CRC

Handbook of Chromatography

Volume II

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PREFACE

Volumes One and Two of the *Handbook of Chromatography* represent over three years of intensive labor by the editors and members of the Editorial Advisory Board. When the Publishers first suggested the production of a Handbook for Chromatographic Data, it seemed to the editors to be just a simple compilation of R_F-tables and retention times or volumes. However, a quick perusal of the literature of chromatography, accumulated during approximately twenty-five years of modern chromatography, revealed that the task would be a formidable one and could not be accomplished in one year, as was first estimated.

The Handbook, as it finally appears, should serve chemists in all fields as a working manual and reference book which should aid in their search for identification of unknowns as well as suggest quantitative methods of analysis.

Volume One of the Handbook contains over 549 tables of chromatographic data expressed in uniform terms of R_F values, retention times and retention volumes as well as other terms used in chromatography. Each table has literature citations which will refer to the primary and sometimes secondary sources. Over 12,000 compounds are cross-indexed to direct the reader to appropriate tables in this volume. Thus, for example, the amino acid alanine will have entries in tables under gas, liquid-column, paper, and thin-layer chromatography.

Volume Two of the Handbook has been designed to give the researcher, even the novice in chromatography, a working knowledge of the theory and practices of the various fields of chromatography–gas, liquid-column, paper, and thin-layer chromatography.

Volume Two also contains two useful sections. One is on detection reagents for paper and thin-layer chromatography, with an alphabetical index for chemical classes and "name" reagents. This section is very helpful for the interpretation of the tables in Volume One. The other section describes selected methods for sample preparations, which will be expanded in future editions of the Handbook. Subsequent sub-sections in **PRACTICAL APPLICATIONS** supply the researcher with useful information on commercial sources of all types of chromatographic supplies.

A Book Directory at the close of Volume Two should serve as a good source for more detailed reading in chromatography.

The editors would be ungrateful if they did not acknowledge the invaluable help and advice from the members of the Advisory Editorial Board.

Special thanks are due to the industrious group of compilers, especially Mrs. Ellen Burton, Miss Susan Rodems, Miss Irene Zweig, Mrs. Frances K. Zweig, and Dr. and Mrs. Coleman Hamel. Special thanks are also due to Dr. Irving Sunshine whose initiative and perseverance are in no small manner responsible for the writing and creation of this Handbook, and to Mrs. Florence Thomas and Mrs. Ruth Pokorney of The Chemical Rubber Co. for their unstinting effort in the production and final editing of the books.

The editors would greatly appreciate suggestions for improvements and additions in future editions from any interested persons.

Gunter Zweig

Joseph Sherma

November 1972

Advisory Board

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PRINCIPLES AND TECHNIQUES

Joseph Sherma

I.I. Introduction

A. HISTORY

A *definition* of *chromatography* similar to one originally stated by Strain is as follows: Chromatography is a separation method in which a mixture is applied as a narrow initial zone to a stationary, porous sorbent and the components are caused to undergo differential migration by the flow of the mobile phase, a liquid or a gas.

The originator of chromatography as it is practiced today was Michael Tswett (1872–1919). In 1906, Tswett, a Russian botanist, published a paper (which has been translated into English and evaluated (*1*)) describing the separation and isolation of green and yellow chloroplast pigments by column adsorption chromatography.

In his original experiments, Tswett tamped a fine powder (such as sucrose) into a glass tube to produce a column of the desired height. After extracting the pigments from leaves and transferring them to petroleum ether, he poured a small volume of the solution onto the column. When the solution had percolated and formed a narrow initial zone beneath the top of the adsorbent, fresh solvent (e.g., petroleum ether) was added and pressure applied to the top of the column. As the solvent flowed through the column the individual pigments moved at different rates and eventually separated from each other. Figure 1 illustrates the development of a chromatogram by Tswett's chromatographic method with a solvent employed by Strain in his extensive studies of chloroplast pigments.

The key features of Tswett's technique were the application of the mixture as a narrow initial zone and the development of the chromatogram by application of fresh solvent. Other early workers had employed procedures based on the phenomena of adsorption or partition, but these lacked Tswett's critical development step and therefore did not yield extensive resolution of the mixtures.

Tswett's original column adsorption chromatographic method has been modified in many ways resulting in the different types of chromatography described in the sections below. The *history* of *chromatography* is outlined by the following chronological listing of some of the key contributions to the development of these modifications:

1848	Way and Thompson	Recognized the phenomenon of ion exchange in solids.
1850–1900	Runge, Schoenbein, and Goeppelsroeder	Studied capillary analysis on paper.
1876	Lemberg	Illustrated the reversibility and stoichiometry of ion exchange in aluminum silicate minerals.
1892	Reed	First recorded column separation: tubes of kaolin used for separation of $FeCl_3$ from $CuSO_4$.
1903–1906	Tswett	Invented chromatography with use of pure solvent to develop the chromatogram; devised nomenclature; used mild adsorbents to resolve chloroplast pigments.
1930–1932	Karrer, Kuhn, and Strain	Used activated lime, alumina and magnesia adsorbents.
1935	Holmes and Adams	Synthesized synthetic organic ion-exchange resins.
1938	Reichstein	Introduced the liquid or flowing chromatogram, thus extending application of chromatography to colorless substances.
1938	Izmailov and Schraiber	Discussed the use of a thin layer of unbound alumina spread on a glass plate.
1939	Brown	First use of circular paper chromatography.

