CHAPTER 18

Gas Chromatography

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18.1 INTRODUCTION

One of the most difficult and frustrating problems encountered in pharmaceutical analysis is that of the simultaneous separation, identification, and quantitation of more than one compound from a complex mixture in a pharmaceutical product. This problem in recent years has been greatly simplified by the development of chromatographic procedures. From the first basic chromatographic technique devised by Tswett[1] for the separation of leaf pigments on columns, there has evolved several sophisticated chromatographic methods of separation. These methods are classified into four different groups: gas-solid adsorption chromatography (GSC); gas-liquid partition chromatography (GLC); liquid-solid adsorption chromatography (LSC); and liquid-liquid partition chromatography (LLC). It is the purpose of this chapter to discuss the first two groups which have been collectively called "gas chromatography."*

Historically gas chromatography developed as a logical extension of the earlier work on liquid chromatography. In the paper describing their Nobel Prize work in this field, Martin and Synge in 1941[2] first suggested the possibility of utilizing gas as a mobile phase rather than the previously used liquids. However, for the next 10 years little attention appears to have been paid to this observation. Between 1941 and 1952, limited contributions to the field of gas chromatography were made by some workers such as Claesson,[3,4] Turner,[5] and Philips.[6] It was in 1952 that Martin in conjunction with James published the first reports[7,8] on the successful separation and elution of organic constituents by a flowing gas as the mobile phase. These publications were quickly followed by important contributions both in GSC and GLC by several workers including Janak,[9] Ray,[10] and Bradford et al.[11] By 1955 the usefulness of gas chromatography as an analytical tool was fully realized and since that time several thousand publications have appeared in the literature.

Chromatography as defined by Keulmans[12] is "a physical method of separation in which the components to be separated are distributed between two phases, one of the phases constituting a stationary bed of large surface area, the other being a fluid that percolates through or along the stationary bed." Gas Chromatography utilizes as the stationary phase a glass or metal column filled either with a powdered sorbent or a nonvolatile liquid coated

* For the discussion of LSC and LLC, the reader is referred to Chapter 11, Volume 1.

on a nonsorbent powder. The fluid or mobile phase consists of an inert gas containing the vaporized mixture of solutes flowing through the stationary phase. In gas-solid adsorption chromatography (GSC) retention of solutes is dependent largely upon differences in adsorption properties of the solutes for the powered sorbent as they pass through the stationary phase. With gas-liquid partition chromatography (GLC) the retention of solutes is dependent largely upon the partition coefficients of the solutes for the nonvolatile liquid of the stationary phase.

To date, the use of GSC has been largely limited to the analysis of gases such as H_2, O_2, N_2, NO, and such low boiling point organic compounds as CH_4, C_2H_4, C_3H_6, and C_3H_8. In contrast GLC has had a much greater application in pharmaceutical analysis, being applicable to most organic constituents which have a measurable vapor pressure at the temperature employed. Because of the limited use of GSC to pharmaceutical analysis, and since extensions of arguments used for partition columns can generally be extended to adsorption columns, all further discussion will refer to GLC unless stated otherwise.

The major advantages of gas chromatography as an analytical tool lie in the high efficiency of separation, the sensitivity in detection of components, the speed of separation, and wide application of the method for most groups of pharmaceutical agents. Most GC applications utilize samples in the range of micrograms, but new preparative columns are now capable of isolating gram quantities of purer materials if necessary.

18.2 FUNDAMENTALS OF GAS CHROMATOGRAPHY

A. COMPONENTS OF A GAS CHROMATOGRAPH

A modern gas chromatograph consists of three basic units: (1) the chromatographic unit, (2) the temperature control and signal amplification unit, (3) the recorder unit. Figure 18.1 shows a schematic diagram of a typical instrument.

1. Chromatographic Unit

This unit is the heart of the gas chromatograph. It is constituted essentially of: the carrier gas source, comprised of a tank of compressed gas usually nitrogen or helium; a gas pressure regulator and flow control system; a sample injection port; a chromatographic column containing the stationary phase; a thermostated column oven; a detector; and a gas exit port. In addition most instruments contain a thermostated injection heater (flash heater) surrounding the injection port and a thermostated detector heater controlling the temperature of the detector.

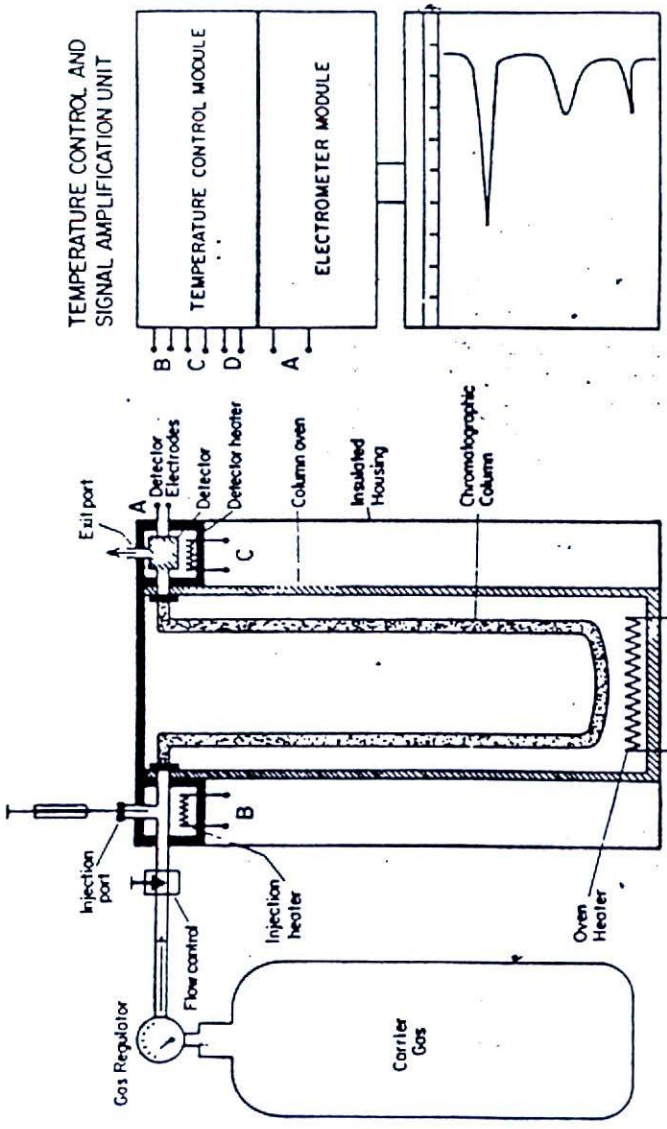

FIGURE 18.1: Schematic diagram of a modern gas chromatograph.

2. Temperature Control and Signal Amplification Unit

Temperature conditions in the chromatographic unit are accurately regulated by heaters and are thermostatically controlled from the temperature control and signal amplification unit. Oven temperature, injection port temperature, and detector temperature are controlled separately. This unit also is frequently equipped with temperature-programming controls whereby the oven temperature may be programmed to increase at a set rate over a limited temperature range as the chromatographic separation proceeds.

The second function of the temperature control and signal amplification unit is to amplify the signal produced by the detector and to transmit this amplified signal to the recorder unit. Amplification is accomplished by means of a specially designed electrometer module placed between the detector and the recorder unit. The electrometer is designed to provide distortion-free amplification. Several ranges of amplification are provided to allow the operator to control the strength of the signal transmitted to the recorder.

3. Recorder Unit

The amplified detector signal from the electrometer is recorded on a millivolt strip recorder to produce a graph of detector signal response against time. The recorder unit may also be equipped with integrators which automatically record the area under each curve.

B. SEPARATION PROCEDURE BY GAS CHROMATOGRAPHY

1. Equilibration of Instrument

The inherent sensitivity of a gas chromatograph to small variations in operating parameters makes it essential that complete equilibration and stabilization of the instrument be attained before the introduction of the sample. Initially, the column packed with the stationary phase is attached to the instrument and the desired flow rate of carrier gas through the column is adjusted by means of the gas regulator system. Column temperature is set and maintained at the desired temperature by the column oven control. Normally both the injection heater and detector heater are then set a few degrees above that of the column oven. If the column is newly packed or has been unused for some time, several hours may be required for conditioning or stabilization of the column. With carrier gas only passing from the column through the detector, the amplified signal from the detector is adjusted by the electrometer to give zero base line on the recorder. Equilibrium conditions exist when no fluctuation of the zero base line occurs over a period of several hours.

