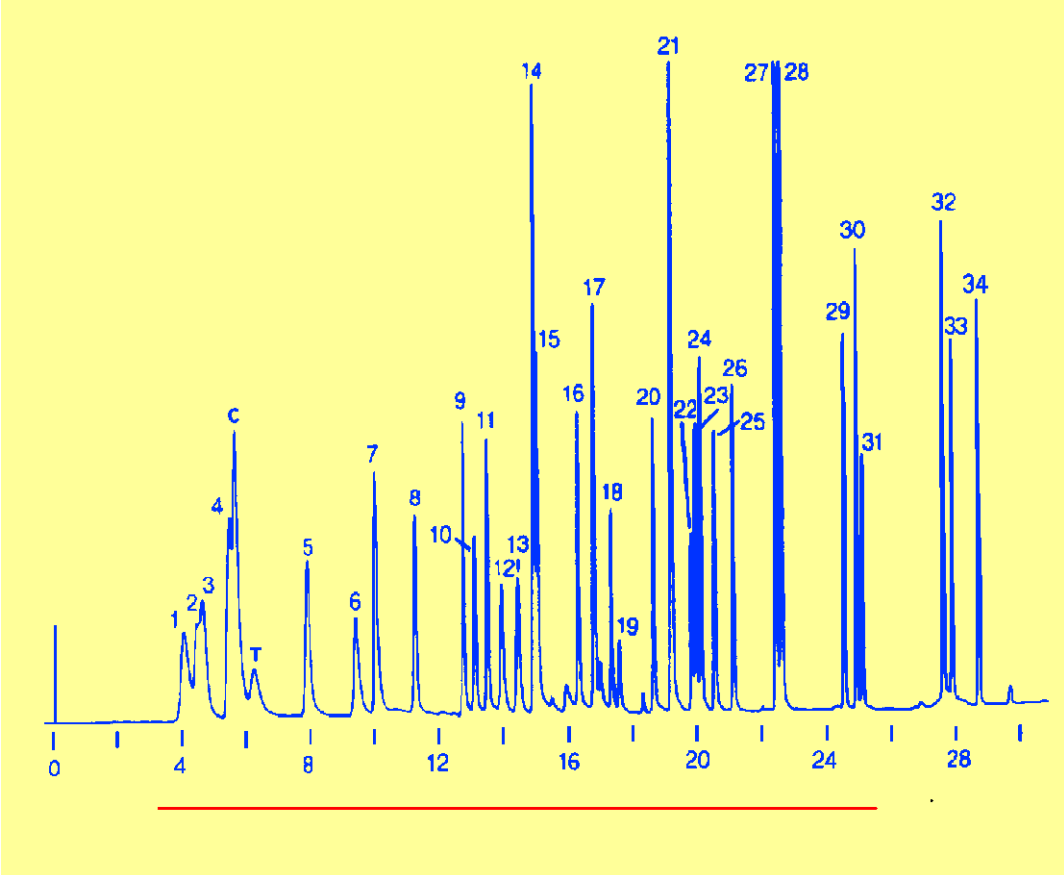


Figure 16. The Static Method for Coating Open Tubular Columns

After filling, one end of the column is sealed, and the other end is connected to a high vacuum pump and placed in an oven and the solvent slowly evaporates and the front retreats leaving a film of

solution on the walls. The solvent then evaporates from this film and the stationary phase remains as a thin coating on the wall.



Courtesy of Supelco, Inc.

1/ Dichlorodifluoromethane
2/ Chloromethane
3/ Vinyl chloride
4/ Bromomethane
5/ 1,1-Dichloroethylene
6/ Methylene chloride
7/ trans-1,2-Dichloroethylene
8/ 1,1-Dichloroethane
9/ cis-1,2-Dichloroethylene
10/ Chloroform
11/ Bromochloromethane
12/ 1,1,1-Trichloroethane
13/ Carbon Tetrachloride
14/ Benzene
15/ 1,2-Dichloroethane
18/ Bromodichloromethane
19/ 2-Chloroethyl vinyl ether
20/ cis-1,3-Dichloropropene
21/ Toluene
22/ trans-1,3-Dichloropropene
23/ 1-Chloro-2-bromopropane
24/ 1,1,2-Trichloroethane
25/ Tetrachloroethylene
26/ Dibromochloromethane
27/ Chlorobenzene
28/ Ethylbenzene
29/ Bromoform
30/ 1,4-Dichlorobutane
31/ 1,1,2,2-Tetrachloroethane
32/ 1,3-Dichlorobenzene

16/ Trichloroethylene
17/ 1,2-Dichloropropane
33/ 1,4-Dichorobenzene
34/ 1,2-Dichlorobenzene

Figure 18. The Separation of Volatile Priority Pollutants

The procedure is continued until all the solvent has evaporated and, except for the stationary phase, the column is empty. This process may take hours to complete. The procedure needs no attention and thus, can be carried out overnight. This procedure is more repeatable than the dynamic method of coating but, produces columns having similar performance to those dynamically coated.

Irrespective of the coating method, column stability depends on the stability of the stationary phase film which depends on the constant nature of the surface tension forces that hold it to the column wall. These surface tension forces can be reduced with an increase in temperature or by the solutes passing through the column. As a consequence, the film can suddenly break up. Thus, it would be highly desirable if the stationary phase was bonded to the column walls or polymerized *in situ*. Such coatings are called *immobilized* stationary phases and can not be removed by solvent washing.

Stationary phases that are polymeric can sometimes be formed on the wall surface by depositing the monomers or dimers on the walls and then initiating polymerization either by heat or an appropriate catalyst. This locks the stationary phase to the column wall and is thus completely immobilized. Polymer coatings can be formed in the same way using dynamic coating. The techniques used for immobilizing the stationary phases are also highly proprietary and little is known of the methods used by companies that manufacture the columns. In any event, most chromatographers do not want the trouble of coating their own columns and prefer to purchase proprietary columns.

Very difficult separations can be achieved using the capillary column, and in a relatively short time. An example of the separation of a complex mixture on a capillary column is shown in figure 17. The column used was designated as a VOCOL column and was 60 m long,

0.75 mm I.D. and carried a film of stationary phase 1.5 micron thick. The column was held a 10˚C for 6 minutes and then programmed to 170˚C at 6˚C per minute. The carrier gas was helium at a flow rate 10 ml/min. The detector employed was the FID. This chromatogram demonstrates the clear advantages of capillary columns over packed column. Not only does the column produce exceeding high efficiencies but they are also achieved with reasonable separation times.

Open Tubular Column Types

Open Tubular columns are broadly split into two classes, *the wall coated open tubular columns* or WCOT Columns (which have already been described and are by far the mot popular,) and the *porous layer open tubes* or PLOT Columns. The two types of column are shown diagramatically in figure 18. The PLOT columns are largely used for gas analysis and the separation of low molecular weight hydrocarbons. The external diameter of PLOT columns range from 320 to 530 μm with a porous layer that can be 5 to 50 μm thick.

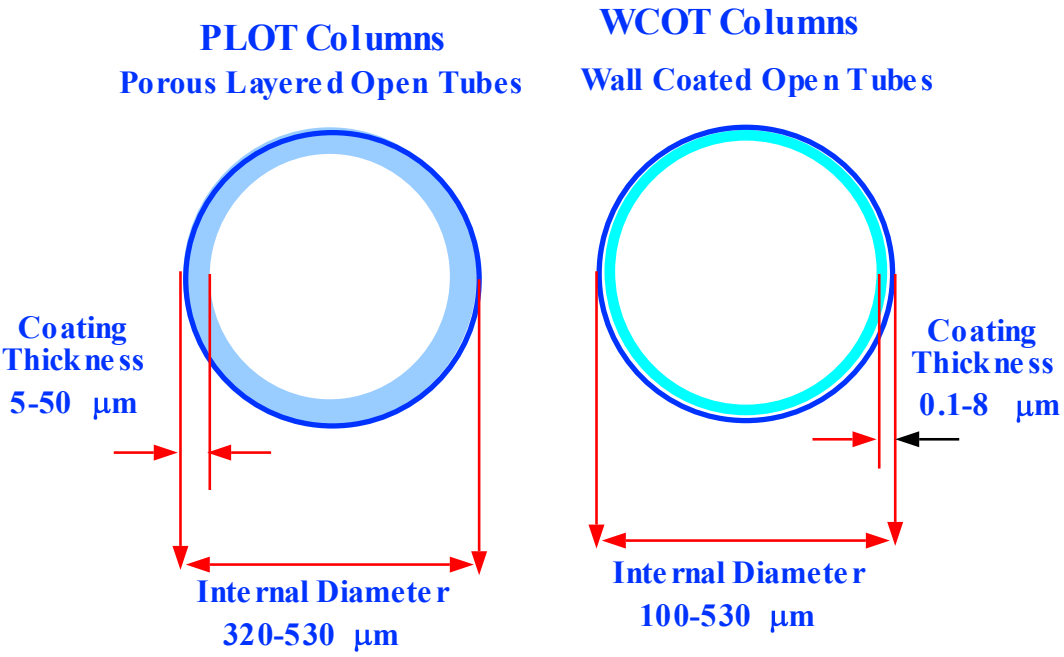


Figure 18. Open Tubular Column Types

The technique of coating the walls with solid particles is again largely proprietary but stable and reproducible columns can be prepared and are commercially available. An example of the use of a PLOT column to separate and determine the impurities in a 2,3-butadiene sample is shown in figure 19. The column was 50 m long, 0.32 mm I.D. and coated with a 5 µm layer of aluminum oxide modified with potassium chloride. The separation was carried out by programming the column temperature from 100 ˚C to 200 ˚C at 6 ˚C per minute. The carrier gas employed was nitrogen and the gas velocity was 35 cm/sec. The sample (100 µl of gas) was placed on the column with a split injector and the detector used was the FID. Figure 20 shows an excellent separation was obtained with near baseline separation for all solutes. Such a separation would allow accurate and precise quantitative assay. The analysis of hydrocarbon gasses is an important and is a control assay in almost all oil refinery quality control laboratories.

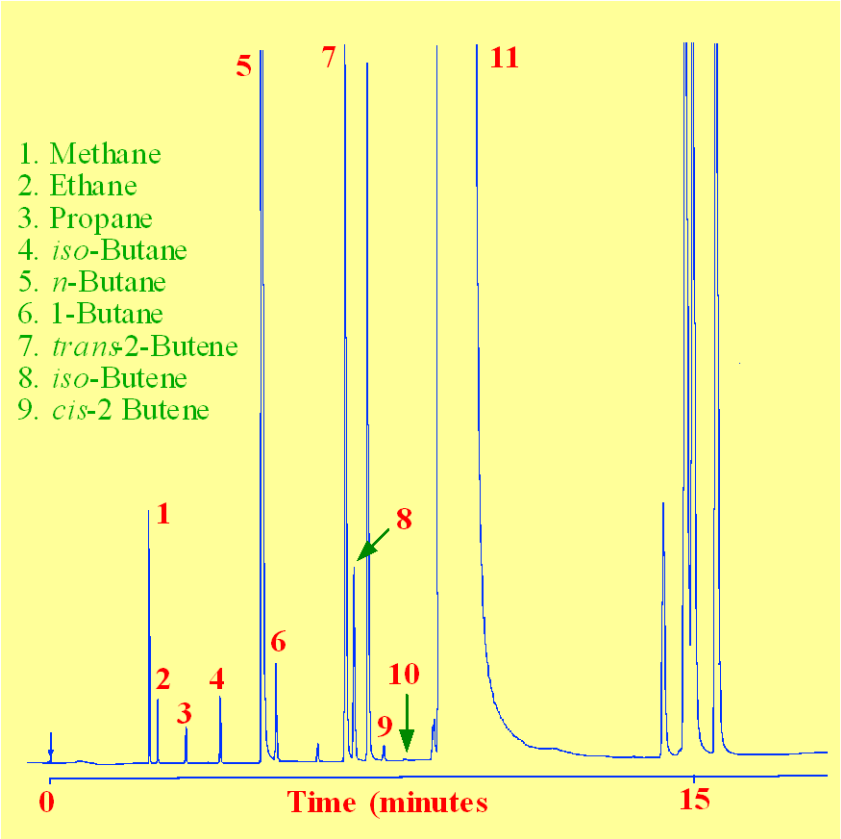


Figure 19. The Separation of the Impurities of 1,3-Butadiene

Chiral Stationary Phases

Modern organic chemistry and pharmaceutical research are becoming increasingly interested in methods of asymmetric syntheses. This enthusiasm has been provoked by the differing physiological activity that has been shown to exist between the geometric isomers of pharmaceutically active compounds. A tragic example being the drug Thalidomide, which was made available as a racemic mixture of N-phthalylglutamic acid imide. The important physiological activity resides in the R-(+)-isomer and it was not found, until too late, that the S-enantiomer was probably tetratogenic and caused serious fetal malformations. The separation and identification of isomers can, clearly, be very important and chromatography can be very effective in the resolution of such mixtures. The use of GC for the separation of asymmetric isomers is not as common as LC, but nevertheless there some very effective optically active stationary phases that can be used in GC for the separation of enantiomers.

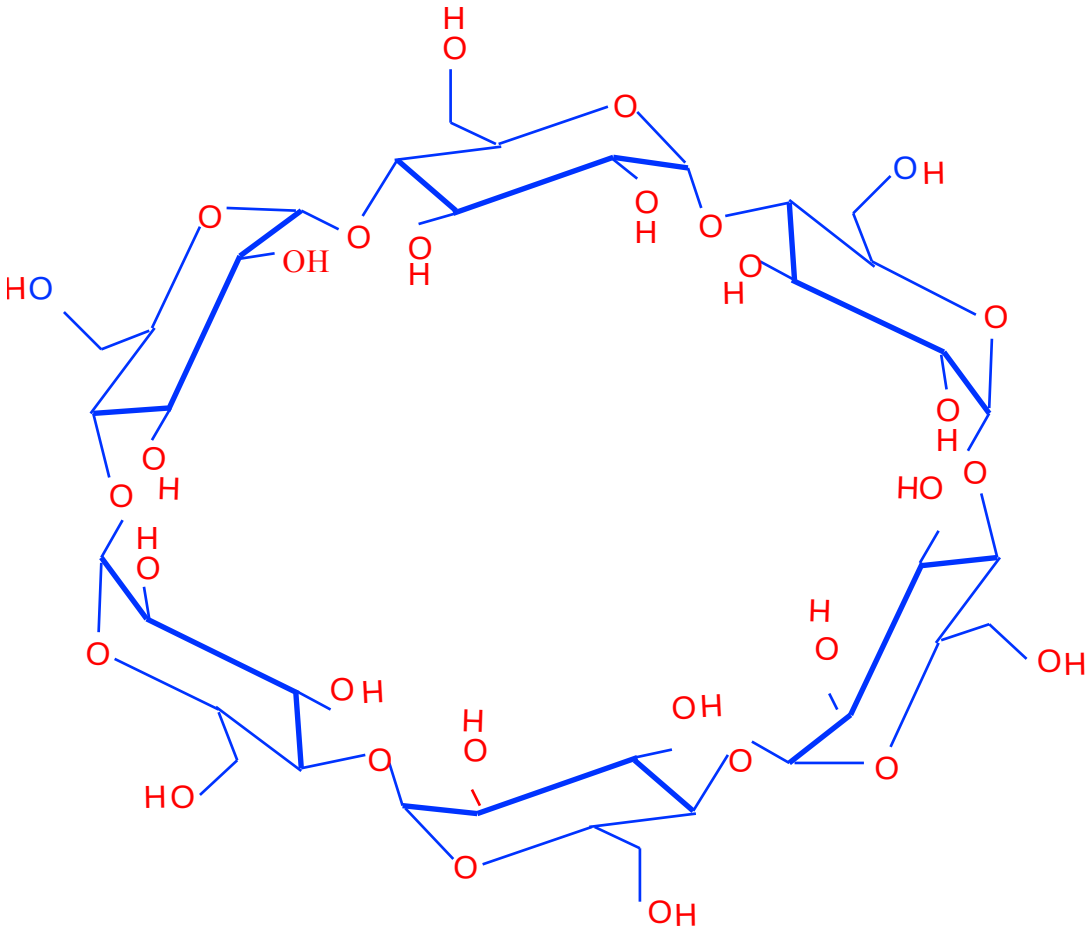
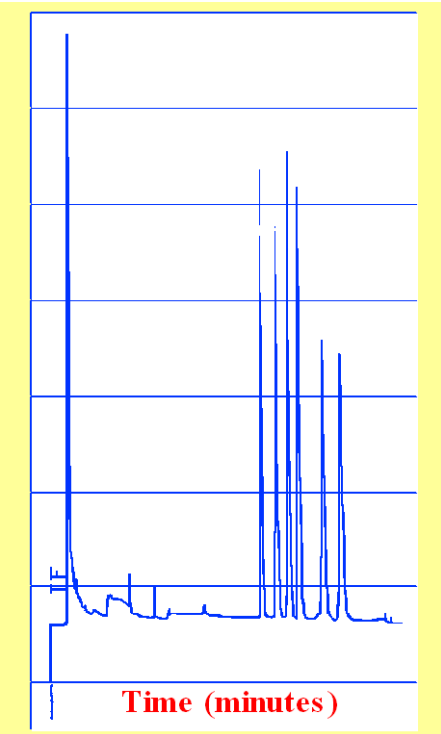


Figure 20. The Structure of α – Cyclodextrin

Some of the more useful GC stationary phases are based on the α- and β-cyclodextrins already described. The α-cyclodextrin structure is depicted in figure 20. The columns are usually 30 or 60 m long 0.25 mm .D. and have an operating temperature range of 30°C to 250°C. Both the α and β forms are commercially available and both have been used very satisfactorily for the separation of the optical isomers of different flavors and fragrances. In order to employ the cyclodextrins as stationary phases for GC the permethylated α- or β-cyclodextrins are often embedded in a siloxane matrix (e.g. 35% phenyl-65% methyl polysiloxane) which is deposited on the walls of fused quartz capillary tubes. The phenyl-methyl-polysiloxane confers onto the column an intermediate level of polarity so the separations are basically *enthalpic* due to the dispersive and polar interactions that take place largely with the polymer but also *entropic* resulting from the chiral selectivity of the cyclodextrins.