1940–1943	Tiselius	Devised frontal analysis and method of displacement development.
1941	Martin and Synge	Introduced column partition chromatography.
1944	Consden, Gordon, and Martin	First described paper partition chromatography.
1947–1950	Boyd, Tompkins, Spedding, Rieman, and others	Ion-exchange chromatography applied to various analytical problems.
1948	M. Lederer and Linstead	Applied paper chromatography to inorganic compounds.
1951	Kirchner	Introduced thin-layer chromatography as it is practiced today.
1952	James and Martin	Developed gas chromatography.
1956	Sober and Peterson	Prepared first ion-exchange celluloses.
1956	Lathe and Ruthvan	Used natural and modified starch molecular sieves for molecular weight estimation.
1959	Porath and Flodin	Introduced cross-linked dextran for molecular sieving.
1964	J. C. Moore	Gel permeation chromatography developed as a practical method.

B. NOMENCLATURE

Tswett is responsible for much of the nomenclature that is used by most chromatographers today. A glass or metal *tube* (or column) is filled with an active solid (*adsorbent*) to form a chromatographic *column*. The mixture to be separated is applied in an *initial zone* and it is washed with the *solvent*, *wash liquid*, or *developer*. The resultant series of zones is the *chromatogram*, and the washing of the initial zone to form the chromatogram is the *formation* or *development* of the chromatogram.

If the separated zones are colorless they must be detected in some way. (See Reference (*2*) for a general discussion of detection methods.) If the chromatogram is treated with a chemical reagent to form colored derivatives, the chromatogram is sometimes referred to as having been *developed*. It is best, however, to reserve this term for the formation of the chromatogram by washing with solvent.

The combination of the solvent, the mixture and the sorbent is termed the *chromatographic system*. Each chromatographic system is composed, then, of a *mobile phase* (the solvent) and a stationary phase (e.g., the column). The generalized term *sorbent* may be used in place of adsorbent, ion-exchange resin, paper sheet, etc., when referring to the stationary phase.

If the components of the mixture are analyzed quantitatively as well as separated, the term *evaluation*, *quantification* or *quantitation* is used. If the separated solute is removed from the sorbent by washing (either in a chromatographic fashion or not) before this analysis, it is said to be *eluted* and the solution to be analyzed is the *eluate*. In ion-exchange and gel permeation chromatography, many workers use this term *elution* for the development of the chromatogram, and *eluant* for the solvent or wash liquid; the liquid emerging from the bottom of the column is then the *elutrient* or the *effluent*.

C. CLASSIFICATION OF CHROMATOGRAPHY

The subject of chromatography may be divided and subdivided as follows to include the great variety of methods which have evolved from Tswett's original chromatographic method.

The two major classifications of chromatography are solution or liquid chromatography (LC) and gas chromatography (GC). In the former a liquid carries the dissolved solutes through the sorbent, which can be a column, paper or thin layer. In the latter an inert wash gas (carrier gas) carries the gaseous mixture through the sorption column.

Within each of these major divisions, subdivisions based on the stationary phase are designated. Thus gas-solid chromatography (GSC) involves a column packed with an adsorbent, and gas-liquid chromatography (GLC) involves a solid coated with a stationary liquid as the sorbent.

Liquid chromatography in columns can be liquid-solid chromatography (LSC) or liquid-liquid (partition) chromatography (LLC). If the solid stationary phase is an adsorbent, the process is called liquid adsorption chromatography. If it is an ion-exchange material, either organic or inorganic, it is termed ion-exchange chromatography (IXC). If it is a nonionic polymeric gel (e.g., polystyrene or Sephadex) the term gel permeation chromatography (GPC), gel filtration chromatography or molecular exclusion chromatography is used.

Other subdivisions of non-column liquid chromatography are paper chromatography (PC) and thin-layer chromatography (TLC). These subdivisions include separations on all kinds of paper and thin layers whether the mechanism of separation is adsorption, partition or ion exchange.

Liquid-liquid chromatography in columns or on paper may be further subdivided into normal-phase partition chromatography (fixed polar liquids) and reversed-phase partition chromatography (fixed nonpolar liquids).