2. Separation of Sample Constituents

Sample size in analytical gas chromatography usually varies from between 10^{-9} to 10^{-2} g. Liquid samples may be applied without dilution, while solids are usually dissolved in a volatile solvent such as hexane, benzene, or carbon disulfide. The sample (0.05 to 100 μliters) is applied to the column by use of a microsyringe inserted into the injection port through a rubber septum covering the port. The injection of sample from the syringe should be instantaneous to prevent a broadening or distortion of the chromatographic peaks resulting. Due to the high temperature of the injection heater, the solutes in the sample are instantaneously vaporized, forming a "plug" of solute vapors which is swept into the gas stream by the carrier gas and onto the chromatographic column.

Separation of individual components within the column is dependent upon two separate factors, the retention of the solutes on the column and the column efficiency. The retention effect establishes the order in which the compounds being separated will elute from the column and is mainly dependent upon the partition coefficients of the solutes between the two phases and upon the temperature. The efficiency of the column determines the degree of broadening of each solute band as it travels down the column. Column efficiency is dependent upon many factors, including the solutes being separated, the physical characteristics of the column, the rate of flow of carrier gas, and the nature of both the solid support media and the liquid phase. The resolution, or the degree of completeness of separation, of two or more solutes on the column then depends directly upon both column efficiency and retention.

3. Isothermal Operation and Temperature-Programmed Operation

The simplest method of temperature control in gas chromatography is that of *isothermal operation*, whereby the column oven is maintained at a constant temperature throughout the chromatographic run. By this procedure the column temperature is selected so that all solutes of interest in the separation mixture will have sufficient volatility to be vaporized at that temperature. Both flash heater and detector temperature are maintained at about 20°C above the column temperature. *Temperature-programmed operation*, in contrast, is a method in which the column oven temperature is programmed to increase at a constant rate from an initial lower level through to an upper limit temperature during the chromatographic run. Both the flash heater temperature and the detector temperature are maintained at about 20°C above the upper limit temperature. Temperature programming is used where the volatilities of the solutes in the mixture may vary over a considerable range. By starting at a low temperature and increasing the temperature during the run it is often possible to separate the components with high volatilities at the low temperature range and components with

relatively low volatility in the high operating temperature range. Temperature programming is most useful when a large number of solutes with a wide range of volatilities is to be separated since the method produces a more even distribution of the peaks along the chromatogram and gives sharper peaks with compounds of low volatility than occurs in isothermal operation. Isothermal operation is most useful where rigidly controlled parameters of operation are required and when the constituents of the solute mixture will separate and elute as relatively sharp peaks at one column temperature i.e., there is a narrow spread in their volatilities.

C. DETECTION AND RECORDING OF SEPARATED COMPONENTS

After resolution of the solutes, each vaporized component emerges in turn from the column and is carried into the detector mixed with the carrier gas. The function of the detector is to "sense" the concentration of impurities in the carrier gas stream, and to transmit a signal to the electrometer which is proportional to the concentration of impurity present. After passage through the detector the gas stream is vented by means of the exit port. The electrometer, upon receiving the signal from the detector electrodes, amplifies and transmits it in turn to the recorder. The record produced by the recorder shows a continuous plot of time vs. detector response i.e., concentration of eluted solutes from the time of injection until the last solute has emerged from the column. This record is referred to as a "differential gas chromatogram." Under ideal operating conditions the same column may be used several hundred times to give reproducible chromatograms with the same sample. Figure 18.2 shows a typical differential chromatogram obtained by separating a mixture of tocopherols and cholestane. The first large unnumbered peak represents the solvent used to dissolve the solutes.

18.3 THEORETICAL CONSIDERATIONS OF GAS CHROMATOGRAPHY

In initiating a discussion on the theoretical concepts involved in separation of solutes by gas liquid chromatography, it is desirable to emphasize that there are two basic considerations involved. The first is the phenomena affecting *retention* or hold-up on the column, sometimes referred to as the thermodynamic aspect. The second phenomena is that of *column efficiency* or the kinetic aspect governing the tendency for a particular solute band to broaden as it travels through column. The *resolution* or extent of separation of any two peaks from a column is dependent upon both retention effects and column efficiency. The following discussion will be largely devoted to these factors.

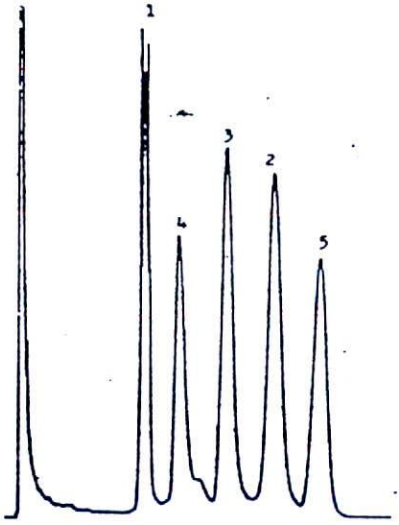

FIGURE 18.2: Chromatogram of the separation of tocopherols by gas liquid chromatography. Cholestane is employed as the internal standard. The large peak on the left is the solvent peak. Courtesy of Hewlett-Packard Company, Analytical Instrument Division, Avondale, Pennsylvania) Key: 1, cholestane; 2, α-tocopherol; 3, γ-tocopherol; 4, δ-tocopherol; 5, α-tocopheryl acetate. Instrument: F and M model 400 Biomedical gas chromatograph. Sample, cholestane; δ-, γ-, α-tocopherol and α-tocopheryl acetate. 2.0 μliters of a 0.1% solution. Column, 4 feet × ¼ in. od, 2% SE-30 on 80–100 mesh Diatoport S. Temperatures, column, 235°C, injection port, 240°C, detector 240°C. Carrier gas, helium, 90 ml/min. Sensitivity, range 10 attenuation × 32.

A. RETENTION

A gas liquid partition chromatography column may be considered to be a tube packed with an enert stationary support material coated with the liquid phase. The *total volume* V_T within this column is the sum of three different volumes, the volume occupied by the solid support medium or the *solid support volume* V_S; the volume occupied by the liquid phase coating the solid support or the *liquid phase volume* V_L; and the volume occupied by the carrier gas filling the interstitital spaces of the column, frequently called the "dead volume" or the *total gas volume* V_G°. Hence

$$V_T = V_S + V_L + V_G^\circ \tag{18.1}$$

Under the operating conditions in the gas chromatograph carrier gas flows through the column with an *average carrier gas flow rate Fc. The absolute inlet pressure* of the carrier gas at the point of entrance to the column is given by P_i, while the *absolute outlet pressure* of the column is given by P_o.

Consider next a differential gas chromatogram represented by Fig. 18.3.

The chromatogram is obtained from the recorder following injection of a sample composed of a mixture of a nonadsorbed gas such as air and a single solute into the gas chromatograph. A katherometer is used for detection. Since the air is not adsorbed by the solid support nor partitioned into the liquid phase it will travel unimpeded at the same rate as the carrier gas. The solute component of the mixture however is carried through the column at a slower rate than the air, depending upon its relative solubility in the liquid phase at the column temperature. As stated earlier this delayed elution of

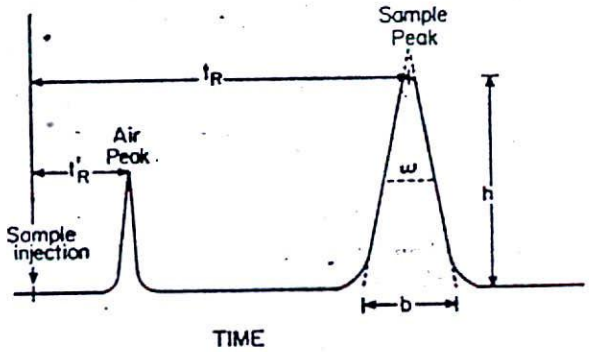

FIGURE 18.3: Schematic differential chromatogram of a pure solute with accompanying air peak.

the solute from the column, or "holdup" of the solute peak, is termed the "retention" of the solute. On the chromatogram represented by Fig. 18.3 the value t_R is a measure of the retention time of the solute, i.e., it represents the time interval between the instant of injection of the solute onto the column and the moment it emerges from the column and causes maximum detector response. Similarly t'_R represents the travel time of the unimpeded air in the sample.

From t_R the *retention volume* (*uncorrected*) V_R may be calculated, where:

$$V_R = t_R F_c \tag{18.2}$$

V_R is then the measure of the volume of gas which passes through the column during time t_R with an average carrier gas flow rate of F_c.

Because of its greater accuracy however, the *corrected retention volume* V'_R is generally employed instead of V_R. Correction of the retention volume is necessary because of the compressibility of the carrier gas, which results in an increased linear velocity of carrier gas along the column length. V'_R is calculated by introduction of a *pressure gradient factor* f to correct for this difference. This pressure gradient factor can be calculated from the column inlet pressure P_i and outlet pressure P_o by the equation:

$$f = \tfrac{3}{2}[(P_i/P_o)^2 - 1]/[(P_i/P_o)^3 - 1] \tag{18.3}$$

The corrected retention volume V_R° then may be determined to be:

$$V_R^\circ = fV'_R = ft_R F_c \qquad (18.4)$$

In an analogous manner it is possible to determine the total gas volume V_G° by determination of the corrected "retention" volume for the air peak. Since the volume of gas displaced ahead of the air peak is in reality the interstitial gas volume of the column, it is equivalent to V_G°. See Fig. 18.3. Therefore

$$V_G^\circ = ft'_R F_c \qquad (18.5)$$

where t'_R is the retention time for the air peak, f is the pressure gradient factor, and F_c is the gas flow rate.