Courtesy of Supelco, Inc.

Solute	Retention Time (min.)
1. R-N-TFA-Amphetamine	10.12
2. S-N-TFA-Amphetamine	10.86
3. S-N-TFA-Methamphetamine	11.41
4. R-N-TFA-Methamphetamine	11.92
5. d-N-TFA--Pseudoephedrine	13.08
6. l-N-TFA-Pseudoephedrine	13.93

Figure 21. The Separation of Some Chiral Amines

Derivatization of the base cyclodextrin structure can introduce groups to which only one enantiomer can interact, while the other(s) are partially or wholly entropically hindered from interaction. This increases the *differential* interaction between the enantiomers and the stationary phase, thus, increasing the separation ratio and hence the resolution.An example of the use of a proprietary modified cyclodextrin in the separation of some chiral amines is shown in figure 21. It is seen that excellent separations were obtained. A G-PN column was used which was 30 m long and 0.25 mm I.D. and operated at 130˚C employing helium as the carrier gas. The basic materials are

patented and the technique of bonding and coating the material onto the column is extremely difficult and involves much proprietary art.

The Column Oven and Temperature Programmer

The column oven should operate over a fairly wide temperature range (*e.g.* from 5˚C to 400˚C). In practice, however, the maximum oven temperature needed is usually less than 250˚C, particularly when synthetic stationary phases are being used, as many of them tend to be unstable and either decompose or volatilize at higher temperatures. Similarly, initial temperatures below 50˚C are also rarely needed. The oven usually has air circulation driven by a powerful fan to ensure an even temperature throughout the oven. The temperature in any part of the oven should be stable to ± 0.5 ˚C and when operating isothermally the column temperature should be constant to ± 0.2 ˚C. The oven should have a capacity of 1-2 cu. ft. and is supplied with fittings to accept more than one column and some switching valves if so desired. Such equipment is needed for multidimensional chromatography.

The temperature programmer (hardware and software) usually has a range of linear gradients from 0.5˚C/min. to about 20˚C/min. Some programmers include nonlinear programs such as logarithmic and exponential, but most GC analyses can be effectively accomplished using linear programs only. The program rate can be changed at any time in the chromatographic development or intermittent isothermal periods can be inserted where necessary in the program. The temperature programming limits are usually the same as those of the oven (*viz*. 5˚C to 400˚C). All connections between the column and the detector, that pass though the column oven wall to the detector oven, are supplied with their own heaters so that no part of the conduit can fall below the column oven temperature. A cool spot in the conduit will cause condensation which can result in broad and distorted peaks.

GC Detectors

A large number of GC detectors have been developed and made commercially available. In general, GC detectors are 4 to 5 orders of magnitude more sensitive than LC detectors and, thus, are ideal for trace analysis and environmental monitoring. The detectors with the highest sensitivity tend to be specific and sense specific types of sample (*e.g*., halogenated substances by the electron capture detector). Conversely, those detectors with a catholic response, although highly sensitive compared to LC detectors (*e.g*. the flame ionization detector) are significantly less sensitive than the specific detectors. The detectors with a catholic response are the most popular and the majority of GC separations are monitored by the flame ionization detector (FID). The most commonly used specific detectors are the nitrogen phosphorus detector (NPD) and the electron capture detector (ECD) The katharometer detector, although having relatively poor sensitivity is widely used in gas analysis.

The Flame Ionization Detector

The FID, invented by Harley and Pretorious (7), and separately by McWilliams and Dewer (8), evolved from the Heat of Combustion Detector developed by Scott (9). The FID detector employs hydrogen as the combustion gas which is mixed with the column eluent (helium, nitrogen or other appropriate gas) and burnt at a small jet situated inside a cylindrical electrode. A potential of a few hundred volts is applied between the jet and the electrode and when a carbon containing solute is burnt in the jet, the electron/ion pairs that are formed are collected at the jet and cylindrical electrode. The current is amplified and fed to a recorder or to the A/D converter of a computer data acquisition system. A diagram of the basic FID is shown in figure 22. During the process of oxidation, oxidized or partially oxidized fragments of the solute are formed in the flame which are thought to generate electrons by thermionic emission. The background current (ions and electrons from the hydrogen flame alone) is very small (1-2 x 10^{-12} amperes) and consequently, the noise level is also commensurably small (about 10^{-14} amperes).

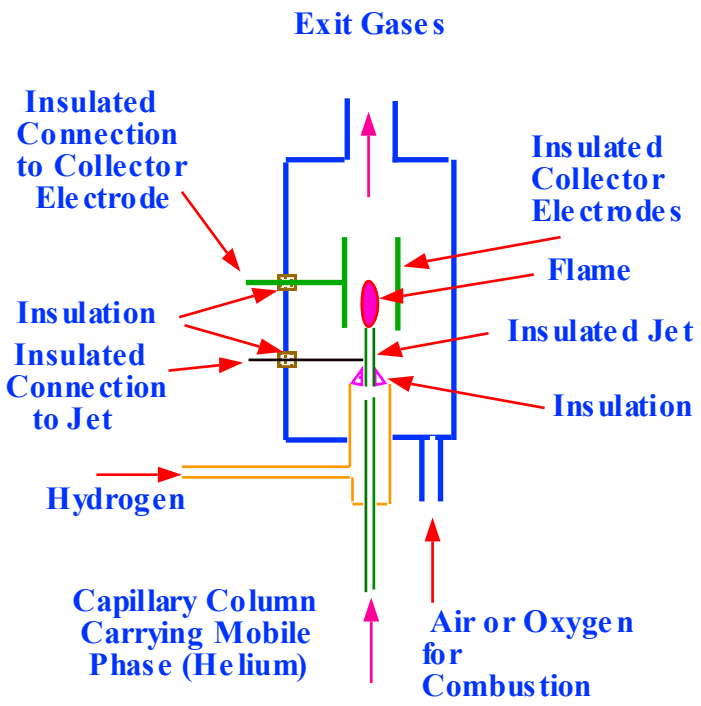
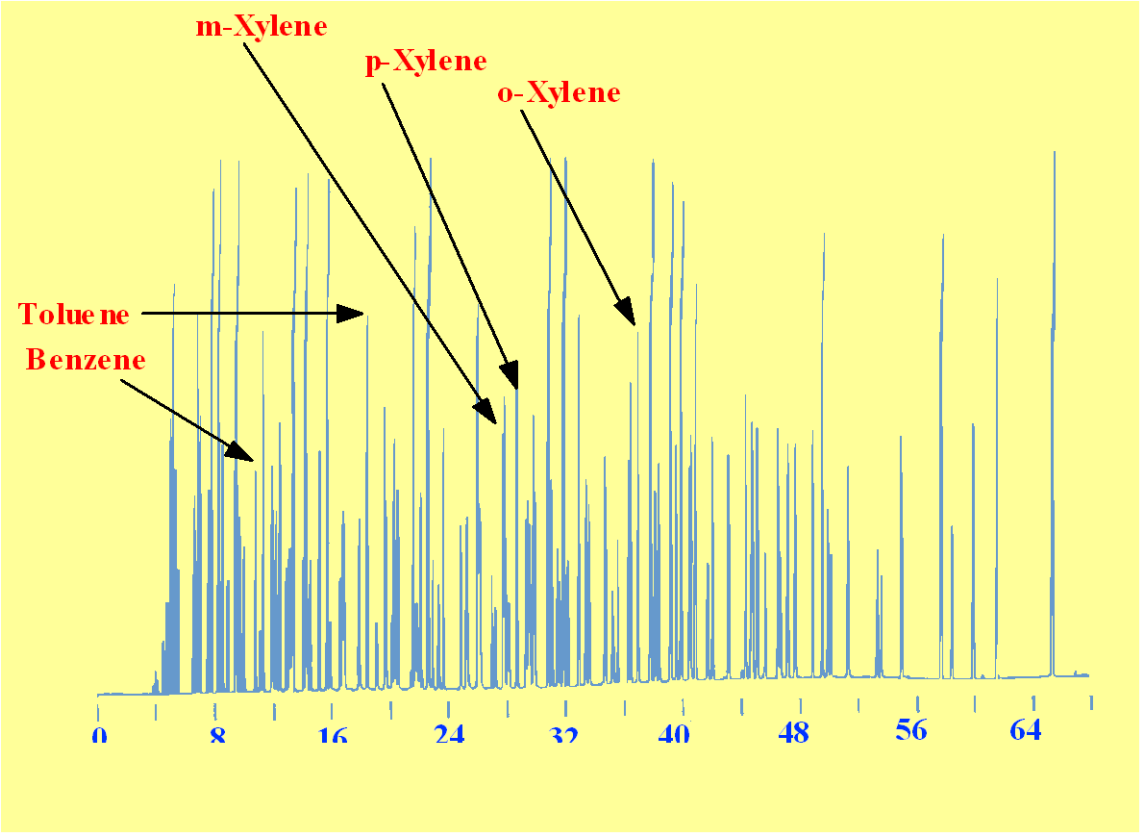


Figure 22. The Flame Ionization Detector

The ionization process is not very efficient, only 0.0018% of the solute molecules produce ions, (about two ions or electrons per 10^5 molecules). Nevertheless, because the noise level is very small, the minimum detectable mass of *n*-heptane is only 2×10^{-12} g/sec. At a column flow rate of 20 ml/min. this is equivalent to a minimum detectable concentration of about 3×10^{-12} g/ml. The detector responds to *mass per unit time* entering the detector, not *mass per unit volume* consequently the response is almost independent of flow rate. This is particularly advantageous and allows it to be used very effectively with capillary columns. Although the column eluent is mixed with the hydrogen prior to entering the detector, as it is mass sensitive and not concentration sensitive, the diluting effect has no impact on the sensitivity. The FID detects virtually all carbon containing solutes, with the exception of a small number of small molecular compounds such as carbon disulfide, carbon monoxide, etc. In fact, due to its diverse and comprehensive response, it is considered a universal detector.

An example of the use of the FID in a *paraffin, isoparaffin, aromatic, naphthene and olefin* analysis of a hydrocarbon mixture (frequently called the PIANO analysis) is shown in figure 23. The column was the

Petrocol DH 50.2, 50 m long and 0.5 mm I.D. and made from fused silica. The column temperature was held a 35ºC for 5 minutes and then programmed up to 200°C at 2°/min. The carrier gas was helium and the mobile phase velocity of 20 cm/sec. Many standard tests carried out in the hydrocarbon and pharmaceutical industries and for environmental testing have been designed to utilize the FID as the detector



Courtesy of Supelco Inc.

Figure 23. The Separation of a PIANO Standard Mixture

The Nitrogen Phosphorus Detector (NPD)

The nitrogen phosphorus detector (NPD), is a highly sensitive but specific detector and evolved directly from the FID. It gives a strong response to organic compounds containing nitrogen and/or phosphorus. Although it appears to function in a very similar manner to the FID, in

fact, it operates on an entirely different principle. A diagram of an NP detector is shown in figure 24.

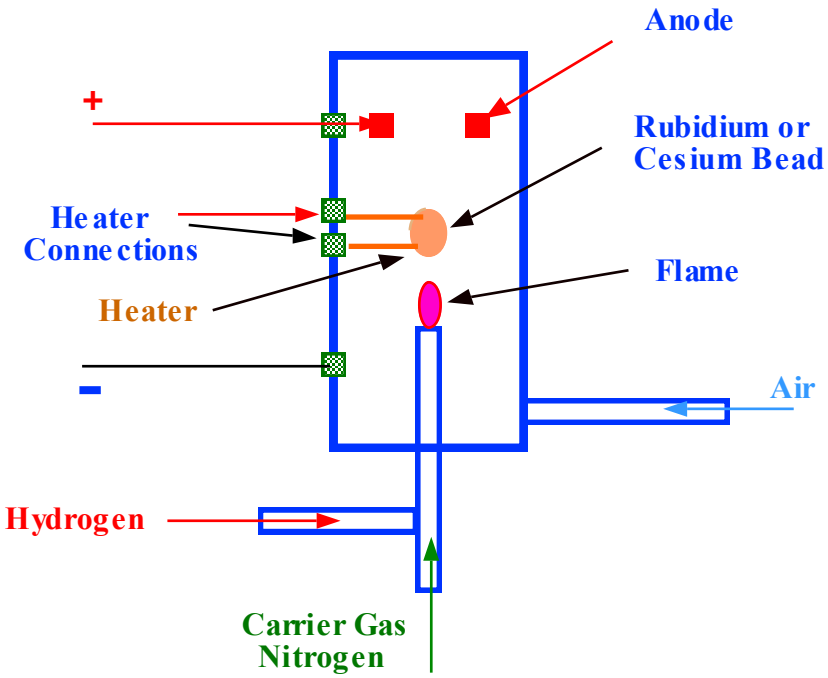
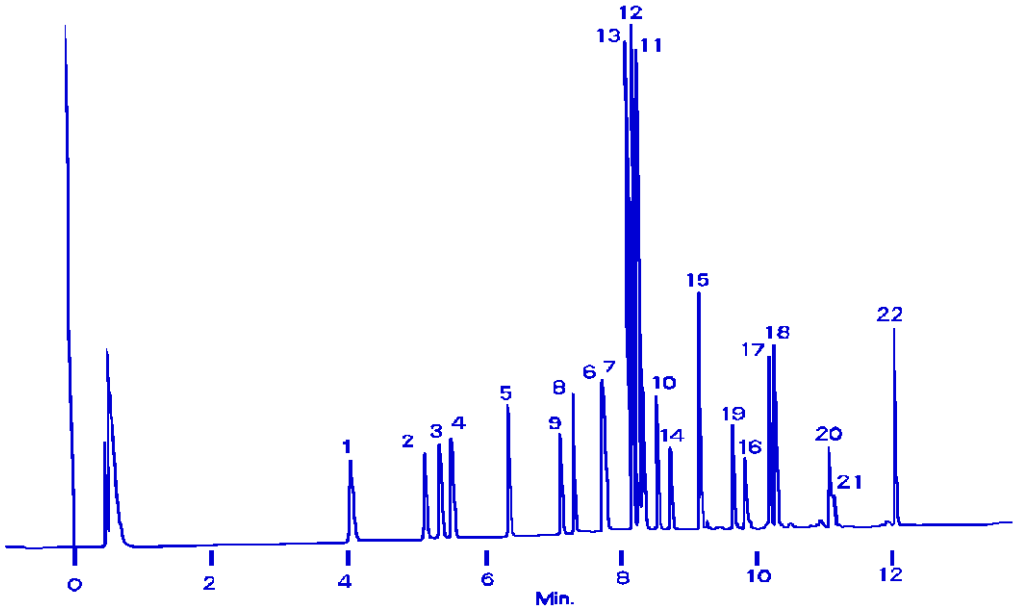


Figure 24. The Nitrogen Phosphorus Detector

The actual NPD sensor is a rubidium or cesium bead contained inside a small heater coil. The helium carrier gas is mixed with hydrogen and passes into the detector through a small jet. The bead is heated by a current passing through the coil which is situated above the jet, and the helium-hydrogen mixture passes over it. If the detector is to respond to both nitrogen and phosphorus, then a minimum hydrogen flow is employed to ensure that the gas does not ignite at the jet. In contrast, if the detector is to respond to phosphorus only, a large flow of hydrogen can be used and the mixture burned at the jet. A potential is applied between the bead and the anode. The heated alkali bead emits electrons by thermionic emission which are collected at the anode and thus produce an ion current. When a solute containing nitrogen or phosphorus is eluted, the partially combusted nitrogen and phosphorus materials are adsorbed on the surface of the bead. This adsorbed material reduces the work function of the surface and, as consequence, the emission of electrons is increased which raises the anode current.