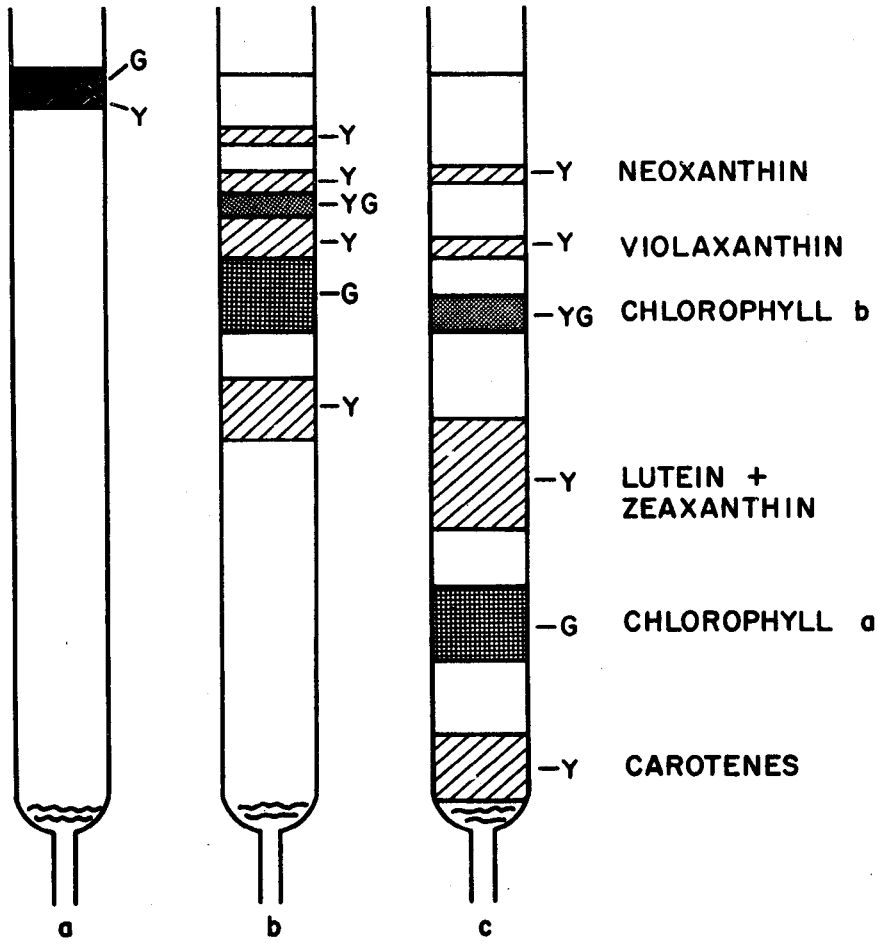

Figure 1. Successive steps in the formation of a chromatogram. Chloroplast pigments of flowering plants, ferns, mosses and lichens and many green algae dissolved in petroleum ether, adsorbed in a column of powdered sugar (a) and washed with petroleum ether plus 0.5 per cent *n*-propanol (b, c). Y = yellow; G = green.

D. MECHANISM OF CHROMATOGRAPHY

The movement of substances during chromatography is the result of two opposing forces, the driving force of the mobile phase and the resistive or retarding action of the sorbent. The driving force acts to move the substances from the origin in the direction of the mobile-phase flow. The resistive action impedes the movement of the substances by dragging them out of the flowing phase back onto the sorbent. Each molecule alternates between a sorbed and unsorbed condition, following a stop and go path through the sorbent. Although the zone moves constantly ahead, only a fraction of the molecules in the zone is moving at any one time. At the end of development, each zone has migrated a certain mean distance and has spread because of the fluctuations in the movement of individual molecules in the zone. The distance travelled by each solute zone in a given time is the resultant of the driving and resistive forces. Substances which move slowly are attracted more strongly to the stationary phase, while those that move quickly spend a smaller fraction of their time in the stationary phase because of less solubility in or affinity for that phase.

The ability to achieve differential migration (i.e., separations) among the mixture components is the result of the selectivity of the chromatographic system. The flow of the mobile phase is nonselective in that it affects all unsorbed solutes equally. As part of the chromatographic system, however, the mobile phase may be selective (e.g., in liquid chromatography) if it helps determine the relative sorbability of the solutes. The sorbent is also part of the chromatographic system and its resistive action (ion exchange, adsorption, etc.) is a selective force. To put this another way, all eluted or nonsorbed components spend equal time in the mobile phase. If there is differential migration, the components spend different amounts of time on the sorbent as determined by the interactions of the chromatographic system.

E. *R* VALUES

The *R* value or retention ratio of a substance indicates its migration relative to that of the mobile phase:

$$R = \frac{\text{solute velocity}}{\text{mobile-phase velocity}}. \quad [1]$$

The *R* value indicates the fraction of time the solute molecules spend in the mobile phase relative to the time in the sorbent.

In paper and thin-layer chromatography it is customary to use migration distances rather than velocities, and to calculate R_F values:

$$R_F = \frac{\text{distance travelled by the center of the solute zone}}{\text{distance travelled by the solvent front}} \times 100. \quad [2]$$

This ratio is similar in idea to *R* as defined above, but R_F values are usually lower than *R* values because the solvent front moves faster over the dry sorbent than does the bulk solvent. The R_F equation also assumes a constant velocity during the run which it does not actually obtain in these techniques.