Another quantity of significance in calculations of gas liquid chromatography data is the *net retention volume* V_N. This value is the volume of carrier gas flowing through the column from the time of injection until the solute peak reaches its maximum, i.e., V_R° less the total gas volume V_G°

$$V_N = V_R^\circ - V_G^\circ \qquad (18.6)$$

The volume of the liquid phase or *liquid phase volume*, V_L is determined at the *column temperature* T_c and is calculated at the time of preparation of the column from the equation:

$$V_L = \frac{W_L}{\rho_L} \qquad (18.7)$$

where W_L is the weight of liquid phase in column and ρ_L is the density of liquid phase at column temperature T_c.

Both W_L and ρ_L are easily determined at room temperature, but since ρ_L varies with temperature the density of the liquid phase at column temperature T_c must be estimated. By the assumption that ρ_L has a reduction coefficient of 10^{-3} per degree centigrade increase in temperature, the value of ρ_L may be approximated under column conditions.

It must be noted that the net retention volume V_N will vary according to the liquid loading, i.e., the per cent liquid phase on the column, and if V_N is utilized in presenting chromatographic data, the amount of liquid phase present must be stated. To obtain an expression for retention volume which is independent of this factor, the quantity *specific retention volumn* V_g is utilized. It is determined from the net retention volume V_N and the liquid phase density ρ_L by Eq. (18.8), where T_c is the column temperature.

$$V_g = \frac{V_N \times 273}{V_L \rho_L (T_c + 273)} \qquad (18.8)$$

The specific retention volume V_g therefore is the net retention volume per gram of liquid phase reduced to standard temperature and pressure. The value V_g is frequently utilized in the literature as a method of reporting solute behavior. The reader is referred to the text of Dal Nogare and Juvet[13] for examples of actual retention calculations.

A further retention value frequently used in the literature is that of *relative retention*, in which retention volumes of all solutes are expressed relative to one standard compound. The primary advantage of this method is that it eliminates the effects of the column size and operating conditions. The disadvantage of this method of reporting is that different authors frequently utilize different standards for different applications. For best results experimental conditions should be identical and constant and it is usual to carry out relative retention calculations on solutes obtained from the same chromatogram or chromatograms run consecutively under identical conditions. Under these conditions,

$$\text{Relative retention } r_{1,2} = \frac{V_{g_1}}{V_{g_2}} = \frac{V_{N_1}}{V_{N_2}} = \frac{V^{\circ}_{R_1}}{V^{\circ}_{R_2}} = \frac{V_{R_1}}{V_{R_2}} \tag{18.9}$$

where subscripts 1 and 2 are those of the standard and compound being studied, respectively.

A solute which is retarded on a gas-liquid chromatography column does so due to its *partition coefficient* K between the stationary liquid phase and the gas phase. Dependent only upon temperature, the partition coefficient is a constant for any particular solute according to Eq. (18.10):

$$K = \frac{\text{weight of solute per ml of stationary liquid phase}}{\text{weight of solute per ml of gas phase}} \tag{18.10}$$

When the solute as a vapor "plug" is introduced into a GLC column there is established over a short period of time a dynamic equilibrium between the solute in the liquid phase and in the carrier gas. The molecules of solute in the vapor phase cannot be swept unhindered through the column in the carrier gas stream and leave behind the solute molecules which have diffused into the liquid phase. Instead, during each unit distance which the solute in the vapor travels, it reestablishes itself in a new dynamic equilibrium with the liquid phase according to its partition coefficient. The overall effect therefore is that the rate of migration of the solute down the column is retarded. A solute with a large partition coefficient will thus travel at a slower rate down the column than a solute having a smaller partition coefficient. Only in the case of a solute such as an insoluble gas in which K approaches O will there be no retardation on the column.

The partition coefficient K is influenced by column temperature but it is independent, within limits, of the size of the column and weight of liquid phase.

It may be shown experimentally or through mathematical derivation that partition coefficient is related to the corrected retention volume V°_{R}, the total gas volume V°_{G}, and the liquid phase volume V_L by Eq. (18.11):

$$K = \frac{V^{\circ}_{R} - V^{\circ}_{G}}{V_L} = \frac{V_N}{V_L} \tag{18.11}$$

Similarly the partition coefficient K may be derived from Eqs. (18.8) and (18.10) and related to the specific retention volume V_g, the liquid density ρ_L, and the column temperature T_c by Eq. (18.12).

$$K = \frac{V_g(T_c + 273)\rho_L}{273} \tag{18.12}$$

B. COLUMN EFFICIENCY

In addition to the retention of the solute as it begins to migrate down the column, there also occurs a widening or spreading of the solute zone. This broadening of the band is due to several kinetic influences in the solute travel including; diffusion, eddy effects, flow rate of carrier gas, and resistance to mass transfer by the liquid phase. The longer the solute is on the column the greater is the tendency for the peak to broaden. This spreading effect may be observed on comparing the solute peak resulting when a short column is used with that obtained when a longer but otherwise identical column is employed. The peak eluting from the short column is generally higher and narrower than that of the longer column, even though the area under the curve is the same in both instances. A comparison of several different solutes eluting from a single column shows also that the first peaks eluted are narrower than those retained for a longer time period; this tendency may be seen in Fig. 18.2.

Column efficiency is determined from the relationship between the peak width and the retention time or retention volume of the solute under examination. The quantitative measure of efficiency is given in terms of the number of theoretical plates n or the height equivalent to a theoretical plate (HETP).

Despite the fact that chromatography is a continuous process, the theoretical plate concept—derived from a discontinuous process—is most frequently utilized for determining column efficiency. In a discontinuous process such as countercurrent extraction, complete equilibration of the two phases takes place in each tube before there is a change of phase. Each such equilibration is equivalent to one theoretical plate. In chromatography however, since the flow of carrier gas is continuous, there is insufficient time for complete equilibration between the liquid phase and the carrier gas phase in any one cross section. What is considered, therefore, is the distance through the column the carrier gas travels before an equivalent distribution of solute between the liquid phase and the gas phase takes place. This distance is called the "height equivalent to a theoretical plate" or "HETP." The HETP in a well packed and efficient column (4 ft × 4 mm) may be as small as 0.3 cm.

One measure of the efficiency of a gas chromatographic column is given by the number of theoretical plates n that a column contains. The equation most frequently utilized in this determination is:

$$n = 16\frac{(t_R)^2}{b} \tag{18.13}$$

The use of this expression relies upon the fact that the elution curves in gas chromatography, in the absence of absorption or overloading on the column, approximate normal distribution curves. The expression is derived on the basis of the binomial distribution theory. Actual calculation of n is carried out from experimental values obtained from the chromatogram. The term b is the base of the triangle formed from the tangents through the inflection points of the curve and with the base line as seen in Fig. 18.3. Both b and t_R must be in the same units since n is dimensionless. Equation (18.13) may also be modified so as to express retention volume V_R or corrected volume V_R° as long as b is also expressed in the same units.

The HETP may be calculated also from experimental values by dividing the number of theoretical plates n by the column length l. Hence

$$\text{HETP} = l/n \tag{18.14}$$

It should be remembered that both n and the HETP apply only to that solute whose peak was measured and not to other solute peaks on the chromatogram. It also should be noted that within limits, n may be increased by lengthening the column, but is reduced somewhat by increase in column diameter.

Application of the plate theory is useful in determining the quantitative measure of column efficiency under standard operating conditions, but it gives no clue as to the best parameters of operation for that column. Van Deemter et al.[14] proposed a rate theory for determination of the HETP, which relates the column efficiency to various column parameters. Three separate phenomena are considered of major importance in determining column efficiency: (1) the eddy diffusion caused by the distance of gas flow through multiple pathways in the column, (2) the molecular diffusion of the solute into the carrier gas, (3) the resistance to mass transfer of solute molecules from the gas phase into the liquid phase.