The sensitivity of the NPD is about 10^{-12} g/ml for phosphorus and 10^{-11} g/ml for nitrogen). Unfortunately, the performance deteriorates with time. Reese (10) examined the function of the NPD in great detail. The alkali salt employed as the bead is usually a silicate and Reese showed that the reduced response was due to water vapor from the burning hydrogen, converting the alkali silicate to the hydroxide.



1/ Eptam®	2/ Sutan®	3/ Vernam®	4/ Tillam®
5/ Odram®	6/ Treflan®	7/ Balan®	8/ Ro-Neet®
9/ Propachlor	10/ Tolban®	11/ Propazine	12/ Atrazine
13/ Simazine	14/ Terbacil	15/ Sencor®	16/ Dual®
17/ Paarlan®	18/ Prowl®	19/ Bromacil	30/ Oxadiazon
21/ Goal®	22/ Hexazinone		

Courtesy of Supelco Inc.

Figure 25. The Separation and Specific Detection of Some Herbicides Using the Nitrogen Phosphorus Detector

At the operating temperature of the bead, the alkali hydroxide has a significant vapor pressure and consequently, the rubidium or cesium is continually lost during the operation of the detector. Eventually all the alkali is evaporated, leaving a bead of inactive silica. This is an

inherent problem with all NP detectors and as a result the bead needs to replaced fairly regularly if the detector is in continuous use. The specific response of the NPD to nitrogen and phosphorus and its high sensitivity, makes it especially useful for the analysis of many pharmaceuticals and in particular in environmental analyses involving herbicides. Employing appropriate columns traces of herbicides at the 500 pg level can easily be determined.

An example of the separation and identification of a series of herbicides employing the NPD is shown in figure 25. An SPB-5 column was used, 15 m long and 0.53 mm I.D. carrying a 0.5 μ film of stationary phase. The column temperature was held at 60^{o}C for 1 minute and then programmed at 16^{o}/min. to 290^{o}C and then held there for 5 minutes. The flow rate was 5 ml/min. and the carrier gas helium. The sample size was 1 μl of ethyl acetate containing 5 ng of each herbicide.

The Electron Capture Detector

The electron capture detector contains a low energy β-ray source which is used to produce electrons for capturing by appropriate atoms. Although tritium adsorbed into a silver foil has been used as the β particle source, it is relatively unstable at high temperatures, the Ni^{63} source was found to be preferable. The detector can be used in two modes, either with a constant potential applied across the cell (the DC mode) or with a pulsed potential across the cell (the pulsed mode). In the DC mode, hydrogen or nitrogen can be used as the carrier gas and a small potential (usually only a few volts) is applied across the cell that is just sufficient to collect all the electrons available and provide a small standing current. If an electron capturing molecule (for example a molecule containing an halogen atom which has only seven electrons in its outer shell) enters the cell, the electrons are captured by the molecule and the molecules become charged. The mobility of the captured electrons is much smaller than the free electrons and the electrode current falls dramatically. The DC mode of detection, however, has some distinct disadvantages. The most serious objection

is that the electron energy varies with the applied potential. The electron capturing properties of a molecule varies with the electron energy, so the specific response of the detector will depend on the applied potential

Operating in the pulsed mode, a mixture of 10% methane in argon is employed which changes the nature of the electron capturing environment. The electrons generated by the radioactive source rapidly assume only thermal energy and, in the absence of a collecting potential, exist at the source surface in an annular region about 2 mm deep at room temperature and about 4 mm deep at 400˚C. A short period square wave pulse is applied to the electrode collecting the electrons and producing a base current. The standing current, using 10% methane in argon is about 10^{-8} amp with a noise level of about 5 x 10^{-12} amp. The pulse wave form is shown in figure 26.

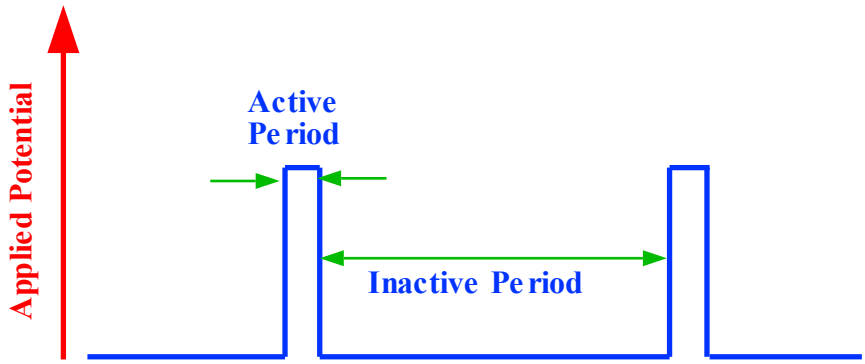


Figure 26. Wave form of Electron Capture Detector Pulses

In the inactive period of the wave form, electrons having thermal energy only will attached themselves readily to any electron capturing molecules present in the cell with the consequent production of negatively charged ions. The negative ions quickly recombine with the positive ions (produced simultaneously with the electrons by the β particles) and thus become *unavailable* for collection. Consequently the standing current measured during the potential pulse will be reduced.

The period of the pulsed potential is adjusted such that relatively few of the slow negatively charged molecules (molecules having captured electrons and not neutralized by collision with positive ions) have time to reach the anode, but the faster moving electrons are all collected. During the "off period" the electrons re-establish equilibrium with the gas. The three operating variables are the pulse duration, pulse frequency and pulse amplitude. By appropriate adjustment of these parameters the current can be made to reflect the relative mobilities of the different charged species in the cell and thus exercise some discrimination between different electron capturing materials. A diagram of an electron capture detector is shown in figure 27.

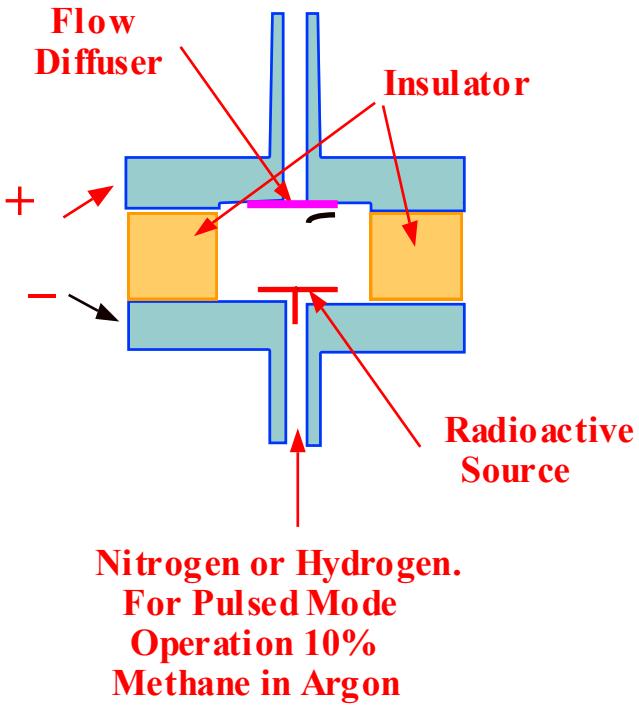
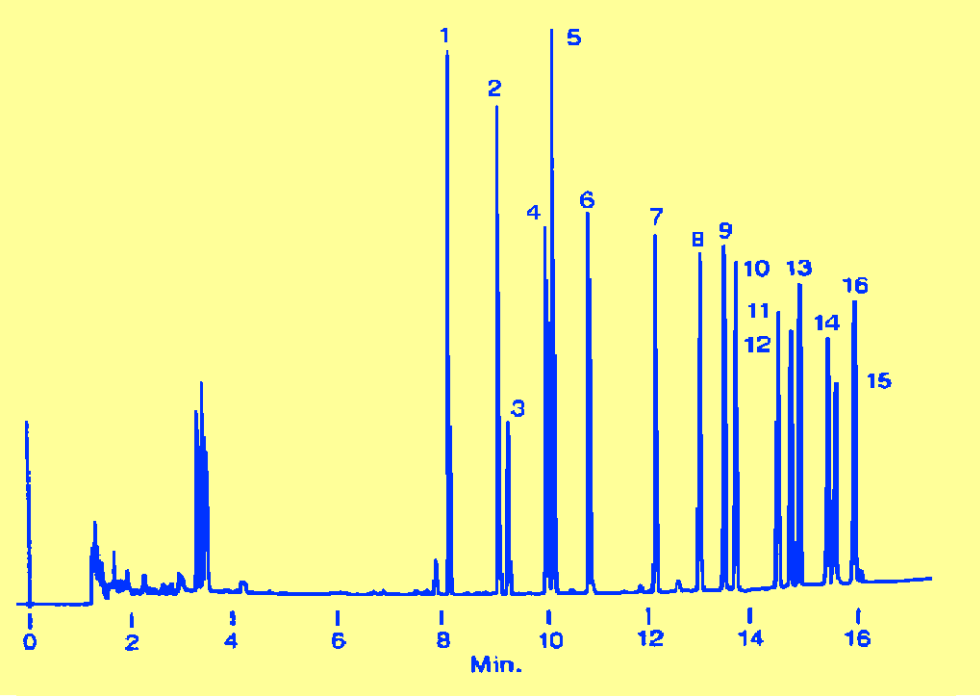


Figure 27 The Electron Capture Detector

The basic electron capture detector consists of a small chamber one or two ml in volume enclosing two metal electrodes. The electrodes may be concentric cylinders or metal discs separated by an insulator. The cell contains the radioactive source, electrically connected to the entrance conduit and to the negative side of the power supply. A gauze

"diffuser" is connected to the cell exit and to the positive side of the power supply. The output from the sensor is processed by suitable electronics and the output passed to either a potentiometric recorder of a computer data acquisition system. The electron capture detector is very sensitive, probably the most sensitive GC detector available (*ca.* 10^{-13} g/ml) and is widely used in the analysis of halogenated compounds, in particular, pesticides. An example of a pesticide analysis employing an electron capture detector is shown in figure 28.



1 α–BHC	2γ-BHC (Lindane)	3 β-BHC	4 Heptachlor
5 δ-BHC	6 Aldrin	7 Heptachlor Epox.	8 Endosulphan
9 p,p'-DDE	10 Dieldrin	11 Endrin	12 p,p'-DDD
13 Endosulphan 11	14 p,p'-DDt	15 Endin Aldehyde	16Endosulp. Sulf.

Courtesy of Supelco Inc.

Figure 28. The Analysis of Priority Pollutant Pesticides

The column used was a SPB-608 fused silica capillary column, 30 m x 0.53 mm I.D. with a 0.5 μ film of stationary phase. The column was programmed from 50ºC at 1º/min. to 150ºC and then to 260ºC at

8º/min. Helium was used as the carrier gas at a flow rate of 5 ml/min. The sample consisted of 0.6µl of a solution of the pollutants in *n*-decane. The mass of each pollutant present was about 120 pg.

The Katherometer Detector

The katherometer detector (sometimes spelt catherometer and often referred to as the *thermal conductivity detector* or *hot wire detector*) is relatively insensitive but has survived largely as a result of its catholic response and, in particular, its response to the permanent gases. Consequently, it is often the detector of choice for gas analysis and environmental testing. Its frequent use in these special types of application, somewhat surprisingly, has made it the fourth most commonly used GC detector. A filament carrying a current is situated in the column eluent and, under equilibrium conditions, the heat generated in the filament is equal to the heat lost by conduction and convection and consequently the filament assumes a constant temperature. At the equilibrium temperature, the resistance of the filament and thus the potential across it is also constant.

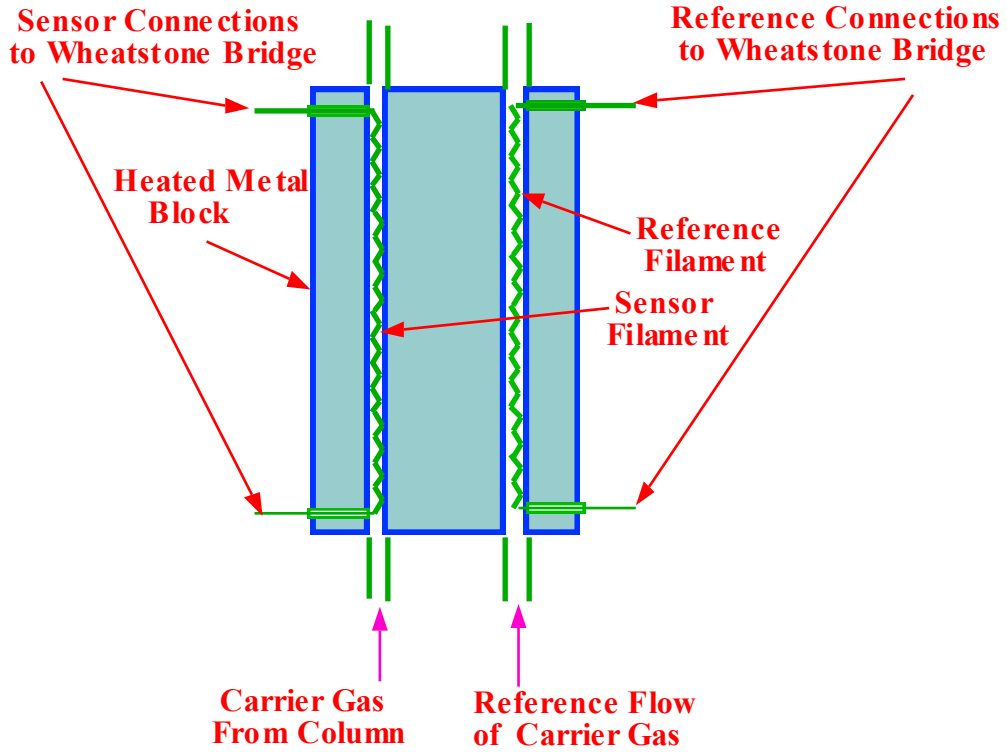
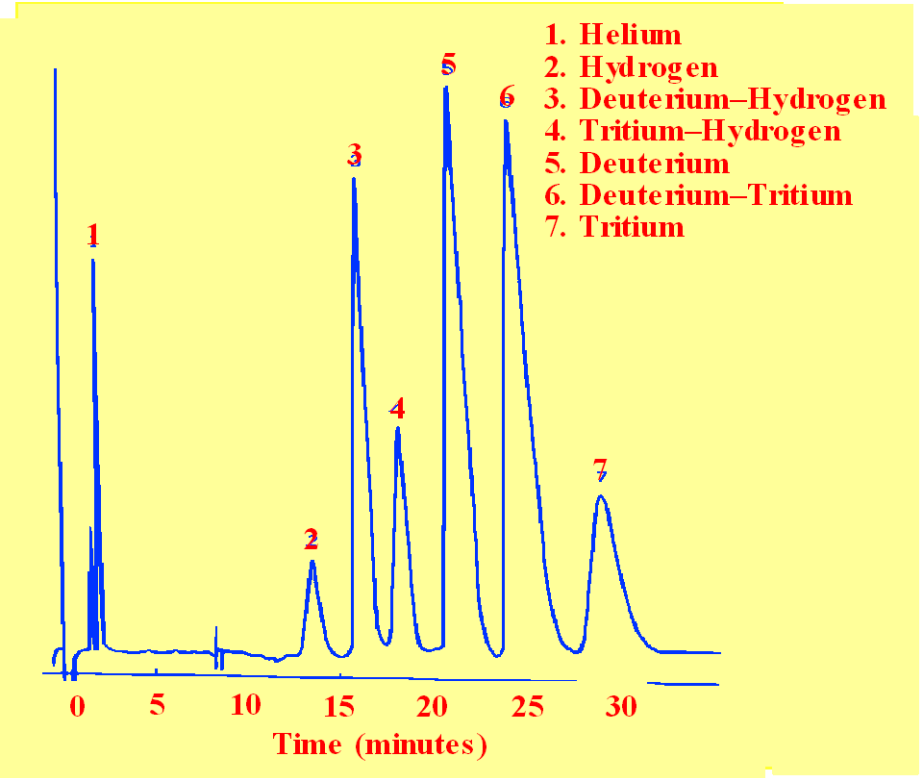


Figure 29. The Katherometer Detector ("In-Line Cell")

The heat lost from the filament will depend on the thermal conductivity of the gas and its specific heat and both these parameters will change in the presence of a foreign gas or solute vapor. The presence of a different gas entering the detector causes the equilibrium temperature to change, producing a change in potential across the filament. This potential change is amplified and fed to a suitable recorder. A diagram of the katherometer is shown in figure 329.