R and R_F values are not chromatographic constants but vary with the experimental conditions employed during the run. All conditions (such as the nature and preparation of the solvent and sorbent, the arrangement and conditions for the development, the sample size, the temperature, etc.) should be stated when recording *R* values, so that they will have maximum significance. Even then, one should not expect to be able to reproduce exactly *R* values reported by others. The major practical use of *R* values is to indicate relative sorbability and to show which systems may be useful for obtaining the separation of a certain mixture.

The dependence of *R* values on experimental conditions can be lessened if solute migration is described relative to a reference standard which is naturally present in the sample or is added to it. This is especially useful for biological samples containing various impurities (e.g., lipids, inorganic salts) which might alter migration behavior. These values are called R_X values (or *R* values relative to *X*):

$$R_X = \frac{\text{velocity of (or distance travelled by) solute zone}}{\text{velocity of (or distance travelled by) compound } X}. \quad [3]$$

F. SIGNIFICANCE, USES AND APPLICATIONS OF CHROMATOGRAPHY

Chromatography is primarily an analytical tool effective for the separation of mixtures and the qualitative and quantitative analysis of the separated substances. Ideally, each component of the mixture will be completely separated from the other components (it will, of course, be mixed with the mobile phase) and each substance will yield a single, well-defined, regularly-shaped zone. In some cases, however, results are anomalous leading to irregularly shaped zones or multiple zonation (a single substance yields more than one zone).

Chromatography is useful for the comparison of substances, for providing clues as to the structure of organic substances, and for the detection of structural changes produced by various chemical reagents or nuclear and biological processes. Chromatography, combined with conventional chemical and instrumental analytical methods, serves to identify chemical species. Chromatography alone is not adequate for positive qualitative identification even when a sample, prepared by mixing the unknown with an authentic standard it is suspected of being, is found to be inseparable under various dissimilar conditions (this procedure is called co-chromatography). In such a case, the unknown and the standard might be either identical or very similar (e.g., isotopes or isomers). If two substances separate chromatographically, this is positive proof that they are not identical.

Chromatographic methods have a nearly unlimited range of applicability. They can be used to separate the smallest molecules (H_2, D_2) as well as the biggest (proteins, nucleic acids). Isotopes can be separated by gas chromatography and less readily by solution chromatography. Because systems making use of all kinds of physico-chemical interactions can be employed, any substance which is either present in a gaseous state at ambient temperature, vaporizable, or soluble is amenable to chromatographic separation under some obtainable conditions. Quantities in the picogram range can be separated and detected by gas chromatography combined with mass spectrometry, while at the other end of the weight range, multigram quantities can be separated and isolated by preparative column chromatographic methods.

Despite significant recent advances in chromatographic theory [see Reference (*3*), for example] which have provided much information about the mechanism of separations and zone migration, the selectivity of chromatographic systems is still incompletely understood so that the conditions required to separate a given mixture cannot usually be theoretically predicted. For most workers, the experimental approach in chromatography is largely an empirical one, based upon analogy, controlled trial and error, and intuition and experience. Therefore, collations of data such as those presented in this Handbook are extremely useful, because the worker who is interested in applying chromatography to a particular problem can benefit from the experiences of others who have worked with the same or similar compounds. By adopting or adapting previously successful systems, effective separation conditions will usually be found. The more successful users of chromatography strive to understand the physico-chemical causes of successful separations and

the reasons for the choice of particular operating conditions, and are thereby better able to determine parameters which may lead to, and improve, separations.

The sections below are designed to introduce the various kinds of chromatography to those who may not be entirely familiar with them and their basic methods. All these modifications are related, because they involve the application of a narrow initial zone of mixture, the presence of a mobile and stationary phase (i.e., a driving force and a resistive action), and the achievement of separations due to differential migration of the components of the mixture.

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2. Polesuk, J., *American Laboratory*, p. 27, May 1970, and p. 37, June 1970.
3. Rony, P. R., *American Laboratory*, p. 10, May 1970.

Section I.II

GAS CHROMATOGRAPHY

In GC the mobile phase is an inert gas and the stationary phase is either a solid or a fixed liquid packed into a column. The solid in gas-solid chromatography (GSC) is commonly alumina, silica gel, charcoal or a molecular sieve, and selective sorption on these solids allows the separation of certain permanent gases and low molecular weight hydrocarbons. Aliphatic and aromatic hydrocarbons have also been separated on salt-modified adsorbents.

Gas-liquid chromatography (GLC) is much more important and will be discussed for the rest of this section, although much of what is written applies to both techniques. The carrier gas drives the mixture through the column wherein the solutes partition between the gas and the stationary liquid. In addition to partition, adsorption of the solutes on the solid support and at the gas-liquid interface is also a factor in many cases. Differential migration results if the distribution coefficients of the solutes are different enough to allow the sorbent to selectively retard them. In gas chromatography, unlike liquid chromatography, the mobile phase is always nonselective. There is, however, the added temperature variable which aids in achieving resolution in GC.