The van Deemter equation is given as:

$$\text{HETP} = 2\lambda d_p + \frac{2\gamma D_g}{U} + \frac{8}{\pi^2}\frac{k}{(1+k)^2}\frac{d_f^2}{D_{liq}}U \tag{18.15}$$

where λ = statistical irregularity of packing
d_p = particle diameter of the support medium
D_g and D_{liq} = solute diffusivity in the gas and liquid phases
γ = a correction factor to express channel tortuosity
k = ratio of the solute in the liquid phase to that in the gas phase
d_f = Thickness of the liquid coating on the support particles
U = average carrier gas velocity
$\frac{8}{\pi^2}$ = a geometry constant in the mass transfer term

Because of the complexity of Eq. (18.15) a simpler expression of it has

been devised:

$$\text{HETP} = A + \frac{B}{U} + CU \qquad (18.16)$$

in which $A = 2\lambda d_p$

$B = 2\gamma D_g$

$C = (8/\pi^2)[k/(1 + k)^2](d_f^2/D_{liq})$

Term A or $2\lambda d_p$ expresses the eddy diffusion effect on peak broadening. Such broadening is produced by the variance in time required by individual solute molecules to travel through the multiple pathways of different lengths within the column packing. The numbers and lengths of these pathways are influenced both by irregularity of packing and by particle size. The terms λ and d_p represent irregularity of packing and particle size, respectively. Uniform packing, i.e., a small λ, decreases the peak width, as does the decreasing particle size d_p down to a lower limit. Very small particle size decreases the HETP, but this is counterbalanced to some extent by the greater irregularity of packing produced by fine powders. There is therefore a practical lower limit to particle size dependent upon the particular stationary phase used and its packing properties. Mesh sizes varying from 20 to 120 are commonly used, depending upon the separation involved.

The second term B/U or $2\gamma D_g$ expresses the peak broadening effect due to molecular diffusion of solute particles into the carrier gas. Unlike A, which is independent of the nature of the carrier gas, the properties of the solute and the carrier gas velocity, B/U is the product of all three effects. The term γ is a correction factor introduced to account for the tortuosity of the gas pathways. D_g or carrier gas diffusivity is related to the density of the carrier gas. The value of D_g is decreased by increasing the molecular weight of the carrier gas. In some instances, significant decrease in B may be achieved by changing to a carrier gas with a higher density, e.g., hydrogen to nitrogen. Different solutes will show different diffusion properties, but this factor is generally impossible to control other than by modification of chemical structure of the solutes separated. The last parameter U or average gas velocity in B/U is of considerable importance. As may be seen from Eq. (18.15), increasing the carrier gas velocity will decrease the value of $2\gamma D_g/U$, thus decreasing the molecular diffusion term. At low gas velocities significant increase in efficiency may be achieved by increasing the gas velocity. If a high pressure drop is necessary to achieve high flow rate however, as may occur in long columns, a loss of efficiency may result instead. In addition, an increase in gas velocity at higher values produce a peak broadening effect in the third term CU.

The final term CU or

$$\frac{8}{\pi^2}\frac{k}{(1+k)^2}\frac{d_f^2}{D_{liq}}U$$

describes the magnitude of the peak broadening due to the resistance to mass transfer of solute molecules from the mobile gas phase to the liquid stationary

phase. The parameters involved in this final expression have significant effects on column efficiency or HETP. The constant $8/\pi^2$ is a factor necessary to account for the geometrical relationships involved in the mass transfer within the column. The ratio of the solute in the liquid phase to solute in the gas phase is given by k and is directly related to the partition coefficient K and the amount of liquid phase present. By choice of a liquid phase that will give a large partition coefficient K, i.e., a high solubility of solute in the liquid phase, the HETP may be decreased. Similarly increasing the amount of liquid phase tends to keep the term $k/(1 + k)^2$ small. The value of d_f^2 also is significant. It can be seen that the magnitude of this value varies as the square of the thickness of the liquid phase on the particle. Therefore by decreasing the coating thickness the HETP will be decreased. This increased efficiency is offset somewhat by the decrease of liquid phase required. A correct balance between the two effects may be achieved by careful control of the per cent liquid phase used in the column. Lightly loaded columns (1 to 5%) are common for many types of separation today. (Note: Too light a liquid coating however may result in decreased efficiency due to incomplete coating of the support particles, causing adsorption effects to occur.) D_{liq} or solute diffusivity into the liquid phase is seen to possess an inverse relationship in the mass transfer value. Thus, increasing values for the diffusion of solute into the liquid phase decreases the HETP. Such an increase may be accomplished by increased column temperature. Again such an advantage may be offset by the decrease in k which results from increased temperature. In general, low molecular weight nonpolar liquids show greater diffusivity than do high molecular weight polar liquids. Once more however the choice of liquid phase must consider the partition coefficient K as well. Increase in the average carrier gas velocity U when considering the resistance to mass transfer term is seen to produce an increase in the magnitude of the term. Thus the effect on the mass transfer term is opposite to its effect on the mass diffusion term. It is evident therefore that there will occur an optimium gas velocity value, on either side of which decreased column efficiency or increased HETP will occur.

A plot of HETP against carrier gas velocity U for a single solute and temperature, as shown in Fig. 18.4, graphically expresses the simplified van Deemter equation and indicates the comparative contribution of each of the three terms to the HETP. The importance of carrier gas velocity is emphasized by the plot. The effect of A is generally small and independent of U, while B and C are seen to be dependent upon U.

C. RESOLUTION

For any two solute peaks on a gas chromatogram the degree of separation or resolution of the peaks is dependent upon both retention effect and column efficiency. The relative retention between any two solutes on a chromatogram

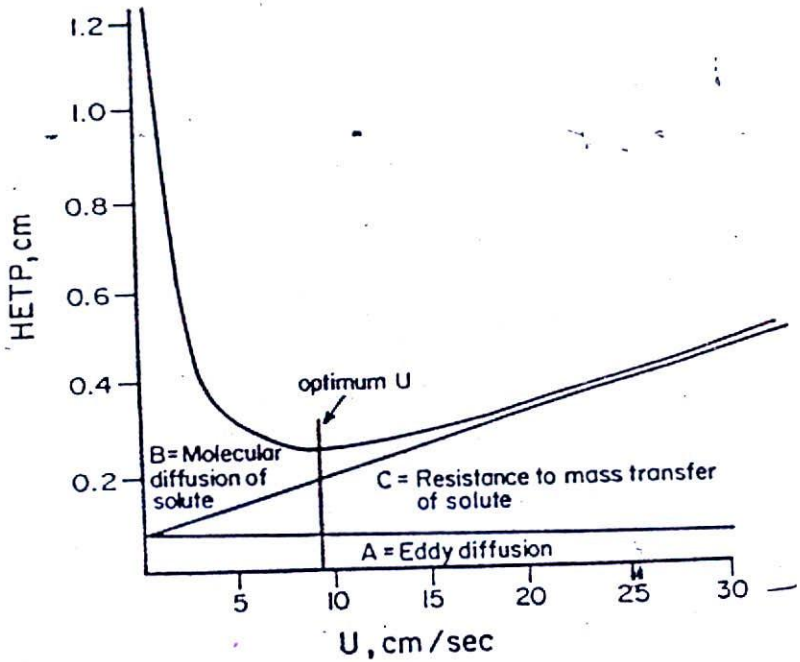

FIGURE 18.4: Plot of HETP against average carrier gas velocity U.

is best given by the *separation factor* α which is the ratio of the retention volumes and thus is a measure of the distances of the apices of the two solute peaks.

$$\alpha = \frac{V_{R_2}}{V_{R_1}} = \frac{K_2}{K_1} \tag{18.17}$$

where V_{R_1} and K_1 are the retention volume and partition coefficient of solute 1 and V_{R_2} and K_2 are the retention volume and partition coefficient of solute 2.

Only if the separation factor differs from unity can the peaks be resolved. The larger the α value is from 1 the better the separation. In many instances however, even through α is significantly larger than 1, the solute peaks may not be resolved completely (Fig. 18.5A). If column efficiency is low, i.e., too few theoretical plates, the solute peaks may still overlap. By modification of parameters to increase column efficiency or by lengthening the column, it may be possible to correct the peak broadening defect (Fig. 18.5B). If resolution is poor however due to a separation factor near 1 it is necessary to change either the column temperature or the liquid phase. By a change

in temperature it may be possible, because of differences in the boiling points of the solutes, to alter their K values thus increasing α (Fig. 18.5C). If the temperature change does not alter the separation factor sufficiently, it is then necessary to change the stationary liquid phase to one with a higher selectivity (see p. 673).