The katherometer may have an "in-line" sensor where the column eluent passes directly over the filament or an "off-line" sensor where the filaments are situated out of the main carrier gas stream and the gases or vapors reach the sensing element by diffusion. Due to the high diffusivity of vapors in gases, diffusion can be considered as almost instantaneous.



Courtesy of the Supelco Inc.

Figure 30. The Separation of the Compounds of Hydrogen, Deuterium and Tritium

The katherometer detector is very *flow* and *pressure* sensitive and the sensor must be carefully thermostatted and fitted with reference cells to compensate for changes in pressure or flow rate. The filaments of the reference and measuring cell are made to form two of the arms of a Wheatstone bridge and the out-of-balance signal amplified and fed to a recorder or computer data acquisition system.The maximum sensitivity will be realized if hydrogen is used as the carrier gas, but, to reduce fire hazards, helium is preferred and can be used with very little compromise in sensitivity. The katherometer sensitivity is about 10^{-6} g/ml with a linear dynamic range of about 500. Although the least glamorous, this detector can be used in most GC analyses that utilize packed columns and where there is no limitation in sample availability. The device is simple, reliable, and rugged and is particularly useful for those with limited experience in GC. It is also often the detector of choice for process monitoring. An example of the separation of the various compounds of hydrogen, deuterium and tritium, employing gas solid chromatography and using a katherometer detector is shown in figure 30. The stationary phase was activated alumina (treated with $Fe(OH)_2$), and the column was 3 m long and 4 mm I.D. The carrier gas was neon, the flow rate 200 ml/min. (at atmospheric pressure) and the column temperature was -196^{o}C.

The four detectors described are well established. reliable and generally simple to operate. They are also, probably the most popular. The FID, ECD, NPD and the katherometer are employed in over 90% of all GC applications. The FID is the most versatile, sensitive and linear, and probably the most generally useful. For details of other GC detectors see Book 4.

Data Acquisition and Processing

Originally, analytical results were calculated from measurements made directly on the chromatogram provided by the chart recorder. This is still true for many chromatographs in use today, but analyses obtained

from contemporary instruments commonly process the results using a computer. The output from the detector (which is only rarely the direct output from the detector sensor) is usually in millivolts and is suitable for direct connection to a potentiometric recorder. This output represents a voltage that is linearly related to solute concentration being measured by the detector sensor and as the sensor response is often nonlinear, the signal usually requires nonlinear processing to provide the equired output. This is carried out by the detector electronics. The FID is an exception to this, as the ion current from the flame itself happens to be linearly related to the mass of carbon passing through it per unit time. A block diagram showing the essential elements of a data acquisition and processing system is given in figure 31.

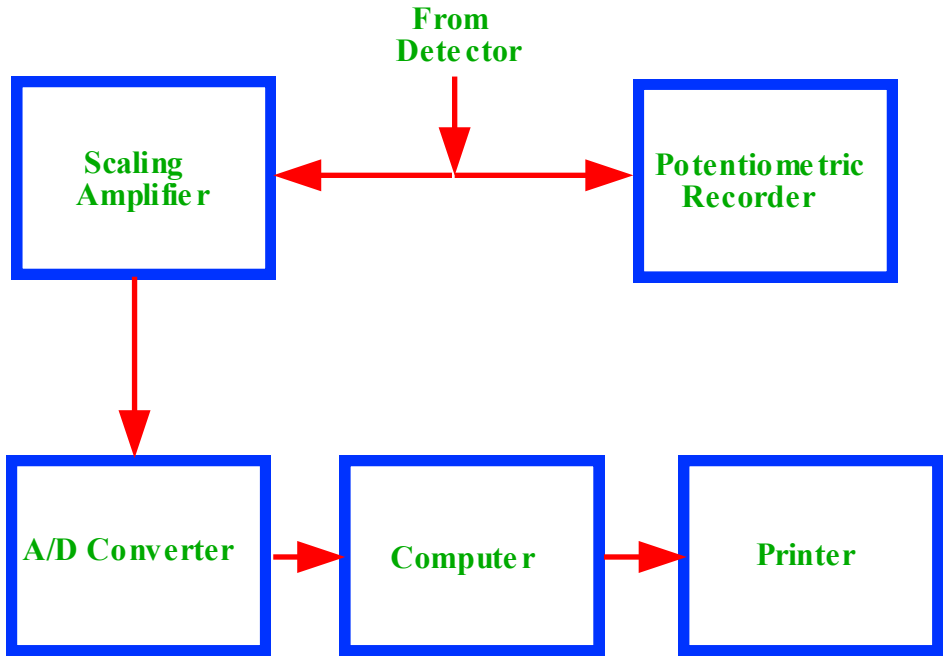


Figure 31. Data Acquisition and Processing System

The Scaling Ampifier

The output from the detector usually passes directly to a scaling amplifier that modifies the signal to a range that is appropriate for the

analog-to-digital (A/D) converter. The output can alternatively pass to a potentiometric recorder and produce the chromatogram in real time. The computer system can also produce a real time chromatogram but, to do so, the data must be processed and the chromatogram presented on the printer. The output from most detectors ranges from 0 to 10 mV? whereas the input required by most A/D converters is considerably greater *e.g*. 0 to 1.0 V. For example, if the FSD of the signal is 10 mv, the instantaneous measurement of 2 mV (assumed from the detector) must be scaled up to 0.2 volt, which is carried out by a simple linear scaling amplifier having a gain of 100.

The A/D Converter

After scaling, the signal must be converted to digital form. There are a number of ways to digitize and only one, the simplest will be described. A diagram showing the operating principle of an voltage/frequenc V/F type A/D converter is shown in figure 32.

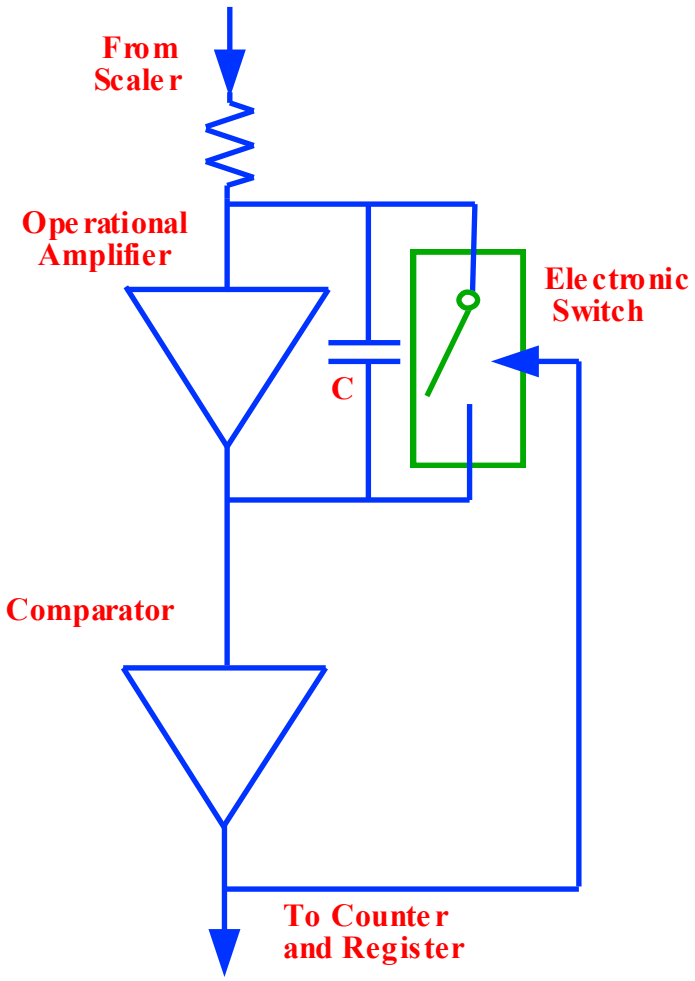


Figure 32. The Basic V/F Analog–to–Digital Converter.

The converter consists of an integrator that can be constructed from an operational amplifier with a feedback capacitor. The capacitor is charged by the voltage from the scaling amplifier through the operational amplifier.The output from the integrator is sensed by a comparator which activates the electronic switch when the potential across the capacitor reaches a preset voltage. The activation of the comparator also causes a pulse to be passed to a counter and at the same time the capacitor is discharged by the electronic switch. The process then starts again. The time taken to charge the capacitor to the prescribed voltage will be inversely proportional to the applied voltage and consequently the frequency of the pulses from the comparator will be directly proportional to the applied voltage. The frequency of the pulses generated by the voltage controlled oscillator is sampled at

regular intervals by a counter which then transfers the count in binary form to a register. The overall system is shown diagramatically in figure 33.

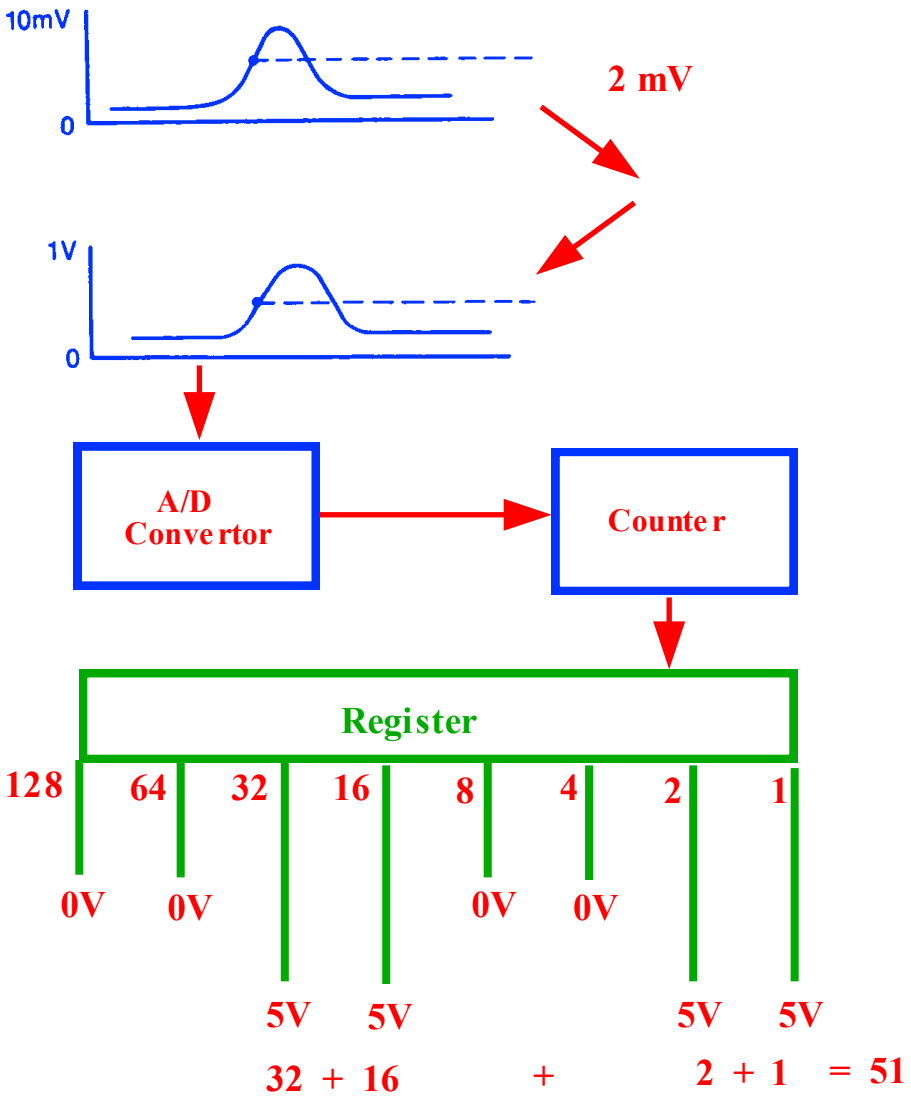


Figure 33. Stages of Data Acquisition

The output from most detectors ranges from zero to ten millivolts and the input range of many A/D converters is from zero to one volt. Thus, the instantaneous measurement of 0.2 mV from the detector must be scaled up by a factor of 100 to 0.2 volts, which is carried out by the scaling amplifier in the manner shown. The A/D converter changes the analog voltage to a digital number, the magnitude of which is determined by the number of "bits" that the computer employs in its

calculations. If, for example, eight bits are used, the largest decimal number will be 255. The digital data shown in figure 33 can be processed backward to demonstrate A/D procedure. It is seen that the third and fourth most significant "bits" (which are counted from the far left) and the two least significant "bits" (which are counted from the far right) are at the five volt level (high), which as shown in figure 32 is equivalent to 51 in decimal notation (32+16+2+1). It follows that the voltage that was converted must be $\frac{51}{255}$ x 1 volt = 0.2 volt**.** It should also be noted that because of the limitation of 8 "bits", the minimum discrimination that can be made between any two numbers is $\frac{1}{255}$ x 100 ≈ 0.4%**.** It follows that 8 bit systems are rarely used today and contemporary A/D converters usually have at least 12 or 16 bit outputs.

The output from the A/D converter is sampled regularly by the computer and the curve relating this data to time will reconstitute the chromatogram. The precision of the chromatogram and any calculations made with the data will obviously depend on the frequency of sampling which is normally user selected.

Data Processing

In the early days of gas chromatography, the associated computers used core storage which was bulky, expensive and had a very limited capacity (*e.g.,* 8 *kilo*bytes was a large memory). The limited memory meant that the programming was limited and had to be written extremely economically (*i.e.* employing the minimum of memory) and much of the data processing was done 'on-the-fly'. This meant that after each peak was eluted, it retention time and height was noted and its area calculated and then the raw data was discarded and only the retention time, peak height and peak area were stored. This economic processing package could not recalculate the data after the separation was complete, it could not reconstitute the chromatogram and it could not employ an alternative algorithm for area measurement if the one

used was not appropriate. These restrictions were entirely a result of the cost and size limitation of computer memory at that time. With the introduction of cheap, compact solid state memory and the high capacity disk memory, the situation has completely changed. 8 *mega*bytes is now a small memory and disk capacities are now measured in *giga*bytes. All the chromatography data can now be stored and reprocessed after the separation as many times as required, chromatograms can be reconstituted (with modified axes if necessary) and quantitative data manipulated as necessary. In addition, because the computer speeds have also increased greatly, on the fly processing can be carried out in parallel with normal data processing if required. The processing can include a variety of fairly sophisticated mathamatical procedures such as base-line correction, peak skimming, and multi-peak deconvolution. Techniques used in data processing will be discussed in more detail in Book 10.

Quantitative Analysis

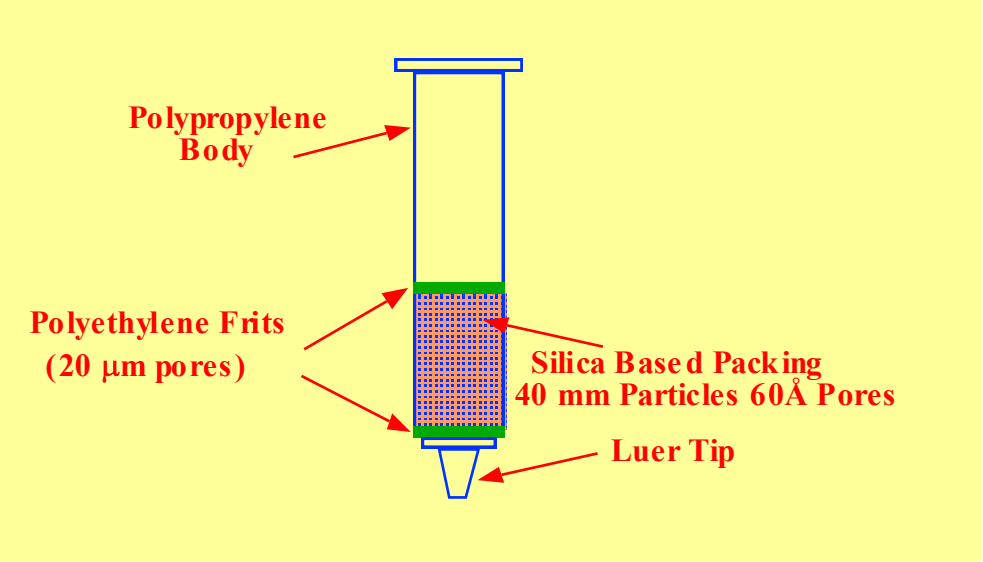
There are three important stages in a GC analysis,

1. The preparation of the sample.
2. The development of the separation and the production of the chromatogram
3. The processing of the data and the presentation of the results.