A. BASIC APPARATUS FOR GC

A schematic drawing of the basic apparatus necessary for GC is shown in Figure 2. The parts include:

1. A high pressure cylinder of carrier gas. The gas chosen must be suitable for the detector employed, and beyond this should be inert, pure, inexpensive and as heavy (high molecular weight) as possible so as to minimize solute diffusion. If speed is important but highest column efficiency is not, a low-molecular-weight gas such as helium or hydrogen may be used.

2. Pressure regulators and flow-control valves are used to obtain a uniform rate of gas flow, which is measured by a flow meter of the float, capillary or soap bubble type. The latter type is placed at the outlet of the column. Typical flow rates are 25–125 ml/min depending upon the size of the column. The optimum flow rate is chosen by making a van Deemter plot and noting the minimum value of HETP (see "Theory" below).

3. Samples are injected quickly onto the column through a self-sealing septum at the injection port. Gas-tight syringes are used for gas samples and liquid syringes for liquids and dissolved solids. Typical sample sizes range from 0.1–10 μl for gases and 0.004–0.5 μl for liquids on $\frac{1}{16}$ in. capillary columns, to 0.05–5 l for gases and 0.02–2 ml for liquids on 1 in. preparative columns. Regular analytical columns ($\frac{1}{8}$ and $\frac{1}{4}$ in. O.D.) usually receive 0.1–50 ml of gases and 0.04–20 μl of liquids. The sample size (column capacity) depends upon the amount of the liquid phase as well as the column size. Small samples are often applied by injecting 1–2 μl of sample and delivering only part of this to the column via a sample splitter.

The recommended sample injection technique for liquids in gas chromatography is the solvent flush method (*1*). Pure solvent is drawn into the syringe barrel followed by an air pocket, then the sample solution, and finally another air pocket. The sample volume is read, and then the sample is injected. The flush solvent behind the sample assures that the entire sample is flushed into the column without hang-up.

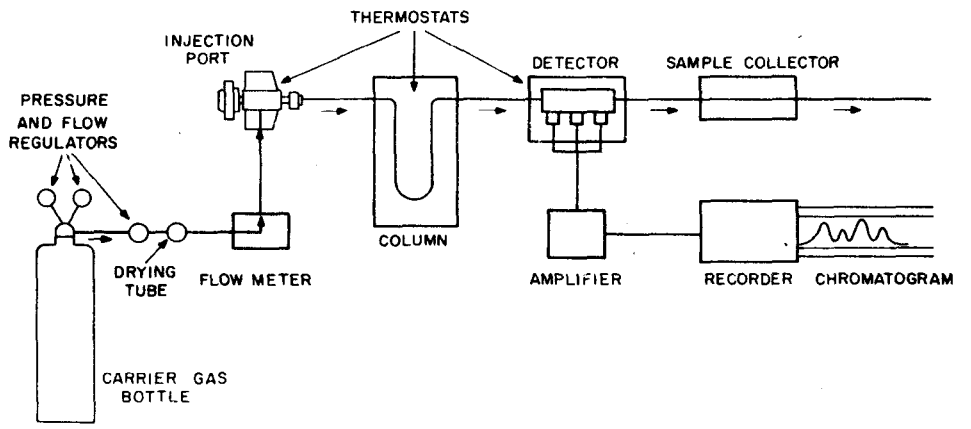

Figure 2. Schematic drawing of apparatus for gas chromatography (not to scale). Arrows show path of gas flow.

4. Columns are made from copper, stainless steel or glass tubing and are straight, bent or coiled. Except for glass, the columns are packed while straight and then bent. General analytical columns are 6–20 ft in length and ¼–¼ in. O.D. Preparative columns can measure up to 2 in. I.D. but are most often ¼–½ in. and 20 ft in length. Capillary columns 0.01–0.03 in. I.D. and 100–1000 ft in length are usual. The nature of the column is discussed further below. Column efficiency generally improves with decreasing column diameter.

5. The detector indicates and measures the solutes in the carrier gas stream. A good detector is highly sensitive, has a linear response over a large concentration range and is relatively insensitive to flow and temperature variations. Either universality or selectivity of response can be advantageous. A further discussion of detectors is found below.

6. Pure samples may be collected from the column effluent in cooled capillary tubing, or in glass collection bottles for larger samples.

7. The signal from the detector must be amplified and fed to a recorder where the chromatogram is drawn on a strip chart. A 1 mv, 1 sec (full scale) recorder is generally useful for gas chromatographs.

8. The temperature must be controlled in three places (Figure 2). The injection port is kept hot enough to vaporize the sample rapidly but not so hot as to decompose. A temperature 10–50° above that of the column is often recommended. The port need not be heated for gas samples. The temperature of the column is kept as low as possible consistent with good resolution but high enough to obtain reasonably fast separations. Isothermal operation is often adequate for mixtures with a narrow boiling range. For mixtures with a wide boiling range, temperature programming is recommended (see below). The temperature of the detector must be high enough to resist condensation of the sample or the liquid phase or any products formed in the detector if ionization is involved. Temperature control of the column within ±2 °C is usually adequate; close temperature control for some detectors (thermal conductivity) is critical but for others (flame ionization) is not.