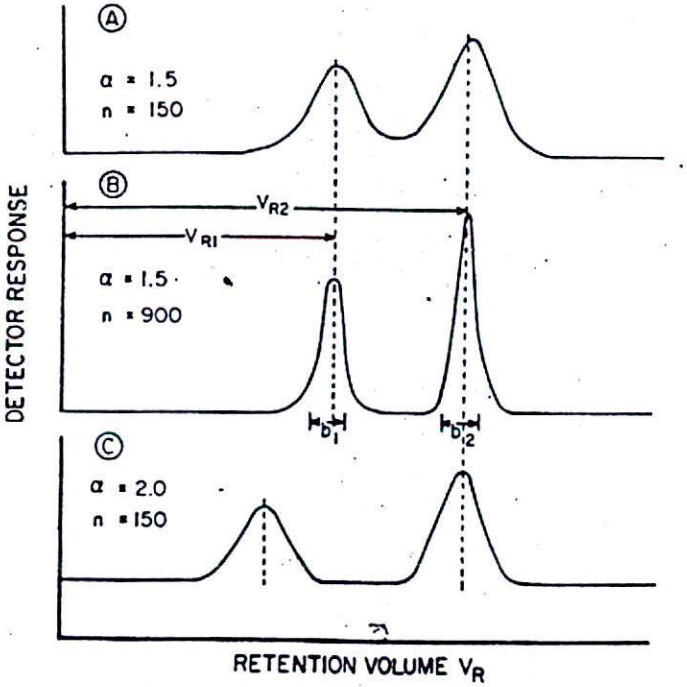

FIGURE 18.5: Schematic diagram of chromatographic separation of two solutes showing effect of retention and column efficiency on resolution. (A) Unresolved solute peaks, (B) peaks resolved by increased column efficiency, (C) peaks resolved by increased separation factor.

An expression of the resolution obtained on a column has been derived based upon peak width and retention volumes.

$$\text{Resolution} = \frac{2(V_{R_2} - V_{R_1})}{b_1 + b_2} \qquad (18.18)$$

Where V_{R_2} is the retention volume from the second peak eluting and V_{R_1} is the retention volume from the first peak eluting. b_1 and b_2 are the peak widths of the first and second peaks, as illustrated in Fig. 18.5B.

The total number of theoretical plates necessary to produce a particular resolution may be determined by expression (18.19)

$$n = \left(2(\text{resolution})\frac{\alpha + 1}{\alpha - 1}\right)^2 \tag{18.19}$$

where α = separation factor, and the resolution is calculated as in (18.18). In general excellent resolution should be possible if the separation factor of the two solutes is 1.2 or above.

18.4 COLUMN TECHNOLOGY

In the consideration of column operation, each unit of the column assembly is of importance. The individual unit parts comprising the complete chromatographic column are the column, the support medium, the liquid phase, and the carrier gas.

A. COLUMN

Depending upon the type of oven module used, chromatographic columns may vary in shape and dimensions. The two most common shapes are the coiled helix and the U-tube. Coiled helical columns have the advantage of being the most efficient shape for columns of 8 ft or longer, but suffer from the disadvangage of being more difficult to shape and pack evenly. Generally columns are packed as long tubes and then coiled. The U-tube column, however, is convenient for columns of short length and is relatively easy to pack and shape. The length of a GC column should be limited to that length just sufficient to carry out the desired separation. The shortest possible column reduces diffusion effects and keeps retention time to a minimum. In most cases, columns of 6 to 12 ft and having 600 to 1000 plates/ft give completely satisfactory separations. The tubing selected is usually glass, aluminum, copper, steel, or stainless steel. Nylon and other synthetic plastic tubings have been utilized, but in most instances the temperature requirements for separation prohibit their use. Copper, stainless steel, and aluminum have proven to be most useful for routine work in helical columns. Glass is frequently employed in U-tube columns and for separation of biological materials. Compounds sensitive to catalytic action of metals also are best separated on glass columns. Most commercial chromatographs are designed to accept tubing of diameters from 1/8 through 3/8 in., connections being made with nuts and ferrules or O-rings. Adapters for various size tubings are also readily available from most manufacturers.

B. SUPPORT MEDIUM

The support medium for the liquid phase within the column has two primary requirements: it must be a poor adsorbent and it must be a finely

divided porous substance having a large surface area. In addition, other requirements include chemical inertness, heat stability, and sufficient mechanical strength to prevent fracturing with normal handling. In regular GLC the materials which meet these specifications are diatomaceous earths, ground fire brick, glass beads, fluorinated resins, and polyaromatic resins. The most commonly used support mediums are the calcined diatomaceous earths.* The latter are frequently treated with silanizing agents such as dimethyldichlorosilane (DMCS) or hexamethyldisilazane (HMDS), following an acid wash treatment. This treatment reduces surface activity of the support media to negligible levels by deactivating and coating all available active sites. "Tailing" of solute peaks due to adsorption effects is diminished considerably by this treatment. Mesh sizes vary according to column conditions and efficiency required by the particular separation problem. Mesh sizes from 20/30 up to 100/120 are commonly employed.

C. LIQUID PHASE

As was stated earlier, a solute with a small partition coefficient K will travel at a faster rate down a column than a large K. It follows therefore that the solute with the greatest tendency to remain in the gas phase will travel down the column at the fastest rate or, stated in another form, the greater the volatility of a solute the shorter the retention time. For two or more solutes, therefore, comparative retention times or volumes are a matter of relative volatility. As in distillation the *relative volatility* may be given as:

$$\alpha_{1,2} = \frac{a_2^0 P_2^0}{a_1^0 P_1^0} = \frac{K_2}{K_1} \tag{18.20}$$

where $\alpha_{1,2}$ is the relative volatility of solutes 1 and 2 and a_1^0 and a_2^0 is the activity coefficient of the solute in the solvent at infinite dilution. P_1^0 and P_2^0 are vapor pressures of pure solutes 1 and 2.

The terms a_1^0 and a_2^0 are comparable to the deviation factor in Raoult's law and are an expression of the factors other than vapor pressure which influence the solute molecules to leave the solution or liquid phase and migrate to the vapor or mobile phase. These solute-solvent interactions determine the *selectivity* of a liquid phase for different solutes. If a liquid phase has little or no interaction with the solutes from hydrogen-bonding, dipole interactions, or complexation effects, then separation of solutes utilizing such a *nonselective liquid phase* will depend upon boiling point alone. The nonpolar liquids such as squalane, paraffin oils, and silicone oils are nonselective stationary phases on which separation depends almost completely on the differences in boiling points of the solutes. With increasing polarity of the liquid phase increased selectivity results and separation may then depend

* Available as Chromosorbs, Johns-Mansville Products.

upon both selectivity of the liquid phase and the vapor pressures of the solutes. A very selective phase for two solutes is one which will resolve two solutes of different molecular type but with the same boiling points. If $P_1^0 = P_2^0$, then Eq. (18.20) becomes:

$$\alpha_{1,2} = \frac{a_2^0}{a_1^0} = \frac{K_2}{K_1} \qquad (18.21)$$

Sample components which have a low polarity will tend to dissolve easily in nonpolar nonselective liquid phases. According to their partial pressures, they will distribute themselves readily between the fixed liquid phase and the gas phase. Because of low a values there is little or no tendency to be held in the liquid phase due to solute-solvent interactions and they will elute from the column in increasing order of their boiling points. For example in the series of saturated hydrocarbons methane, ethane, propane, butane, and isobutane elution from the nonselective liquid phase hexadecane is in the order of increasing boiling points. However, if the sample components and liquid phase have different polarities, the solubilities of the solute in the liquid phase will be much less. This results in higher vapor pressures than would be predicted from the molar concentrations involved. As a result, the solutes will be eluted again in order of their boiling points, but at a faster rate than when both solute and liquid phase are nonpolar. For example the polar halogenated hydrocarbons such as the chloroalkanes will travel through a nonselective hexadecane column faster than will the corresponding nonpolar nonhalogenated alkanes. Similarly if nonpolar alkanes are chromatographed on both a nonpolar liquid phase such as hexadecane and a polar liquid phase such as benzyl ether, the polar liquid phase will show shorter retention times than the nonpolar liquid phase. In both phases elution order will be the same and in increasing order of boiling points.

Selectivity becomes of major importance only when there is significant polarity or other solute-solvent interaction between the liquid and solute phase. Of the different interactions hydrogen-bonding plays a very significant role. Hence separation of polar solutes on a polar stationary phase may be as dependent upon hydrogen-bonding interactions as upon boiling points. In other situations, interactions between permanent or induced dipoles in the solutes with polar groups in the liquid phase may be the critical factor in effective separations on selective columns. In still different applications, weak complexation interactions may be utilized to influence the selectivity of the liquid phase and hence to improve separation of components, e.g., alkenes on silver-treated columns.

In selecting a stationary phase from the hundreds of liquids available there are various practical limitations which must be considered. Of prime importance is the upper temperature limit of the liquid. This limit is governed both by the liquid's volatility and by its stability. At high operating

temperatures *column bleed* may occur. Column bleed is indicated by a rising base line with rise in temperature and indicates either a detectable volatility or partial decomposition of the liquid at the temperature involved. Another practical factor is that of purity of the liquid. Contaminants within the liquid phase frequently produce extraneous peaks or give an uneven base line. Solubility of the liquid phase in various solvents also must be considered when coating the support medium. Heavy viscous liquid phases may have too low a solubility in the solvent chosen and may prevent a sufficiently high percentage loading of liquid phase onto the column.