Each stage is equally important and if not carried out correctly the results will be neither precise nor accurate. Sample preparation can be very simple involving no more that diluting a known weight of sample with mobile phase or be much more complex including an extraction procedure followed by derivatization and then dilution. For some samples the preparation can be the most time consuming and difficult part of the whole analysis. Details of sample preparation is the subject of Book 18 but an example of one of the more complex sample preparation methods will be given to illustrate some of the procedures that may be necessary.

Liquid extraction is a clumsy procedure, particularly when used on the micro scale which is often necessary in sample preparation. An

alernative procedure is solid phase extraction. The procedure is relatively simple and involves the use of a short tube packed with an appropriate adsorbent such as silica, reversed phase silica or, for some applications, macro porous polymer beads. The adsorbent must be capable of removing the substances of interest from the liquid medium. Extracting trace materials from water (*e.g.*, pollution analysis) a reversed phase would be appropriate. Then the substances could be displaced into solvents such as n-hexane, methylene dichloride etc. A diagram of a simple solid phase extraction tube is shown in figure 34.



Courtesy of Supelco Inc.

Figure 34 A Solid Phase Extraction Tube

The extraction tubes are usually made of an inert plastic such as polypropylene and have a range of capacities of 1, 2, or 5 ml. The tube is one fifth filled with adsorbent and contained by plastic frits at either end. The upper part of the tube, above the packing, acts as a funnel or container for the liquid to be extracted. The liquid sample is allowed to percolate through the adsorbent bed. Sometimes the lower end of the tube is connected to a vacuum or the top to a gas supply to increase the flow of sample through the bed. The adsobed material is then desorbed with an appropriate solvent, the sample diluted to a known volume and

an aliquot used for analysis. If necessary the extract can be concentrated by evaporation and the total concentrate employed for analysis.

To avoid breakdown of labile materials, a totally inert extraction apparatus can be constructed from Teflon. A diagram of such an apparatus, produced by Alltech, is shown in figure 35 which even includes a Teflon hypodermic needle.

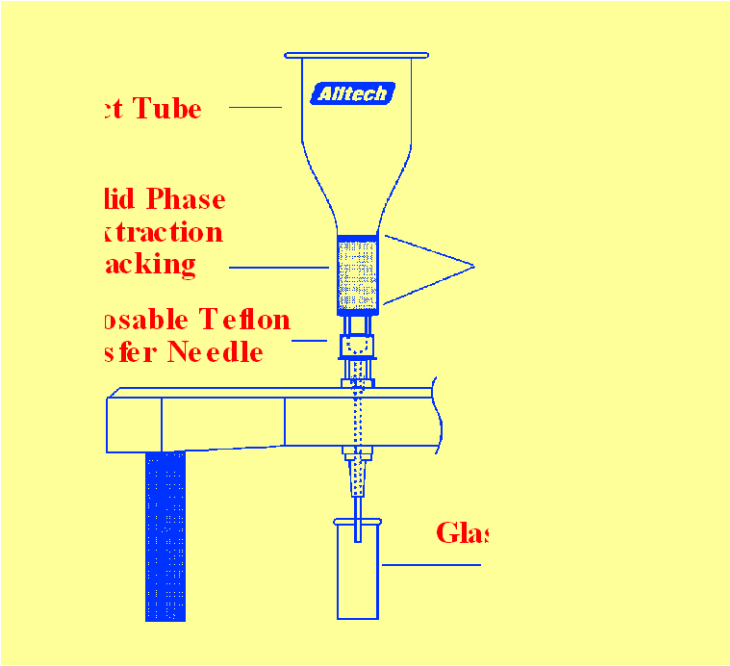
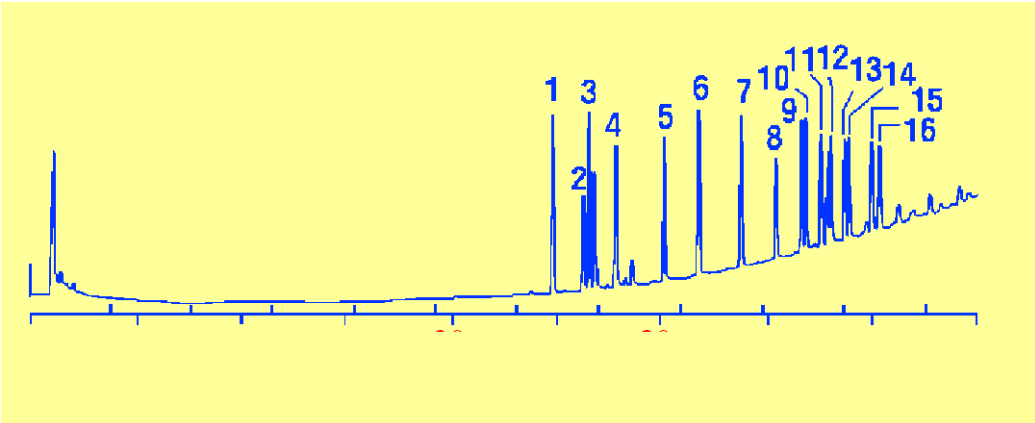


Figure 35 An All–Teflon Solid Phase Extraction Apparatus

This type of extraction system is useful for biotechnology samples. An example of the use of solid phase extraction to determine trace amounts (5 ppb) of some chlorinated pesticides in drinking water is shown in figure 36. The extraction tube was designated as the Novo-Clean C18. It was 47 mm tube long which included the membrane manifold. The materials were removed from the water sample by dispersive interactions between the solutes and the C18 reversed phase. The tube was conditioned before use with 10 ml of methanol, 10 ml of methyl-tributylether (MTBE), 15 ml of methanol and finally 125 ml of deionized water.



Courtesy of Alltech Inc

1. Lindane–α
2. Lindane–β
3. Lindane–γ
4. Lindane-Δ
5. Heptachlor
6. Aldrin
7. Heptachlo Epoxide
8. Dieldrin
9. *p,p'*-2-chlorophenyl,2-*p*-chlorpenyl 1,1,dichloroethylene
10. Endrin
11. *p,p'*-2,2-*bis p* chlorpheny1 chlor-ethylene
12. Endrin aldehyde
13. Ensodulfan Sulfate
14 *p,p*'-1'1'1-trichlor, 2,2-*bis p* chloro phenyl ethane
15. Endosulfan II

Figure 36. Separation of Some Chlorinated Pesticides Removed from Drinking Water by Solid State Extraction.

The water sample was pumped through the extraction tube at a rate of 100 ml/min. The solutes removed were displaced from the extraction tube with 10 ml of methanol followed by 10 ml of (MTBE) and dried over anhydrous sodium sulfate. It is seen that all the chlorinated pesticides were extracted and concentrations down to 1 ppb could be easily identified.

Derivatization

GC samples are usually derivatized to render highly polar materials sufficiently volatile so that they can be eluted at reasonable temperatures without thermal decomposition or molecular re-

arrangement. Examples of such materials that need to be derivatized are the organic acids, amides, poly hydroxy compounds, amino acids etc. In order to render such materials more volatile, they are either esterified, silanated or acetylated using one of a number of different methods of derivatization.

Acids can be esterified by treating them with an appropriate alcohol using an inorganic acid to catalyze the reaction. Hydrochloric acid was popular for this purpose because it's strength was adequate and any excess could be easily removed. Sulfuric acid is not very suitable as it can cause charring and any excess is difficult to remove. Other catalysts that have been found effective are trifluoroacetic acid, dichloroacetic acid, benzene sulphonic acid, *p*-toluene sulphonic acids and suphuryl and thionyl chlorides. A volatile acid is recommended such as hydrochloric acid or thionyl chloride. However, the derivative must be must be sufficiently involatile not to allow loss when removing the excess alcohol and where appropriate the catalyst itself. A general method would be to treat one or two milligrams of the acid contained in a small with 125 μl of either methanol or ethanol that contains 3M hydrochloric acid and heat at 65˚C for about 35 minutes. A stream of nitrogen is bubbled through the reaction mixture to remove the alcohol. It is clear that the derivative must be sufficiently involatile, (*i.e., has* an adequately low vapor pressure) to prevent any loss during the removal of the alcohol.

Amino acids are more difficult to derivatize but can also be esterified in a comparable manner. A few milligrams of the amino acid mixture is mixed with 2 ml of 4M alcoholic methanol and heated at 70˚C for 2 hours. Any excess methanol is then removed by evaporation in a stream of nitrogen. Any remaining water is removed by adding a little dichloromethane (*ca* 150 μl) and repeating the evaporation process. The derivative is in the form of the hydrochloride and the free base must be liberated without causing the ester to be saponified.

Another useful catalyst for esterification is thionyl chloride but the thionyl chloride must be purified by distilling from linseed oil before

use. 10 to 50 mg of acid is placed in a stoppered vial and 200 µl of dry methanol is added and cooled in a solid carbon dioxide-acetone bath. 20 µl of thionyl chloride is added with shaking and the vial is the warmed to 40˚C and maintained at that temperature for two hours. The solution is evaporated to dryness in a stream of nitrogen.

The Lewis acid boron trifluoride or the equivalent reagent boron trichloride is also very useful for forming ester derivatives. Boron trifluoride is supplied as a 14% solution in methanol. Boron trifluoride catalyzed reactions are very fast and can be complete in a few minutes. 1 to 15 mg of the acid are placed in a vial fitted with a ground glass stopper and 1 ml of 14% boron trifluoride in methanol added. The mixture is heated on a water bath for 2 minutes and then cooled. The esters can be extracted with *n*-heptane with vigorous shaking. Care must be taken to extract all the derivative.

The complimentary form of derivatization would be the esterification of an involatile alcohol. The normal reagent used for this purpose is an acid anhydride which also removes the water as the esterification proceeds. There is some competition between the alcohol and the water for the anhydride if the conditions are optimized, the anhydride reacts preferentially with the water. 10 to 100 mg of acid are placed in a stopped vial and 1 molar equivalent of the alcohol is added together with 1.2 to 1.4 equivalents of trifluoroacetic anhydride. The mixture is warmed and the reaction proceeds rapidly to completion in about 10 minutes.

One very popular esterifying reagent is diazomethane.

However, diazomethane is carcinogenic and can be extremely unstable. All reactions should be carried out in a fume hood and any stored solutions of diazomethane in diethyl ether should *be restricted to a maximum* of 100 ml and kept in a refrigerator. The materials must *never be overheated* as there is a risk of explosion.

Despite the dangers, the reagent is very effective. Providing its use is restricted to microscale reactions and sensible precautions are taken, it is normally safe to use.

Diazomethane is a yellow gas but is used in the form of an ethereal solution. Its reacts with an organic acid in the following manner,

$$R—COOH + CH_2N_2 \longrightarrow R—COO–CH_3 + N_2$$

When the reaction is complete, the yellow color persists and thus the reagent acts as its own indicator. An apparatus, developed by Schlenk and Gellerman (11) for esterification with diazomethane is shown in figure 37.

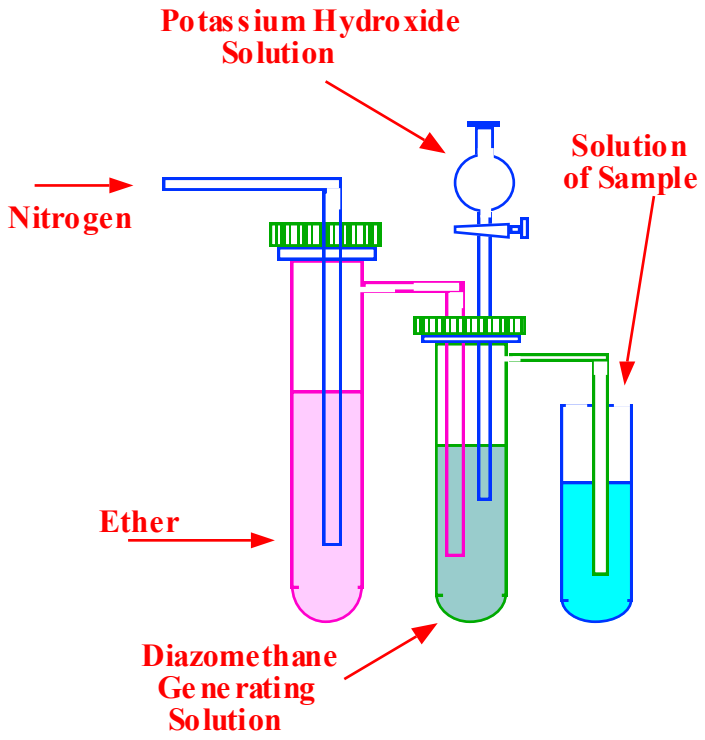


Figure 37 Apparatus for Generating Diazomethane for Esterification

Nitrogen is bubbled through ether so that the gas is saturated with ether and then passed through a vessel containing a solution of *N*–methyl–*N*–Nitroso–*p* toluene sulfonamide. When potassium hydroxide, is added through a dropping funnel, diazomethane is generated. The nitrogen

containing the diazomethane is passed into a solution of the sample until a yellow color persists. The sample solution consists of 0.5 to 30 mg of acid dissolved in 2 ml of a 10% solution of methanol in diethyl ether. About 4 ml/ min. of nitrogen is used and after the reaction is complete, the solvent is removed by evaporation. Due to the methylation of the hydroxyl groups the procedure does not work well with phenolic acids. Diazoethane can also be used employing a similar technique.

Silyl reagents will react with both alcohols an acids to form trimethylsilyl ethers and trimethylsilyl esters respectively. These derivatives are volatile and for the most part, are easily separated. The two most popular reagents are N,N–bis(trimethyl–silyl)trifluoro-acetamide (BSTFA) and bis(trimethylsilyl)–acetamide (BSA). Each react rapidly with organic acids to give high yields; the latter reagent is often used when an electron capture detector is employed. A few milligram of the acid is placed in a vial and about 50 µl of BSA or BSTFA added. Reaction can be expected to be complete directly on solution, but the mixture can be heated for 5 to 10 min. at 60˚C to ensure that reaction is really complete. The reaction mixture can be injected directly into the gas chromatograph.

For GC/MS analyses Tert-butyldimethylsilyl esters (TBDMS) are recommended. The TBDMS esters are prepared by dissolving about 5 mg of the acid in 100 µl of dimethylformamide containing 20 µmol of imidazole and 10 µmol of TBDMS. The mixture is then heated to 60˚C for about 15 minutes, an equal volume of 5% NaCl is added and the esters extracted with 1 ml of ether.

Acylation Reactions

Acylation is also a popular reaction for the production of volatile derivatives of highly polar and involatile organic materials. The technique, however, has a number of other advantages. In addition to improving volatility, acylation reduces the polarity of the substance and thus can improve the peak shape and, reduce peak tailing. As a

consequence amide esters are usually well separated with symmetrical peaks. By inserting protecting groups into the molecule, acylation also improves the stability of those compounds that are thermally labile. Acylation can render extremely polar materials such as sugars amenable to separation by GC and, consequently, are a useful alternative to the silyl reagents. Acylation is frequently used to derivatize amines, amides, alcohols, thiols, phenols, enols, and glycols etc..

A typical example of anacylation is the reaction between acetic anhydride and an alcohol

$$R–CO–CO-R + R'–O–H = R–CO–O–R' + R-CO-O-H$$

About 5 mg the acid is dissolved in 5 ml of chloroform together with 0.5 ml of acetic anhydride and 1 ml of acetic acid and is heated for 2-16 hours at 50˚C. The excess reagents are removed under vacuum and the residue dissolved in chloroform and injected directly onto the column. Sodium acetate can be used as an alternative to acetic acid in which case, 0.3 ml of acetic anhydride is added to 12 mg of sodium acetate. The reaction is carried out at 100˚C for about an hour, excess reagent is removed by evaporation and the residue taken up in a suitable solvent for analysis. An excellent handbook describing a wide range of procedures used to produce derivatives for chromatographic analysis has been compiled by Blau and Halket (12).