B. THEORY

The theory of gas chromatography will be given in some detail in this section. The theory of the other chromatographic methods is essentially the same although there are some differences in terms and actual differences due to variations in the techniques.

The separation of solute peaks in chromatography depends upon the separation of the peak centers and the degree of spreading of the peaks. Obviously, complete separation of narrow peaks can be obtained with the peak centers closer together than if the peaks are spread (Figure 3).

The location of the peak centers depends upon the solute's sorptive equilibrium as determined by the respective distribution (partition) coefficients and the temperature. The interactions between the solutes and the sorbent leading to dissimilar partition coefficients are hydrogen bonds, Debye forces, Van der Waals' forces and specific chemical interactions (the partition coefficient K = the amount of solute per unit volume of liquid phase/the amount of solute per unit volume of gas phase). [4]

Peak width or spread is determined by mass transport and kinetic processes and is related to the column efficiency. Column efficiency is measured by the number of theoretical plates in the column or better by the resolution of the column. The longer a peak is in a column, the broader it becomes (under isothermal conditions).

The number of theoretical plates (N) is measured from the actual chromatogram. Tangents are drawn to the peak at the points of inflection (about two-thirds of the height). Then, referring to Figure 4, N for the first peak is given by

$$N = 16\left(\frac{X}{W_1}\right)^2, \qquad [5]$$

where X is the distance from the point of injection to the peak maximum and W is the length of base line enclosed by the two tangents. Both the numerator and denominator must be in the same units, most conveniently in cm measured along the chart paper.

HETP or height equivalent to a theoretical plate is the column length divided by N. A good column has a large number of theoretical plates and a small HETP (less than one mm for GC). The HETP may be different for each solute on a given column. It should be emphasized that HETP is a function not only of the width of the peak but of its retention time. Therefore a wide peak with a high retention time can represent the same HETP as a narrow peak which is eluted earlier.

The separation of two adjacent peaks is measured by the resolution (R) (see Figure 4):

$$R = \frac{2d}{W_1 + W_2}. \qquad [6]$$

When recording resolution, the specific compounds being considered should be stated. As defined in equation [6], an R of 1 indicates "perfect" (near base-line) separation.