TABLE 18.1: Commonly Used Stationary Phases

Stationary phase	Common name	Relative polarity[a]	Maximum operating temperature, °C	Solvent[b]
Squalane	—	NP	125	1 or 2
High vacuum grease	Apiezon L	NP	300	1 or 2
Methyl silicone rubber gum	SE 30	NP	375	1 or 2 (hot)
Fluoro silicone rubber gum	QF-1 or FS-1265	IP	250	1 or 2
Silicone oil	DC-550	IP	275	1 or 2
Phenyl methyl silicone rubber gum	SE 52	IP	300	1 or 2 (hot)
Diethyleneglycol succinate polyester	DEGS	P	190	
Polypropyleneglycol	Ucon 50 HB-2000	P	200	1 or 2
Polyethylene glycol	Carbowax 20M	P	225	1 or 2
Butenediol succinate polyester	—	P	225	1 or 2

[a] NP, nonpolar; IP, intermediate polarity; P, polar.
[b] 1, methylene chloride; 2, chloroform.

While hundreds of stationary phases are available, the majority of GLC separations employ only a relatively few liquids. Table 18.1 lists ten of the most frequently utilized stationary phases. In addition it gives the relative polarity, the maximum operating temperature, and the suggested solvents for preparing the columns. It will be noticed that the common name is frequently a trade name or an abbreviation. The reader is referred to various GLC supply catalogues for a complete listing of stationary phases.

D. CARRIER GAS

The most commonly utilized carrier gases in GLC are nitrogen, helium, hydrogen, and argon. Special carrier gases such as propane and acetylene have been utilized for special separations, but are not generally employed.

From a theoretical standpoint the type of carrier gas is the least critical parameter in column operation. All of the common carrier gases are insoluble in the stationary phase and have little influence upon the selectivity of the liquid phase. As mentioned in the discussion of the van Deemter equation [Eq. (18.15)], the value of D_g or carrier gas diffusivity is decreased by increasing molecular weight of the carrier gas. Because of this fact, the more dense the carrier gas the less the peak broadening due to molecular diffusion. If possible therefore the most dense carrier gas is used. The most important criteria for choice of carrier gas is the type of detector to be used. With the thermal conductivity detector the lightest gases, hydrogen and helium, are utilized. The thermal conductivity of a gas is inversely proportional to the square root of the molecular weight. Because the sensitivity of the thermal conductivity is dependent upon the difference in thermal conductivity between the carrier gas and the more dense solute vapors, the least dense carrier gas produces maximum detector sensitivity. With argon beta-ionization detectors, argon is employed as carrier gas since operation of the detector depends upon the presence of argon. Similarly the carrier gas nitrogen or an argon-methane mixture is utilized as the source of slow electrons in the electron capture detector and is thus required as the carrier gas. With the flame ionization detector any nonorganic carrier gas may be employed, and because of the molecular diffusion effect the more dense nitrogen is generally preferred.

E. PREPARATION OF THE CHROMATOGRAPHIC COLUMN

In the preparation of a chromatographic column it is first necessary to prepare the stationary phase by coating the liquid phase over the stationary support medium. If several identical columns will be needed, it is advisable to prepare a sufficient quantity of stationary phase at one time since column characteristics may change slightly from batch to batch. From 50 to 500 g may be prepared by the following procedure:

a. Weigh out the required weight of solid support media of the correct mesh sizes and place in a rotary evaporation flask.

b. Calculate and weigh the amount of liquid phase to give the correct liquid loading. Liquid loading on the support media may vary from 1 to 35%, depending upon the separation desired.

c. Dissolve the liquid phase in a sufficient volume of solvent (see Table 18.1) to just wet the solid support media.

d. Add slowly the dissolved liquid phase to the support media in the flask with stirring until an even slurry is formed. Stir until thoroughly mixed.

e. Attach the flask to a rotary evaporator and allow all solvent to evaporate while rotating under vacuum. Continue rotation until an even coating of liquid phase is insured.

f. Select a suitable length of column tubing and plug one end with glass wool. With a funnel add the stationary phase to the open end. Evenly packed columns are best prepared by use of an electric vibrator along the column or by constant tapping during phase addition. When the column is completely packed, the open end is plugged with glass wool. For preparation of helical columns the tube may be packed straight and then coiled around a suitable object or precoiled columns may be packed with the aid of vibration and a vacuum applied at the bottom end. U-tubes are packed by filling from each end toward the center with vibration.

g. The column is now fastened into the chromatograph and conditioned by purging with carrier gas. Purging is continued for several hours at a temperature about 20°C above maximum operating temperature. A well-prepared conditional column will maintain a constant zero base line on the chromatogram with a minimum of attenuation of detector signal output.

18.5 DETECTORS

The detector may be considered to be the brain center of the gas chromatograph. The impulse received from the eluate of the column in the form of the solute vapor is "sensed" by the detector. It in turn converts this impulse into an electrical signal proportional to the concentration of the solute in the carrier gas. This signal is then amplified and recorded as a peak on the chromatograph.

A good detector should have several important characteristics: stability against the effects of extraneous noise in the detector system; a rapid and linear response to changes in solute vapor concentration as the column effluent passes through the detector; reproducibility of response under designated operating conditions; sensitivity to a wide range of solute vapors.

Many different types of detectors have been produced. Some are extremely versatile, while others have very limited application. While it is beyond the scope of this chapter to deal with all detectors in detail, a brief discussion will be carried out on the four most commonly used detectors.

A. THERMAL CONDUCTIVITY DETECTORS

Thermal conductivity detector—katharometer—is the most commonly used detector and comes closest to fulfilling the basic characteristics of the ideal detector. The detector is simple, applicable for a broad range of solutes, is stable, does not destroy the sample, and is operable over a wide range of temperatures. It is however less sensitive than the other detectors, requiring in the range of 10^{-6} to 10^{-2} g of samples.

The operation of the thermal conductivity detector is based upon differences which occur in the thermal conductivity of the effluent gases from the

chromatographic column. The carrier gas has a constant thermal conductivity and is used as a reference. The thermal conductivity of the binary mixture of solute vapor and carrier gas varies proportionally to the concentration of solute vapor present. Because of these changes in the thermal conductivity of the gas mixture as it flows over the heated filament in the detector, there is produced a similar proportional change in resistance within the heated filament. Due to circuit design these resistance changes cause a similar proportional voltage drop which is then recorded on the chromatogram.

B. β-RAY IONIZATION DETECTORS

β-Ray ionization detectors—argon detectors—utilize a radioactive source of radium 226, strontium 90, or tritium as a source of β rays and employ argon as the carrier gas. Argon is excited to a metastable state by the beta ionization of the radioactive source. On the entrance of the solute vapor molecules to the detector the metastable argon reacts with the solute vapor molecules, producing sufficient solute ions to alter the standardized background current. The change in current is proportional to the amount of solute molecules present.

The principle advantage of the beta ionization detector is the increased sensitivity over the thermal conductivity detector. It also is considerably less sensitive to temperature changes than is the thermal conductivity detector. The argon detector has the disadvantage of requiring a radioactive source and care must be taken not to overheat the detector. Further disadvantages lie in the limited sample size that may be used, i.e., 10^{-7} g, and its insensitivity to certain compounds.

C. FLAME IONIZATION DETECTORS

The flame ionization detector has largely replaced the beta ionization detectors because of its simple construction and its linearity over a dynamic range of 1 million. It requires no radioactive source and can utilize inexpensive carrier gases such as nitrogen. This detector is insensitive to temperature change and is relatively insensitive to fixed gases such as CO_2, H_2O, and H_2S. Because of its insensitivity to temperature change it is ideally suited for temperature programming. The flame ionization detector is exceptionally versatile since it is sensitive to all organic compounds in the range of 10^{-3} to 10^{-9} g. Its chief disadvantage lies in the fact that the sample is destroyed. In principle the flame detector is simple. A hydrogen flame in the detector burns the solute molecules as they enter the detector from the column. During the burning process electrons and ions are formed and are collected at an anode. The electrical current produced is directly proportional to the amount of solute molecules consumed.

D. ELECTRON CAPTURE DETECTORS

This detector has become of considerable importance because of its very high sensitivity to certain molecules. It is especially recommended for alkylhalides, conjugated carbonyls, nitriles, nitrates, and organometallics. Its selective sensitivity to halides makes it especially useful for insecticide analysis. In certain instances, quantities down to the picogram level (10^{-12} g) have been detected. In contrast it is insensitive to a large number of other organic compounds such as hydrocarbons, amines, aldehydes, and ketones. Other disadvantages of the detector are its detector temperature limitation (220°C) and requirement for a very pure nitrogen or an argon-methane mixture for a carrier gas.