Preparative Gas Chromatography

Gas chromatography has not been used extensively for preparative work although its counterpart, liquid chromatography, has been broadly used in the pharmaceutical industry for the isolation and purification of physiologically active substances. There are a number of unique problems associated with preparative gas chromatography. Firstly, it is difficult to recycle the mobile phase and thus large volume of gas are necessary. Secondly, the sample must be fully vaporized onto the column to ensure radial distribution of the sample across the

column. Thirdly, the materials of interest are eluted largely in a very dilute form from the column and therefore must be extracted or condensed from the gas stream which is also difficult to achieve efficiently. Finally, the efficient packing of large GC columns is difficult. Nevertheless, preparative GC has been successfully used in a number of rather special applications; for example the isolation of significant quantities of the trace components of essential oils for organoleptic assessment.

The layout of a preparative gas chromatograph is shown in figure38

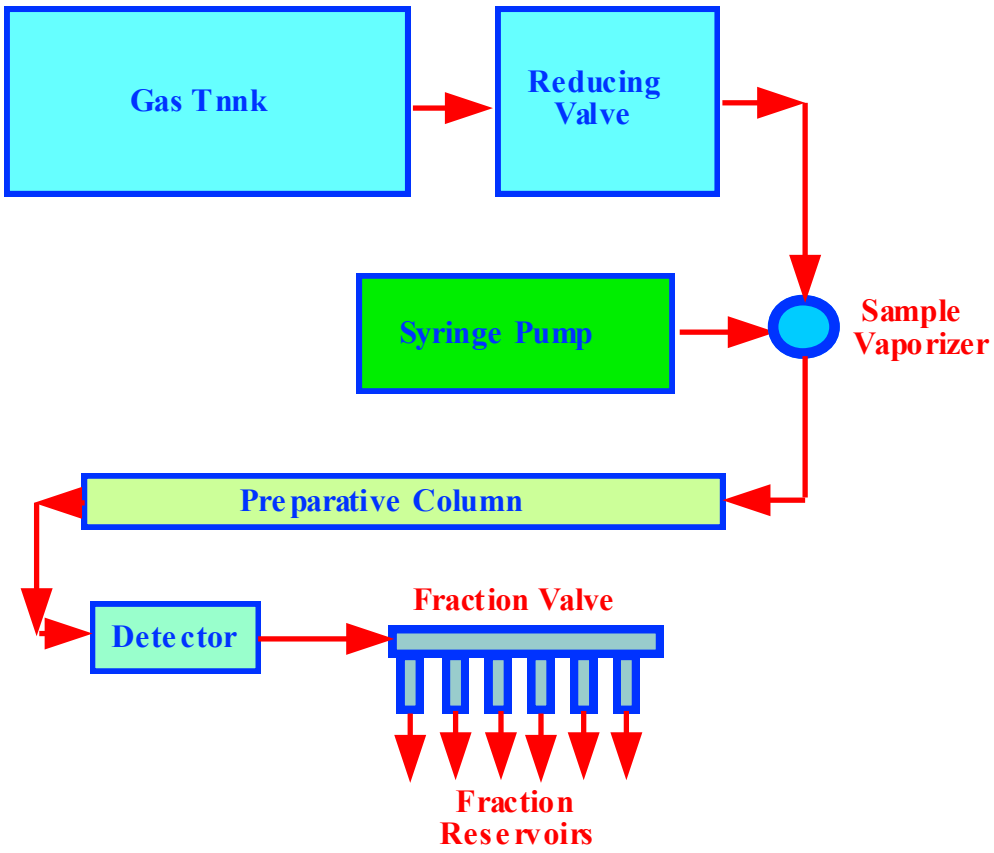


Figure 38 Layout of Preparative Gas Chromatograph

Air can not normally be used as the mobile phase due to likely oxidation and so either a gas tank or a gas (*e.g.,* nitrogen) generator must be used. As the flow rates can be large, more than one generator operating in parallel will often be necessary. The sample is usually

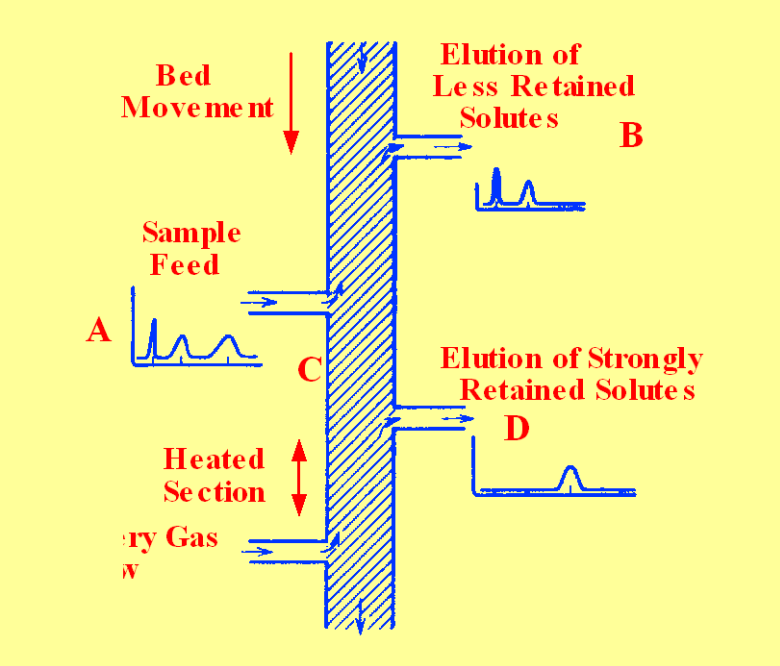
placed on to the column with a syringe pump and rapidly vaporized in a suitable heater. Passing the gas in vapor form onto the column helps evenly distribute the sample radially across the column. The detector that is used must have specifications that are almost opposite to those of an analytical detector. It should function well at high concentrations of solute, have a generally low sensitivity, if in-line it must be non-destructive and have minimum flow impedance. It need not have a particularly linear response. The katharometer is one of the more popular detectors for preparative GC. The column outlet is passed to a selection valve that diverts the eluent to its appropriate collecting vessel. The collecting vessel may be cooled in ice, solid carbon dioxide or if necessary liquid nitrogen (liquid nitrogen can only be used if a low boiling gas such as helium is employed as the carrier gas). In some cases the solutes contained in the eluent can be extracted into an appropriate liquid or onto the surface of a suitable adsorbent. the desired fractions are then recovered by distillation or desorption.

The maximum pressure that can be tolerated by large diameter columns is considerably less than their analytical equivalents. Thus to allow adequate gas flow rate through the column, the particle diameter of the packing must be relatively large. In turn, this means that the efficiency obtainable from preparative GC columns is relatively low and, thus, for effective separations, the stationary phase must be chosen to have the maximum selectivity for the solutes concerned. Compared with analytical GC, preparative GC can be far more difficult, The challenge is to achieve both adequate resolution and a satisfactory throughput.

The Moving Bed Continuous Chromatography System

The concept of the moving bed extraction process was originally introduced for hydrocarbon gas adsorption by Freund *et al*. (13) and was first applied to gas liquid chromatography by Scott (14). A diagram of the moving bed system suitable for GC was proposed by Scott and is shown in figure 39.

The feasibility of this process was established for a gas chromatographic system, subsequently, its viability was also confirmed for liquid chromatography which will be discussed in Book 19. The moving bed system takes a *continuous* sample feed and operates in the following way. The stationary phase, coated on a suitable support, is allowed to fall down a column against and upward stream of carrier gas. In the original device of Scott, the packing (dinonyl phthalate coated on brick dust) was contained in a hopper at the top of the column and was taken off from the bottom the column by a rotating disc feed table and returned to the hopper by a simple air-lift device.



Courtesy of Butterworths Scientific Publications Ltd. [Ref. 10]

Figure 39 The Moving Bed Continuous Chromatography System

By suitable adjustment of the relative rates of upward carrier gas flow and downward stationary phase flow (contained on the falling support) some components were arranged to move upward with the carrier gas, and others move downwards with the stationary phase. Referring to figure 39, if the ordinary chromatogram of the mixture is that depicted at (A), the relative speed of the carrier gas and the stationary phase

defines an imaginary line on the chromatogram. Those components to the left of the line, move up with the carrier gas (B) and those components to the right of the line, move down with the stationary phase (C). The components that move down in the stationary phase are stripped out by arranging a portion of the column to be heated and a second stream of gas elutes them through a second port (D). Scott and Maggs designed a three stage moving bed system to extract pure benzene from coal gas. Coal gas contains a range of saturated aliphatic hydrocarbons, alkenes, naphthenes and aromatics (benzene, toluene and xylenes). The separations they obtained are shown in figure 40.

It is seen that the material stripped from the top section contained the alkanes, alkenes and naphthenes and very little benzene. The material stripped from the center section consisted of almost pure benzene. The residue striped from the lower section contained the toluene, the xylenes and even the thiophene which elutes closely to the benzene. To eliminate the thiophene, however, it was necessary to loose some benzene to the lower stripping section. Nevertheless the separation clearly demonstrates the effective use of the moving bed extraction technique.

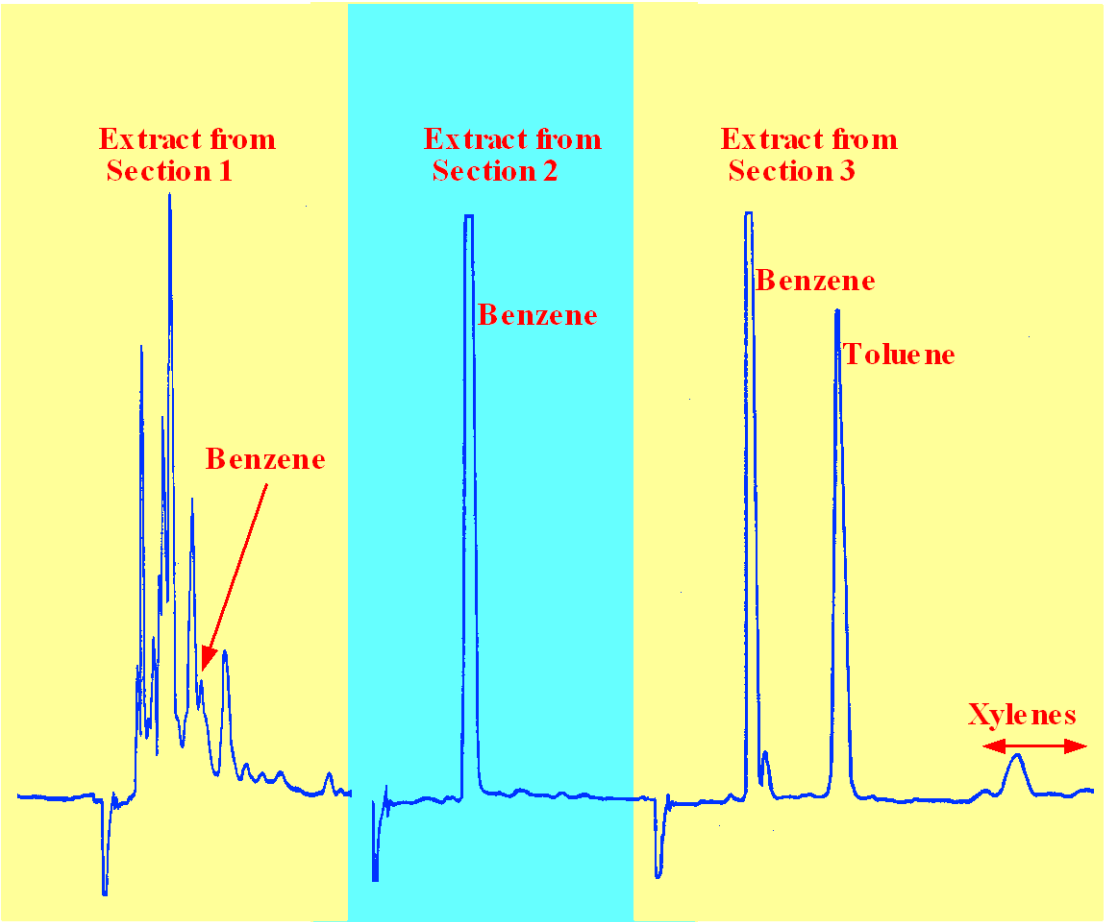


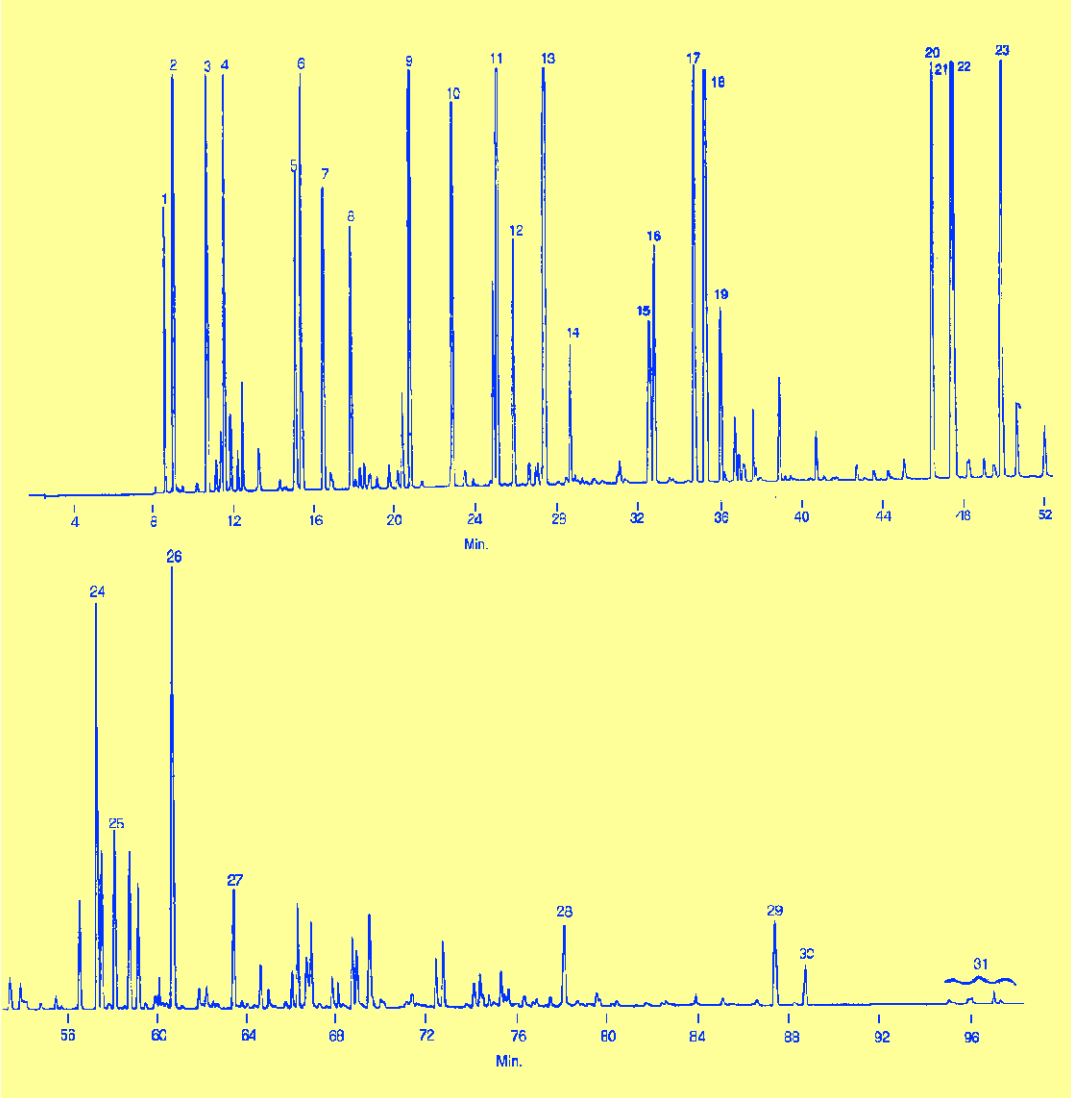
Figure 40 The Extraction of Pure Benzene from Coal Gas by continuous Extraction Using a Moving Bed Technique

Applications

Gas chromatography has a very wide field of application but its first and main area of use is in the separation and analysis of multi component mixtures such as essential oils, hydrocarbons and solvents. Intrinsically, with the use of the flame ionization detector and the electron capture detector (which have very high sensitivities) gas chromatography can quantitatively determine materials present at very low concentrations. It follows, that the second most important application area is in pollution studies, forensic work and general trace analysis.