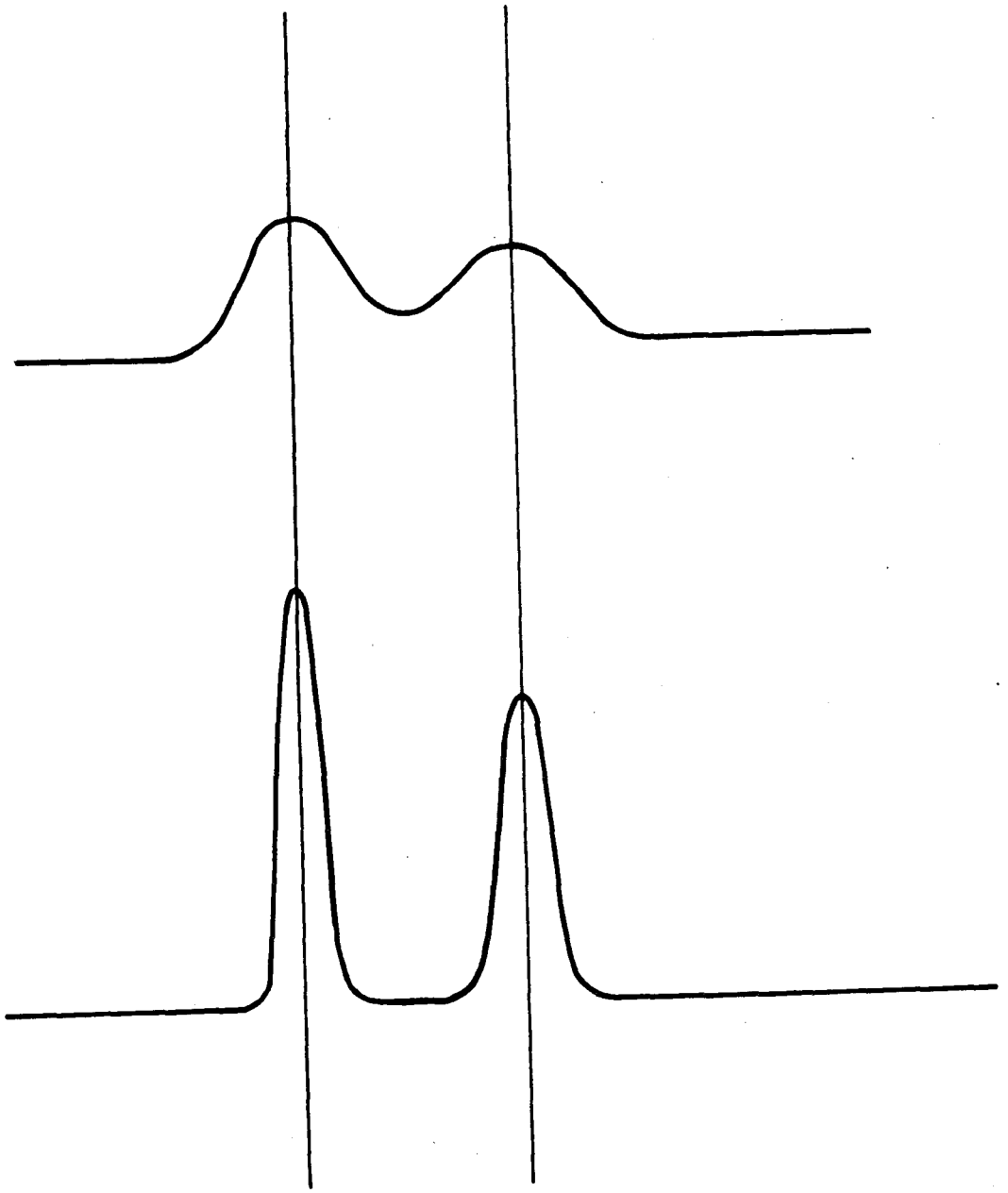

Figure 3. Two chromatograms showing the effect of increased column efficiency on the degree of separation obtained.

For describing the performance of a column, both relative retentions and plate numbers are important considerations. That is, one can obtain a good separation of two components whose relative retentions are significantly different even on a column with a low number of plates. However, if the relative retentions are very similar, even a column with many plates may not provide the desired separation.

Another parameter often used to evaluate column performance is the *Separation Factor*, which is the ratio of the adjusted retention times or volumes (Section F3) for two adjacent peaks.

HETP can be theoretically calculated by means of the van Deemter equation. This equation treats chromatography as the flowing system it is, and allows one to see what conditions should be adopted in order to optimize the system. According to this equation

$$\text{HETP} = 2\lambda dp + \frac{2\gamma D_g}{\mu} + \frac{8k'd_f^2}{\pi^2(1 + k')^2 D_l}\mu. \qquad [7]$$

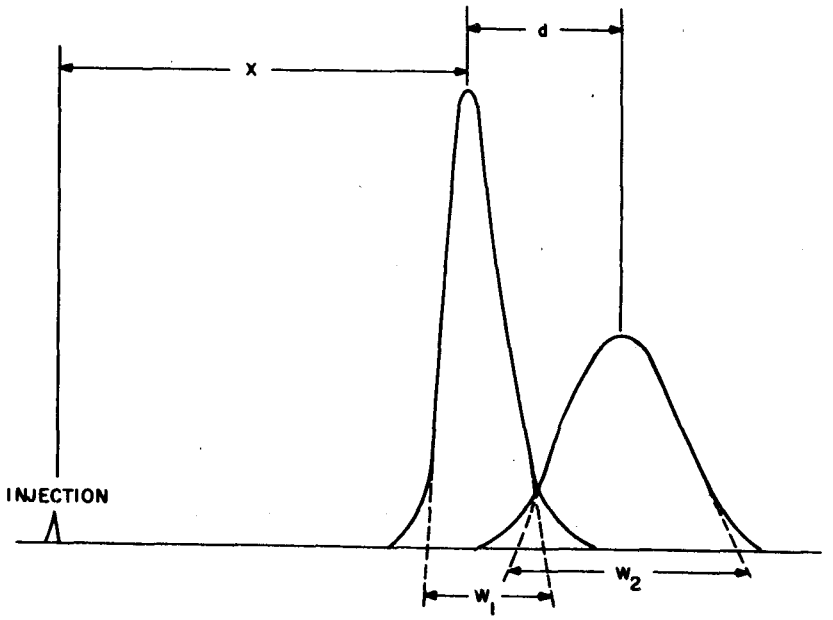

Figure 4. Calculation of N and R from a chromatogram.

where

λ = a constant measuring packing irregularities
dp = the average particle diameter of the support
γ = tortuosity factor
D_g = coefficient of solute diffusion in the gas phase
D_l = coefficient of liquid diffusion
μ = the average linear gas velocity
k' = ratio of the capacity of the liquid phase to that of the gas phase
d_f = average thickness of the liquid film coated on the support particles

Equation [7] can be reduced to

$$\text{HETP} = A + \frac{B}{\mu} + C\mu. \qquad [8]$$

The A term is the multiple path or Eddy diffusion term which is independent of flow rate. It describes peak spread due to molecules taking different paths through the packed column. To decrease HETP (and increase column efficiency), one should use particles as small (low dp) and uniform (low λ) as possible, consistent with an adequate gas flow and a low pressure drop. The B term accounts for the normal longitudinal molecular diffusion of the solute in the carrier gas due to the concentration gradient in the zone. Diffusion is inverse to flow rate and decreases when a high-molecular-weight carrier gas is used (low D_g). The C term involves resistance to mass transfer and is proportional to flow rate. To decrease this term, a thin, uniform film of low-viscosity liquid should be used as the stationary phase (low d_f and high D_l). Lowering d_f changes k' and this complicates prediction of the optimum value of this term.