The electron capture detector, unlike the other detectors discussed, measures a loss of signal rather than an increase in current. A tritium source is used as an ionizing source and in contact with the carrier gas it ionizes the molecules producing electrons. These electrons migrate to a positively charged anode and produce a current which is amplified. When a solute sample composed of electron-absorbing molecules such as alkylhalides enters the detector, some of the electrons are absorbed onto the solute molecules producing a decrease in current proportional to the number of solute molecules present. This decrease in current is recorded on the chromatogram.

18.6 ANALYTICAL DETERMINATION BY GAS CHROMATOGRAPHY

A. QUALITATIVE ANALYSIS

Gas-liquid chromatography is an extremely useful tool for qualitative analysis, being of special value in the identification and comparison of compounds of closely related structure. Its versatility in resolving solute mixtures and simultaneously aiding in their identification has been responsible for much of its popularity. It must be remembered however that GLC data should not be used to provide positive identification by itself in the absence of other supporting information.

Most qualitative determinations are accomplished by comparison of the chromatographic characteristics of the unknown sample with those of known standards. Since the retention of a solute on a chromatographic column is a physical constant when determined under defined conditions, the comparison of corrected retention volumes $V_{R_1}^{\circ}$, net retention volumes V_N, or relative retention values $r_{1,2}$ may be used for identification purposes. Of these values probably the relative retention value $r_{1,2}$ is most convenient since, when the values are determined under identical conditions, the effects of operating conditions are eliminated as discussed on p. 665. If the relative retention value of an unknown solute and a known standard are found to be identical for one column, it is evidence that the compounds are the same.

The occurrence of identical relative retention values for both known and unknown solutes on a second column with a different polarity greatly strengthen this view. With sufficient prior information from other methods a reasonably accurate identification of the unknown may be made by this method.

An alternative method for identification of an unknown compound is to add a known standard compound to an aliquot of the solution of the unknown. When previous evidence has indicated that the unknown is the same compound as the standard chosen, both the original unknown solution and the "spiked" aliquot are chromatographed under identical conditions and the resulting chromatographic peaks are compared. If the unknown compound is different from the standard, two resolved or partially resolved peaks with different retention values are seen in the chromatogram of the "spiked" sample. If both the unknown and standard compounds are identical, a single but larger peak than that occurring with the unknown compound will be found on the chromatogram from the "spiked" sample. Again, a repeat run on a column with different polarity should add further confirmation as to the unknown's identity.

A graphical method of identification of compounds by GLC is that of homologous series plots. In this method, a solute mixture containing several members of a homologous series is chromatographed under specific operating conditions. A plot is made of the logarithm of either the net retention volume or of the relative retention value against the number of carbon atoms. Since the logarithm of either the net retention volume or the specific retention value of a homologous series is proportional to the number of carbon atoms—or other property showing a stepwise increase with carbon content—a straight line is produced. The slope of the line is dependent upon the nature of the stationary phase. Identification of an unknown compound belonging to this homologous series is possible by repeating the experiment with the unknown compound and identifying it from its position on the standard curve. A more positive method of identification than that just described is by the method of two-column homologous series plots. By this procedure several members of two or more different homologous series are each chromatographed on two columns of different polarities. Relative retention times are determined against a known standard of that chemical class for each series. Log-log plots for each series are then made of relative retention times for one column against relative retention time for the second column. Straight-line plots with differing slopes should result for each series. The identification of an individual unknown of one of the series may be made by following the same chromatographic procedure and determining on which straight line the relative retention value will fall. The chemical class of the compound is determined by noting on which homologous series plot it falls, and the number of carbons it contains is determined from its position on the line.

When used in combination with other qualitative methods, the scope of GC is increased still further. By the use of sample collection attachments or direct attachment of the effluent stream to a second instrument it is possible to analyze resolved compounds by further instrumental procedures. By such methods separated components may be examined by infrared, ultraviolet, or NMR spectroscopy. Radioactive effluents may be studied or mass spectrometry may be used by direct attachment of the column by a gas-flow counter to the ion beam of a mass spectrometer.

B. QUANTITATIVE ANALYSIS

Several inherent advantages are possessed by gas chromatography when applied to quantitative analysis. Its ability to simultaneously separate several constituents in one run, its applicability for microgram quantities of sample, its speed and its accuracy make it an exceptional analytical procedure.

In quantitative determinations it is necessary to measure the peak area or the peak height of each compound of interest on the chromatogram. The values so obtained are then correlated with the quantity of solute required to produce each peak.

Determination of peak height is a less accurate method of measurement than is peak area determination and usually is satisfactory only for narrow peaks which elute early in the chromatographic run. More accurate determination is by peak area calculations. In this procedure the area under the peak graphically represents a measure of the solute volume or weight which elutes from the column into the detector. Peak area may be determined by one of several methods:

a. Height times width of half height. The area under a chromatographic peak is calculated in this procedure by multiplying the peak height h by the width of the curve w at one-half the height h (see Fig. 18.3). The calculation gives good results with symmetrical peaks, but errors arise with unsymmetrical curves.

b. Planimeter determination. The use of a planimeter for calculating the peak area is accurate if carefully done. Accuracy varies between operators and errors rise as peak size diminishes.

c. Weighing of the paper cut from the chromatogram. With careful cutting of the peak from the chromatogram the weight of the paper may be obtained. From a determination of weight per unit area of paper the area under the curve may be calculated. The method is tedious and demands careful work.

d. Determination of peak area by integrators. A number of integrating devices have been manufactured which attach directly to the recorder and integrate the area as the chromatogram is recorded. These devices are more convenient and accurate than the other methods described. Electronic integrators giving direct printout of detector response are employed by

some laboratories and offer the maximum accuracy in quantitative determination.

Correlation of peak area and concentration of solute is dependent not only upon the size of sample injected but also to a considerable extent upon the linearity of response of the detector over the concentration range of sample being examined. Factors which influence detector operation and sensitivity also influence detector response and therefore peak area. Such factors as detector and column temperature, gas flow rate, anode voltage, and current flow in the detector must be considered and compensated for if necessary.

Different methods of quantitative determination are utilized to correlate peak area accurately to solute concentration. Three of these methods are given here.

1. Quantitative Determination by Internal Standardization

The most accurate method for quantitative determination of a known solute is the method by which a known amount of a standard sample is added to the solute being examined and the peak area produced by the standard is compared with that of the solute being determined. The internal standard is a compound preferably of the same chemical type, but with different structure than the solute under examination. The standard should have a retention time close to, but completely resolved from, the unknown. The peak area of the standard should approximate that of the quantitative unknown. By the use of an internal standard most of the apparatus variables and differences in operating parameters are cancelled out. In addition, by this method the sample size for injection is not critical so long as it is within the response range of the detector.

Measurement by internal standardization is accomplished by first determining the *sensitivity* or *response factor* of the detector for the quantitative unknown relative to the internal standard. An accurately weighed sample of the internal standard is added to an accurately weighed reference sample of the pure compound being assayed. Accurate dilution with solvent is carried out if necessary and the solution is chromatographed. The response factor is calculated from Eq. (18.22):

$$F_s = \frac{A_s W_u}{W_s A_u} \tag{18.22}$$

where F_s = sensitivity or response factor
A_s = peak area of internal standard
W_s = weight of internal standard added to sample
A_u = peak area of reference sample being analyzed
W_u = weight of reference sample being analyzed

Quantitative determination of the unknown sample is then accomplished by adding an accurately weighed amount of internal standard to a known

weight of sample being examined. The mixture is then run under the identical chromatographic conditions used previously. The volume of the injected mixture does not have to be accurately known.

The calculation of the per cent weight of unknown is determined by the equation:

$$\text{weight per cent of unknown} = \frac{F_s A_u W_s \times 100}{A_s W_x} \qquad (18.23)$$

where W_x is the weight of sample being assayed. When repeated samples are being run, F_s should be determined at regular intervals and at least once a day.

2. Quantitative Determination by Area Normalization

The area normalization method of quantitization is at best a semiquantitative procedure. It is based upon the assumption that equal weights of all substances contained in the sample produce equal responses from the detector and that the response is linear over the range of weights being examined. Under these conditions the summation of all peak areas equals 100 per cent and the per cent concentration of any one substance equals the ratio of that peak area to the sum of the total peak areas multiplied by 100. Therefore:

$$\text{weight per cent of unknown} = \left[\frac{A_u}{(\sum A_u + \cdots A_n)}\right] \times 100 \qquad (18.24)$$

where A_u is the peak area of substance being determined. This method has the advantage of not requiring an accurate sample size, but it gives rise to errors due to different response factors for different compounds.