Gasoline is a multicomponent mixture containing a large number of hydrocarbons, many of which have very similar molecular weights and

all are almost exclusively dispersive in interactive character. The structure of many of the hydrocarbons are also very similar and there are many isomers present. As a consequence, due to their interactive similarity the separation factors between individual components is very small. It follows that columns of very high efficiency will be mandatory to achieve an effective separation. It is clear that open tubular columns are ideal for this type of separation problem. In fact, it would be impossible to separate the components of gasoline efficiently with a packed column, even one that is 50 ft long, and even if the inherent long analysis times could be tolerated. In addition this type of separation demands the maximum number of theoretical plates and therefore not only must open tubes be used but tubes of relatively small diameter to produce the maximum number of theoretical plates. In fact, several hundred thousand theoretical plates will be necessary and so the column must also be very long. As has already been discussed, it is necessary to use small radius open tubular columns with a split injection system. Furthermore, as a result of the wide range of molecular weight of the components present, gasoline has a relatively wide boiling range and so will require a temperature program that will heat the column to 200 ˚C or more. A thermally stable stationary phase must be employed. The individual gasoline components are present over a wide concentration range and thus, for accurate quantitative results, the linear dynamic range of the detector must also be large. These latter demands mandate that the detector must be the FID. The separation of the gasoline components is shown in figure 41. The stationary phase used was Petrocol (the trade name for a special poly(dimethysiloxane)) that is actually intra-column polymerized and thus bonded to the surface and, as a result, is very thermally stable. The alkane chains in the polymer contribute strong dispersive properties to the stationary phase. The necessary high efficiency was obtained by using a column, 100 m long, 250 μm I.D. carrying a film of stationary phase 0.5 μm thick.

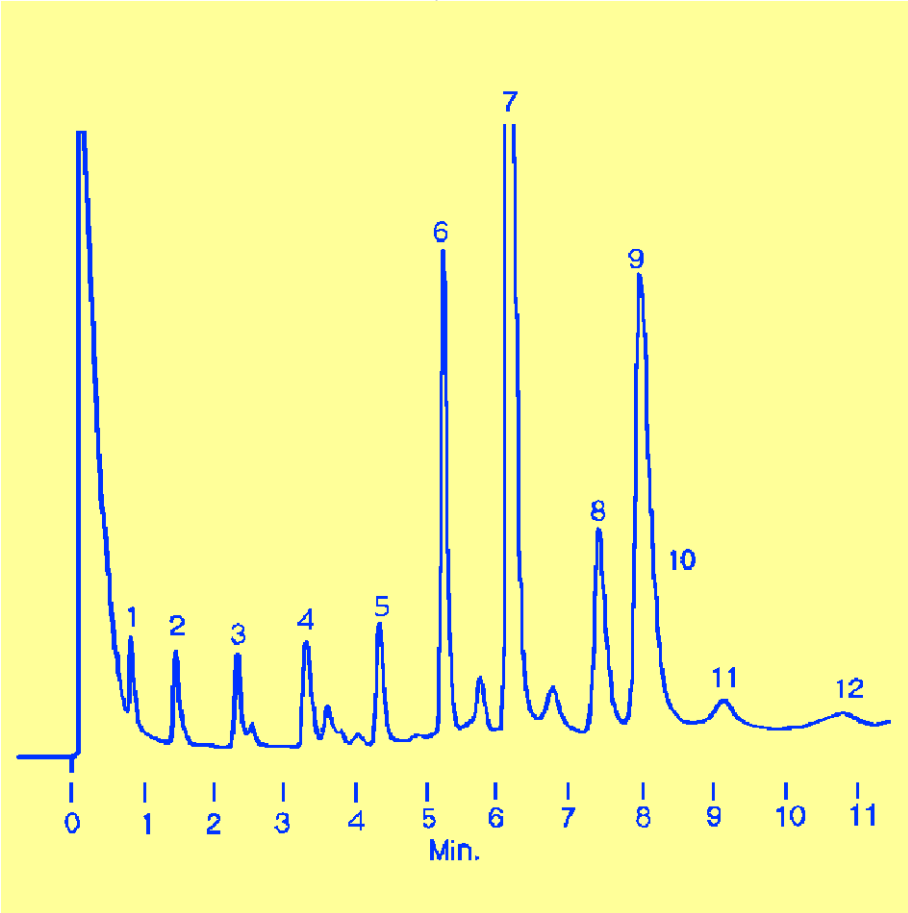


Courtesy of Supelco Inc.

1/ Isobutane	2/ n-Butane	3/ Isopentane
4/ n-Pentane	5/ 2,3-Dimethylbutane	6/ 2-Methylpentane
7/ 3-Methylpentane	8/ n-Hexane	9/ 2,4-Dimethylpentane
10/ Benzene	11/ 2-Methylhexane	12/ 3-Methylhexane
13/2,2,4Trimethylpentane	14/ n-Heptane	15/ 2,5-Dimethylhexane
16/ 2,4-Dimethylhexane	17/2,3,4Trimethylpentane	18/ 2,3-Dimethylhexane
19/ 2,3-Dimethylhexane	20/ethylbenzene	21/ m-Xylene
22/ p-Xylene	23/ o-Xylene	24/ -Me-3-Ethylbenzene
25/ 1,3,5TriMe-benzene	26/ 1,2,4TriMe-benzene	27/ 1,2,3TriMe-benzene
28/ Naphthalene	29/ 2-Methylnaphthalene	30/ 1-Methylnaphthalene
31/ Dimethylnaphthalene		

Figure 41. A Chromatogram of Gasoline

The column was held at 35ºC after in injection for 15 min. and then programmed to 200ºC at 2ºC/min and finally held at 200ºC for 5 min. To ensure that there was no condensation in the detector, the FID was held at 250 ºC (50ºC) above the maximum column temperature . The sample size was 0.1 µl which was split 100-1 onto the column and so the total charge on the column was about 1 µg. Helium was used as the carrier gas at a linear velocity of 20 cm/sec. The value of the open tubular column is clearly demonstrated. An example of the use of the packed column in natural product analysis is the separation and determination of the free fatty acids in whole milk.



Courtesy of Supelco Inc.

1/ n-Valeric Acid	2/ n-Caproic Acid	3/ n-Caprylic Acid
4/ n-Capric Acid	5/ n-Lauric Acid	6/ n-Myristic Acid
7/ n-Palmitic Acid	8/ n-Stearic Acid	9/ n-C_{16}-1 ene
10/ n-Oleic Acid	11 /n-Linoleic Acid	12/ n-Linolenic Acid.

Figure 42. The Separation of the Free Fatty Acids from Milk

An example of such an analysis is shown in figure 42.

This analysis requires a rather lengthy procedure for sample preparation but, at the same time, avoids a derivatization procedure that can easily give incorrect, low values for the fatty acid content. Due to their relatively high volatility, the lower fatty acids can be lost as vapor during the procedure. Losses can also occur as a result of their incomplete derivatization. The sample preparation developed by Supelco involved mixing 10 ml of the milk with 10 ml ethanol, 3 ml of 28% ammonium hydroxide, 25 ml of petroleum ether and 25 ml of diethyl ether. The mixture is then well shaken and allowed to stand for about 20 minutes. The ether phase is separated and carefully evaporated to dryness under a stream of nitrogen. The residue is treated with 3 ml of 0.5n NaOH in methanol and heated on a steam bath for 15 minutes. 5 ml of water is added and then 2N HCl until a pH of about 2.0 is reached. The fatty acids are then extracted with a mixture of 5 ml of petroleum ether and 5 ml of diethyl ether. If a quantitative estimation is required, then an internal standard would be added and the solution diluted to a known volume and an aliquot placed on the column.If an external standard is used, then the extract is merely diluted to a known volume (*e.g., 10* ml) and an aliquot placed on the column. This method could be considered as typical of the preparation procedures used in GC. It is clear that there can be considerably more time spent on the sample preparation than on the actual separation itself. This type of separation, however, lends itself to automation either appropriately designed hard-wired equipment or by the use of a laboratory robot. The hard wired device is generally inflexible, the laboratory robot, on the other hand, can be programmed to carry out many different types of analysis.

The separation itself has some interesting properties. Free acids are very readily adsorbed onto active sites on the support which can result in very asymmetric peaks and, as a result of the strong adsorption, significant quantitative losses can occur. In the above example, the effect of the adsorptive sites on the support is reduced by blocking

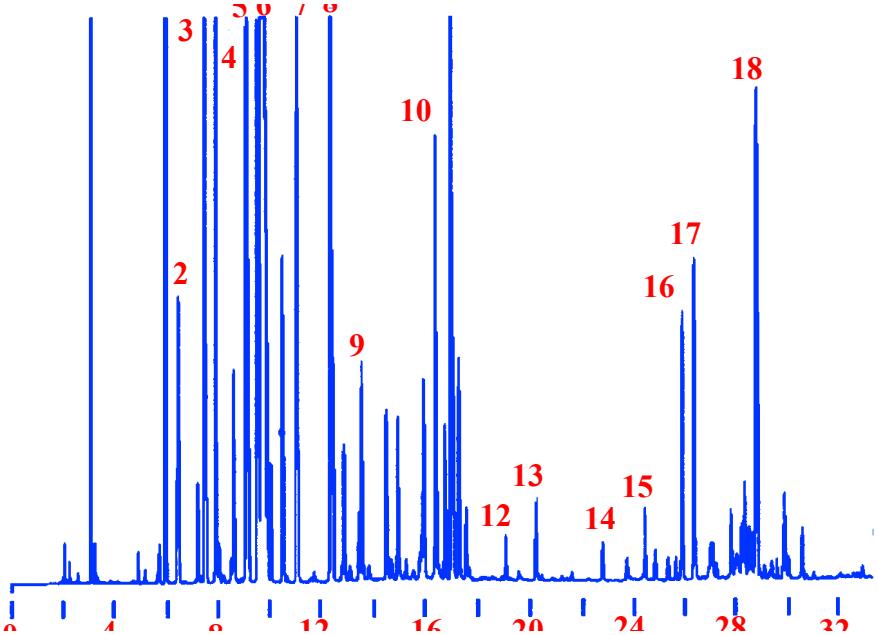
them with phosphoric acid. Phosphoric acid is very involatile and thus can tolerate the high temperature and although it is active enough to block the adsorption sites it is not active enough to cause sample decomposition. It is seen that the peaks exhibit excellent symmetry for free acids. Teraphthalic acid has also been used for this purpose to deactivate the support. The column was glass, 3 m long and 2 mm in diameter and packed with a silicone polymer, SP-216-PS on 100/120 mesh Supelcoport which is a proprietary support that has already been deactivated and treated with phosphoric acid. The column was temperature programmed from 130^{o}C to 200^{o}C at 15^{o}C/min. Nitrogen was used as the carrier gas at a flow rate of 20 ml/min.. The separation is effective, relatively rapid, and accurate quantitative results should be easily obtainable from the system. This analysis also demonstrates the need for rapid sample preparation techniques as well as rapid separations. Fast chromatography is of little use if the chromatograph is idle for long periods between samples while they are being prepared.

Lime Oil

The use of modern GC techniques to separate a sample of lime oil is shown in figure 43. A SB–5 column, that contained poly(5%diphenyl-95%–dimethylsiloxane) as the stationary phase was used to carry out the separation. It is largely a dispersive stationary phase, although the diphenyl group will contribute some induced polarizability capability to interact with polar solutes. As a consequence substances are eluted roughly in order of their boiling points (excepting very polar solutes).

The introduction of the diphenyl groups contributes more to phase temperature stability than it does to solute selectivity. The column was 30 m long, 250 μm I.D. carrying a film 0.25 μm thick of stationary phase. Helium was used as the carrier gas at a linear velocity of 25 cm/sec(set at 155˚C). The column was held isothermally for 8 min. at 75˚C and then programmed up to 200˚C at 4˚c/min. and finally held at 200˚C for 4 min. The sample volume was 0.5 μl which was split at 100:1 ratio allowing about 5 μg to be placed on the column. It is seen from figure 43 that a very good separation is obtained that

convincingly confirms the complex nature of the essential oil. In practice, however, the net flavor or odor impact can often be achieved by a relatively simple mixture of synthetic compounds.



1. *a*–Pinene
2. Camphene
3. β–Pinene
4. Myrcene
5. *p*–Cymene
6. Limonene
7. γ–Terpinene
8. Terpinolene
9. Linalool
10. Terpinene–4–ol
11. α–Terpineol
12. Neral
13. Geraniol
14. Neryl Acetate
15. Geranyl Acetate
16. Caryophyllene
17. *trans*–α–Bergamotene
18. b–Bisabolen

Courtesy of Supelco Inc.

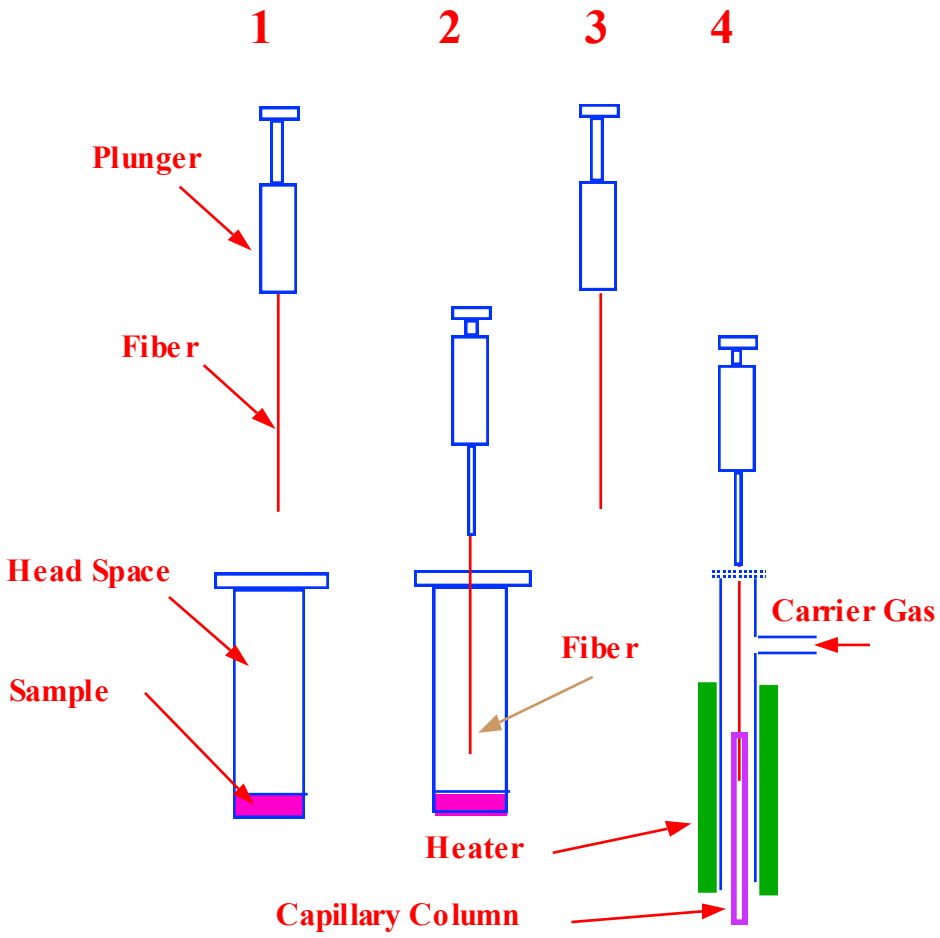
Figure 43 A Chromatogram of Lime Oil

The Head space Analysis of Tobacco

Tobacco is a herbaceous plant, the leaves of which are harvested, cured and suitably prepared for smoking, as cigars or cigarettes, or

alternatively, chewing or taken as snuff. Its main component, nicotine is habit forming and other compounds produced by pyrolysis during smoking are carcinogenic and can cause a number of other health problems. Tobacco is an extremely valuable export in the United States despite the health concern, and its quality is carefully monitored. Tobacco can be flue cured, air cured, fire cured or sun cured, but the quality of the product can often be monitored by analyzing the vapors in the head space above the tobacco. The head space over tobacco can be sampled and analyzed using a Solid Phase Micro Extraction (SPME) technique. The apparatus used for SPME is shown in figure 44.

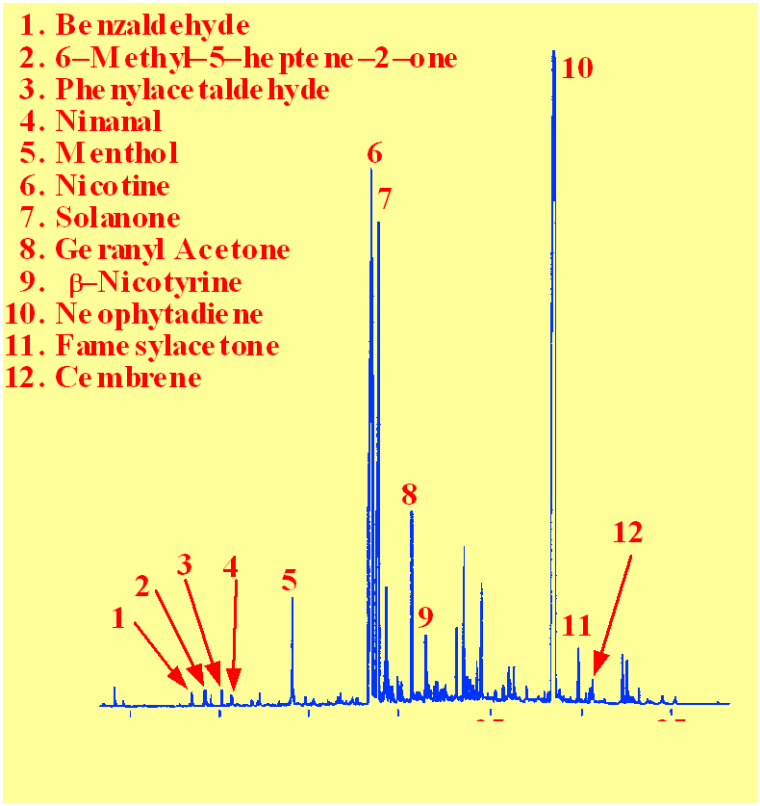
The extraction apparatus consists of a length of fused silica fiber, coated with a suitable polymeric adsorbent, which is attached to the steel plunger contained in a protective holder. The steps that are taken to sample a vapor using the apparatus are represented in figure 44.



Courtesy of Supelco Inc.

Figure 44. The Solid Phase Micro Extraction Apparatus

The sample is placed in a small head space vial and allowed to come to equilibrium with the air (1). The needle of the syringe containing the fiber is the made to piece the cap, and the plunger pressed to expose the fiber to the head space vapor. The fiber is left in contact with air above the sample for periods that can range from 3 to 60 minutes, depending on the nature of the sample (2). The fiber is then removed from the vials (3) and then passed through the septum of the injection system of the gas chromatograph into the region surrounded by a heater (4). The plunger is again depressed and the fiber, now protruding into the heater is rapidly heated to desorb the sample onto the GC column. It is advisable to arrange for the column is kept cool so the components concentrate on the front of the column.



Courtesy of Supelco Inc.

Figure 45 A Chromatogram of Tobacco Head Space

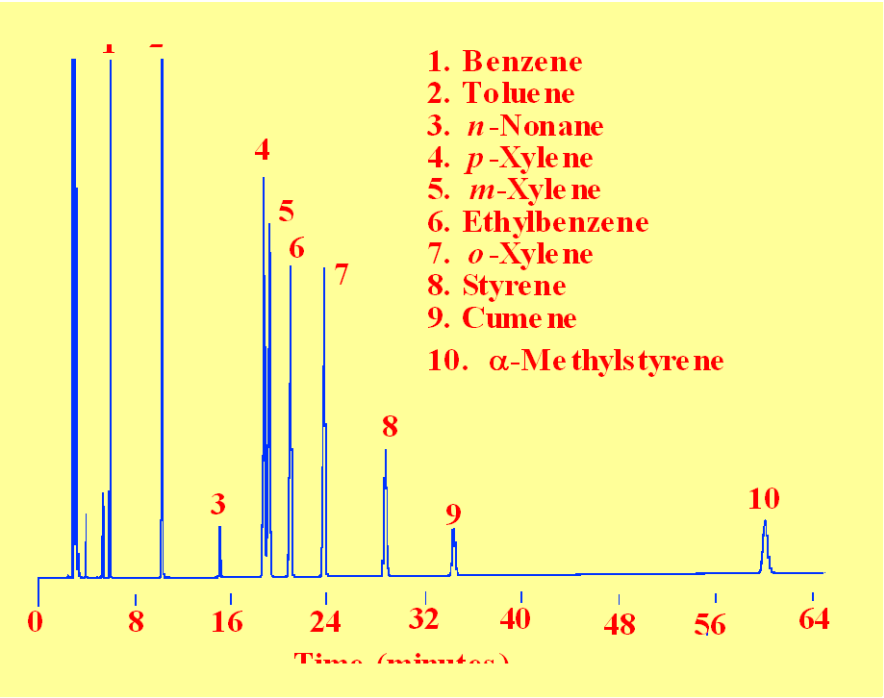
When desorption is complete (a few seconds) the column can then be appropriately temperature programmed to separated the components of the sample. A chromatogram of the head space sample taken over tobacco is shown in figure 45. The procedure as outlined by Supelco Inc. is as follows. 1 g of tobacco (12% moisture) was placed in a 20 ml head space vial and 3.0 ml of 3M potassium chloride solution added. The fiber was coated with polydimethyl siloxane (a highly dispersive adsorbent) as a 100 μm film. The vial was heated to 95˚C and the fiber was left in contact with the head space for 30 min. The sample was then desorbed from the fiber for one minute at 250˚C.The separation was carried out on a column 30 m long, 250 μm I.D. carrying a 0.25 μm thick film of 5% phenylmethylsiloxane. The stationary phase was predominantly dispersive with a slight capability of polar interactions with strong polarizing solute groups by the polarized aromatic nuclei of the phenyl groups. Helium was used as the carrier gas at 30 cm/sec. The column was held isothermally at 40˚C for one minute and the programmed to 250˚C at 6˚C/min. and then held at 250˚C for 2 min. It is seen that a clean separation of the components of the tobacco head space is obtained and the resolution is quite adequate to compare tobaccos from different sources, tobaccos with different histories and tobaccos of different quality.

Food and Beverage Products

Due to the likely contamination of food and beverage products with pesticides, herbicides and many other materials that are considered a health risk, all such products on sale today must be carefully assayed. There is extensive legislation controlling the quality of all human foods and drinks, and offensives carry very serious penalties. In addition, the condition of the food is also of great concern to the food chemist, who will look for those trace materials that have been established to indicate the onset of bacterial action, aging, rancidity or decomposition. In addition, tests that identify the area or country in which the food was processed or grown may also be needed. The source of many plants

(herbs and spices) can often be identified from the peak pattern of the chromatograms obtained directly from head space analysis. Similarly, unique qualitative and quantitative patterns from a GC analysis will often help identify the source of many alcoholic beverages. Unfortunately, food analysis involves the separation and identification of very complex mixtures and the difficulties are compounded by the fact that the components are present at very low concentrations. Thus, gas chromatography is the ideal (if not only) technique that can be used successfully in food and beverage assays and tests.

The potential carcinogenity of the aromatic hydrocarbons make their separation and analysis extremely important in environmental testing. However, the aromatics can pose some serious separation problems (for example, the *m*- and *p*-xylenes) due to the closely similar chemical structure and characteristics. The xylene isomers differ in structure (although not optically active) have similar spatial differences to pairs of enantiomers. It follows, chiral stationary phases that separate enantiomers can also be used for separating spatial isomers that are not necessarily optically active. Nevertheless, the separation ratios of such isomeric pairs (even on cyclodextrin stationary phases) is still very small, often in the 1.02–1.03 range. As a consequence, the use of high efficiency capillary columns is essential, if reasonable analysis times are also to be maintained.

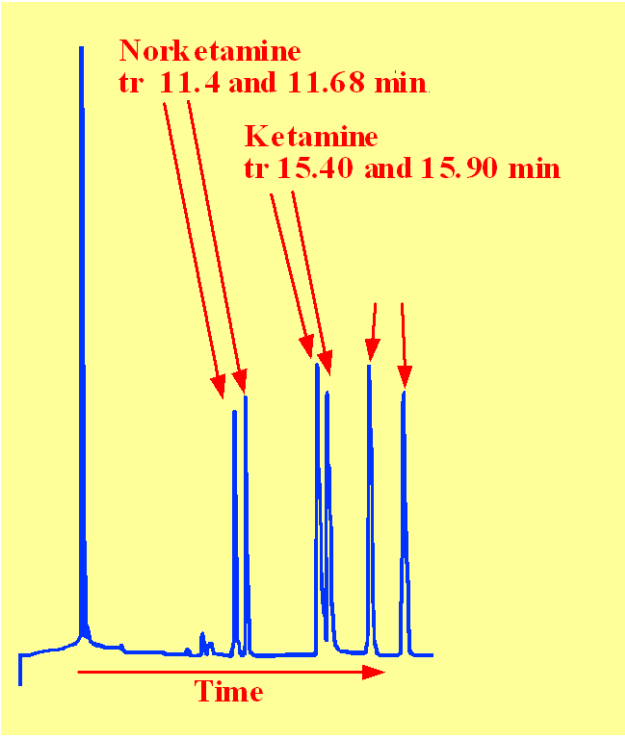


Courtesy of Supelco Inc.

Figure 46 The Separation of Some Aromatic Hydrocarbons

The separation of some aromatics contained in a mixture of hydrocarbons is shown in figure 46. A column 30 m long, 0.25 mm I.D., carrying a film of permethylated β-cyclodextrin 0.25 μm thick, was used by Supelco for the separation. The column was operated isothermally at 50˚C and helium was use as the carrier gas at a flow velocity of 20 cm/s. It is seen that baseline separation is achieved for the *m*- and *p*-xylenes and that the separation ratio for the two isomers was about 1.03.

Chiral analysis in the drug industry is now extremely crucial. There are two factors that have contributed to the importance of chiral GC in drug analysis. Firstly, the critical nature of the enantiomeric character of a drug has now been well established (largely arising from the thalamide disaster). The Food and Drug Administration, as a consequence, has mandated that the physiological effect of both or all enantiomers of any drug that can exist in chiral form must be determined.



Courtesy of Astec Inc.

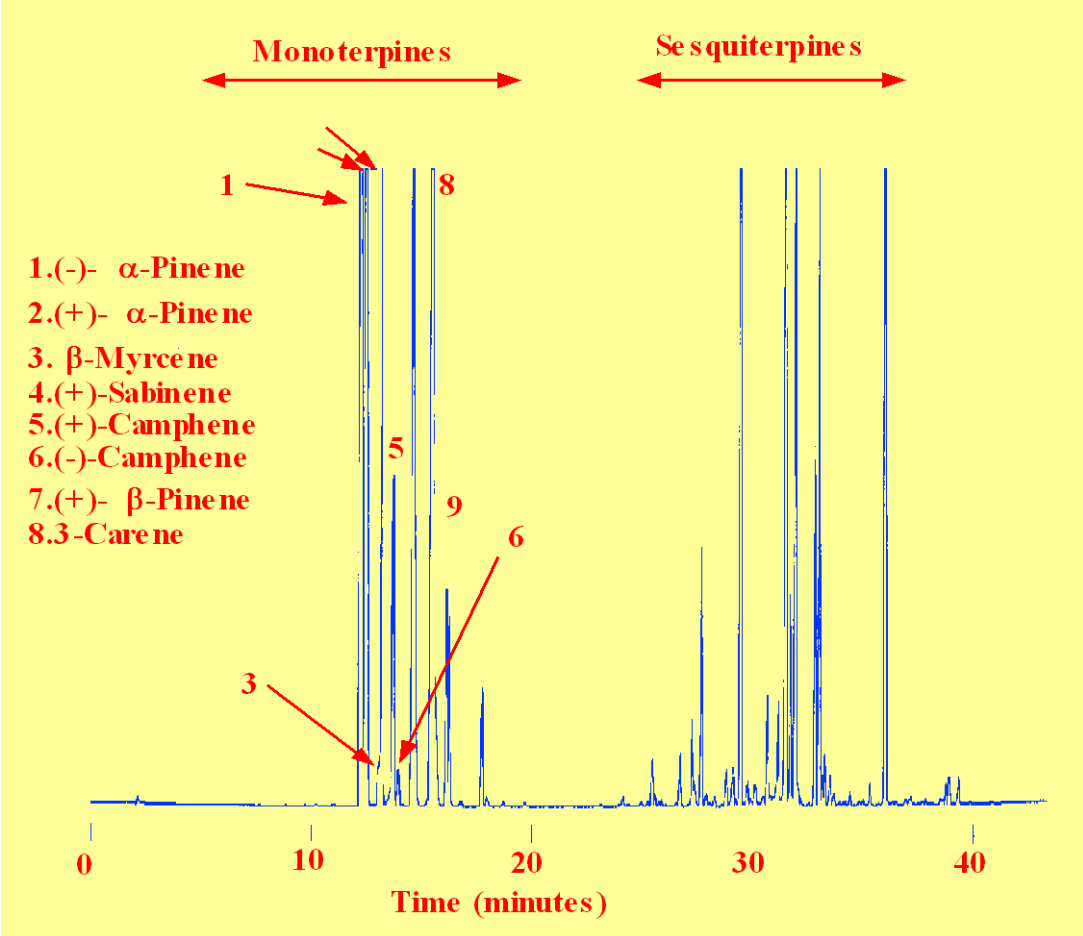
Figure 47 The Separation of the Enantiomers of Ketamine and its Metabolites Norketamine and Dehydro-norketamine

Moreover, the chiral purity of any commercially available drug must also be monitored and controlled. GC is a natural technique for this type of work as many modern drugs have relatively small molecular weights and consequently are volatile or can easily be made into volatile derivatives. In addition, GC capillary columns can easily provide the high efficiencies necessary to separate very similar compounds with relatively small separation ratios. Ketamine, was recently investigated as a potential drug that would reverse the problem of protein metabolism in AIDS patients. Unfortunately, the determination of the drug distribution in various body fluids by GC analysis was complicated by the presence of two chiral metabolites. The analysis was successfully achieved using a 30 m long, 250 μm I.D. (a Chiraldex G-TA column) operated isothermally at 160˚C using helium as the carrier gas with an inlet pressure of 3 Kg/cm^2. The method could separate all 6 enantiomers as their trifluoryl acetyl derivatives as shown in figure 47. The high efficiencies and the general

versatility of this stationary phase, that provides strong dispersive and polar interactions, makes it especially useful for the separation of substances with multiple chiral centers and in the presence of metabolites. The use of a 5m retention gap method of injection (see page 19) allowed the direct injection of 7 μl of plasma.

Essential oils (flavors and perfumes) also contain many chiral compounds and one enantiomer may be entirely responsible for a particular taste or odor whereas the complementary enantiomer has an entirely different olfactory effect. It is clear that the use of chiral chromatography can be one of the more useful techniques for the analysis of essential oils. A chromatogram of the essential oil vapor from White Pine leaves is shown in figure 48.

A head space sample was taken, employing the method previously described using 0.5 g of pine leaves contained in a 7 ml vial. The solid state extraction procedure employed a glass fiber coated with a polysiloxane film which was exposed to the sample vapor at 40˚C for 20 minutes. Using the special applicator, the fiber was withdrawn from the sample vial and placed in a unique capillary column sample device. The fiber was then heated to 250˚C for one minute and the vapors passed onto the column using a split injector with a 100:1 split. The column used was 30 m long 0.25 mm I.D. and carried a film of β-DEX 0.25 μm thick and was programmed from 40˚C to 220˚C at 4˚C/min. Helium was used as the carrier gas at a velocity of 35 cm/s. It is seen that the sample is broadly separated into two groups, the monoterpenes and the sesquiterpenes. The enantiomers of α-pinene and camphene are cleanly separated. As these compounds contain *no polar groups*, the chiral selectivity must be based entirely on differential dispersive interactions with the derivatized cyclodextrin.



Courtesy of Supelco

The columns were 30 m long, 0.25 mm I.D., carrying a film of stationary phase 0.25 μm thick of β-DEX™. The column was programmed from 40˚C to 220˚C at 4˚C/min. The helium flow velocity was 35 cm/s.

Figure 6.9 Chromatogram of the Essential Oil From White Pine Leaves

It should be noted that whereas the (–)-α-pinene is the first eluted enantiomer of α-pinene it is the (+)-camphene that is the first eluted of the camphene enantiomers. This tends to indicate that there is no rational procedure for predicting the order of elution of an enantiomeric pair.

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