3. Quantitative Determination by External Standardization

External standardization is based upon the comparison of the peak areas of chromatograms prepared from known standard concentrations of the compound under assay and those of the unknown concentration. Separate chromatographic runs are made for each known concentration and these peak areas are used as standards. Calibration curves may be prepared or direct calculation may be made with the unknown sample. The method may be accurate to 1 % providing very careful control of all chromatographic parameters is maintained. Best results are obtained when both standards and unknowns are chromatographed during a single time period of constant operating conditions.

The disadvantages of the external standardization procedure are related to errors introduced because of changes in operating conditions, instrument variation, and errors in measurement of the sample. Each type of detector varies with respect to the effect of operating conditions on signal output

sensitivity. Thermal conductivity detectors for example show changes in sensitivity with changes in gas flow rate, detector temperature, and filament current. During a single operating period, errors from these sources may be minimized, but if the instrument is used at varying intervals, restandardization is usually necessary each time. Probably the greatest single error involved in the external standardization procedure is due to the measurement of injection volumes. Since sample volumes are frequently in the range of fractions of microliters, small errors in estimating sample volumes introduces sizeable errors in results.

It may also be pointed out that when more than one sample component is being measured on a single chromatogram it is necessary to prepare standard calibration curves for each component individually. This is required because of the possible differences in sensitivity or response to each component by the detector and is comparable to determining the response factor as mentioned previously.

18.7 EXPERIMENTS

In analysis with gas chromatography it is imperative that the operator be thoroughly familiar with the instrument and its operation. The following experiments are designed to accomplish this purpose as well as to illustrate principles discussed in the text.

A. QUALITATIVE DETERMINATIONS OF *n*-HYDROCARBONS

Column Operation Data: column, 4 ft × ¼ in. OD, 10% SE30 on 60/80 mesh Chromosorb *W*;* temperature, isothermal operation: 180°C column, 300°C detector, 300°C injection port; detector, thermal conductivity or flame ionization; carrier gas, helium, 50 ml/min.

1. Identification from Relative Retention Values

Set up the chromatograph for isothermal operation with the operating conditions given previously. Prepare 100-μl standard samples of the following binary mixtures, using 50 μl of each standard hydrocarbon:† octane-dodecane; decane-dodecane; tetradecane-dodecane. For each standard sample prepare a chromatograph by injecting 0.5 μl of the sample into the injection port with a microsyringe. At the instant of injection move the recorder pen to mark the point of injection. If necessary adjust the signal attenuation to give satisfactory peak heights. From each chromatogram

*Johns-Manville Products, New York, N.Y.

† Available in Polyscience Kit 82-00500-800 Qualitative Kit 210 Varian Aerograph, Walnuf Creek, California.

obtained measure the retention times for each of the two hydrocarbon peaks. Retention time is calculated by dividing the chart speed into the distance the chart has traveled from the point of injection to the center of the hydrocarbon peak. See Fig. 18.3. Determine the relative retention values, $r_{1,2}$ for octane, decane, and tetradecane, using dodecane as the internal standard. Since the retention time t_R is proportional to the retention volume under the conditions employed, Eq. (18.9) becomes:

$$r_{1,2} = \frac{V_{R_1}}{V_{R_2}} = \frac{t_{R_1}}{t_{R_2}} \tag{18.25}$$

where t_{R_1} and V_{R_1} are the retention time and volume of dodecane and t_{R_2} and V_{R_2} are the retention time and volume of the second hydrocarbon.

To 50 μl of unknown samples provided add 50 μl of dodecane and mix. Each unknown will contain one or more of the C_8, C_{10}, or C_{14} hydrocarbons. Repeat the chromatographic procedure described previously with the unknowns. Calculate the relative retention values for each unknown peak and compare with those of the standards. From the data obtained determine the hydrocarbons in each unknown.

2. Identification from Homologous Series Plots

From the relative retention data obtained previously plot on semilog paper the relative retention times of the standard *n*-hydrocarbons on the semilog scale against carbon number. A straight-line plot should result. Identification of each unknown may then be determined by plotting its position on the curve and locating the *n*-hydrocarbon it falls closest to. By extrapolation of the line, larger or smaller carbon-containing *n*-hydrocarbons may be determined.

B. DETERMINATION OF COLUMN EFFICIENCY FOR DECANE AND TETRADECANE

Maintain the same column conditions as in part A. Inject 0.3 μl of standard decane solution, marking the chromatogram at the instant of injection. It is important that the injection of solute be made rapidly and smoothly so that the application of solute to the column is as nearly instantaneous as possible. When the chromatogram is completed, make a second chromatogram with 0.3 μl of tetradecane.

From each chromatogram determine the number of theoretical plates on the column for that hydrocarbon as follows: for each hydrocarbon peak measure the retention time t_R. Determine the base width b for both the hydrocarbon peaks by drawing tangents through the inflection points of the curves and extending the tangents to form a triangle with the base lines as shown in Fig. 18.3.

Calculate the number of theoretical plates from Eq. (18.13):

$$n = \frac{16(t_R)^2}{b} \tag{18.13}$$

Compare the number of theoretical plates for both hydrocarbons. A good column should have from 300 to 600 HETP's per foot of column. From the data obtained previously calculate the HETP for each hydrocarbon, where HETP = l/n (18.14).

C. SEPARATION AND IDENTIFICATION OF *n*-HYDROCARBONS BY TEMPERATURE PROGRAMMED OPERATION

Except for the column temperature conditions, the operating parameters are those given in Section 18.7A. Set program controls at an initial column temperature of 100°C and the upper limit temperature control at 300°C. The rate of temperature rise is set at 15°C/min. Prepare a sample of *n*-hydrocarbon containing equal volumes each of octane, decane, dodecane, and tetradecane. Inject 1.0 μl of the sample and start programmed operation. Determine the elution time for each peak. Repeat the procedure with 0.5 μl of each of the binary standard hydrocarbons in Section 18.7A. Determine the elution temperature for each standard component. From the results obtained previously identify each peak in the original mixture.

D. DETERMINATION OF ALCOHOL CONTENT OF NITROMERSOL TINCTURE

Column Operation Data: Column, 6 ft × ¼ in. OD, 20% polyethylene glycol 400 on 60/80 mesh Chromosorb W. Temperature, isothermal operation, 100°C column; 120°C detector; 160°C injection port. Detector, thermal conductivity preferred. Carrier Gas, helium 60 ml/min.

Pipette exactly 10 ml of nitromersol tincture into a stopped flask and add exactly 4.0 ml of methylethyl ketone (MEK) as an internal standard. Prepare about 50 ml of 50% alcohol solution and accurately determine the alcohol content.* Pipette triplicate samples of exactly 10 ml each of the known alcohol solution into stoppered flasks and add exactly 4.0 ml of MEK to each as an internal standard. Utilizing identical column operating conditions, make individual chromatographic runs on each known alcohol sample and on the nitromersol solution. Each injection should be sufficiently large—1 to 5 μl—to enable an accurate measurement of peak height. Measure all peak areas accurately in square centimeters by integrator or by

* The alcohol content is found by determination of the specific gravity at 25°C and referring to alcoholmetric tables.

the method of peak height times width at half-height. Calculate the sensitivity or response factor F_s of the detector for each of the 10-ml samples of known alcohol solution from the equation:

$$F_s = \frac{A_s W_u}{W_s A_u}$$

where A_s = peak area of MEK internal standard
W_s = volume of MEK in known alcohol
A_u = peak area of alcohol in known alcohol sample
W_u = volume of alcohol in known alcohol sample, i.e., 10/100 × alcohol per cent

From the three values of F_s obtained find the average F_s value. For accurate results F_s values must be determined each time the instrument is started up.

With the average F_s value obtained calculate the per cent alcohol in the nitromersol tincture by the following equation.

$$\frac{F_s A_u W_s}{W_x A_s} \times 100$$

where W_x is the volume of nitromersol solution used in sample.

QUESTIONS

Q18.1. Two types of detectors have been utilized in GC, the differential and the integral detector. By referring to other literature sources discuss the basic principle upon which each type is based. Make a sketch of a typical integral and differential chromatogram.

Q18.2. Capillary columns are frequently employed in the petroleum industry. They consist often of several hundred feet of capillary tubing coated on the inside with liquid phase. Discuss the relative merits and disadvantages of this type of column.

Q18.3. By consideration of the van Deemter equation discuss the effect on column efficiency produced by: increasing the percentage of the liquid coating on the support medium, changing the carrier gas from nitrogen to hydrogen and increasing the carrier gas velocity when it is already at a high flow rate.

Q18.4. Assuming that a halogenated hydrocarbon RCl and its parent hydrocarbon R have nearly the same boiling point and will chromatograph under the conditions employed, which compound would you expect to be eluted first from a squalane column? From a DEGS column? Explain.

Q18.5. Discuss the derivation of Eq. (18.23).

$$\text{Weight per cent of unknown} = \frac{F_s A_u W_s \times 100}{A_s W_x}$$

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