The optimum carrier-gas flow rate is determined by plotting HETP *vs* μ and choosing a value at or slightly above the minimum in the curve. Since the outlet pressure is usually one atmosphere, the flow rate chosen will dictate the inlet pressure to be used.

C. THE COLUMN

The choice of the column packing is the most critical in GC. In GLC, the column can be one of two types, either packed or capillary. Packed columns contain an inert solid support with a thin coating of the liquid phase. As seen above, small, uniform particle sizes (40–60 to 100–120 mesh) give the highest efficiency columns. The support is usually diatomaceous earth (kieselguhr), e.g., the Chromosorbs (see Section II.III, Table 2). In order to reduce tailing and decomposition of certain classes of compounds (steroids), the support surface must be made entirely inert by reacting surface hydroxyl and oxide groups with a compound such as dimethyldichlorosilane.

The liquid phase (see Section II.III, Table 1) should have a low viscosity and a high and differential solubility (leading to different distribution coefficients) for the mixture components. A loading of 2–10% of the liquid is generally used; less-loaded columns give faster separations at lower temperature but have lower sample capacity and may require inactive solid supports (e.g., VarAport 30 or Teflon). Retention times decrease (at a given temperature) as the loading becomes lighter, and adjacent peaks become narrower with centers which are less separated. The liquid should neither react with the support or the solutes nor "bleed" appreciably from the column during the run. There is a recommended maximum column temperature for each liquid phase.

Liquid phases can be classified according to their polarity: the most polar liquids are capable of forming strong hydrogen bonds (e.g., FFAP, Carbowaxes, Hallcomid, etc.) while the least-polar can interact only by forming weak Van der Waals' bonds (e.g., SE-30, OV-1, squalene, etc.). Solutes can be classified in the same way, ranging from polar (alcohols, acids, phenols) to nonpolar (saturated hydrocarbons). Liquid phases which are similar to the components retard these components compared to liquid phases which are not similar. Separations are best achieved by matching the solute and liquid types; for example, hydrocarbons are best separated on squalene, alcohols on Carbowax, and fatty acid methyl esters on polyesters (see Section II.III, Table 1). For mixtures containing solutes of different polarity, it is best to choose the liquid to match the most polar solutes. It should be recalled that "polarity" is a function of temperature and that dipole interactions become very weak at high temperature.

Some liquid phases react chemically with certain solutes and are very selective for these compounds. Selective liquid phases give large differences in retention times and require fewer plates (shorter columns) to achieve a desired separation.

Porapak porous polymer resins (Section II.III, Table 2) are used for GLC with no liquid phase. The mechanism of separations on Porapak is apparently a combination of partition, adsorption, and sieving based on size.

Capillary (or open tubular) columns originally contained a liquid film of ca. 0.5 μ thickness coated onto the inside wall of a glass or metal tube. Lately, solid-coated capillary columns containing a layer of coated support around the inside tube wall but still having an open center have been employed. These latter columns are superior because more sample can be applied and the optimum flow rate is higher (about 2–5 ml per min rather than 1 ml per min). Because more sample can be applied, analytical sensitivities are greater and the use of a sample splitter (or other special splitless injection techniques) might be avoided. Support-coated capillary columns must be purchased commercially, whereas wall-coated columns can be prepared in the laboratory. Workers often do not specify in the literature the exact percentage composition of the solution they used to coat a capillary column, but instead state only the phase and the solvent. Ten percent coating solutions are generally used for coating open tubular capillary columns by the dynamic coating method, and two percent solutions or less for the static coating method (*2*).

The number of plates per foot with either type of capillary column is similar to packed columns, but the maximum total number of plates is much higher (several hundred thousand) because very long capillary columns can be employed in the absence of a pressure drop. A disadvantage is that the void volume of capillary columns is so large relative to packed columns that the capacity ratios are significantly smaller. As a result, resolution in the two types of columns is not too different.

Capillary columns with a complete loose packing have also been described (*3*), as have sandwiched capillary columns consisting of one or more carbon threads inserted in a glass capillary and coated with the stationary liquid (*4*). The performance of sandwiched capillary columns is between open tubular and classical packed columns. Their advantage lies in the ease of preparation and lower pressure drop corresponding to a minimum plate height.

A new type of stationary phase developed by Halasz consists of organic molecules attached chemically to the surface of an inorganic support. [See Reference (*5*) for a review of these phases.] The stationary phase is then like a brush with organic bristles on the inorganic surface. Such a material may be produced by esterifying the surface hydroxide groups of silica gel with an alcohol. Changing the esterifying agent results in a change in the polarity of the organic bristle. These brushes (available commercially from Waters Associates under the trade name Durapak) are useful in both gas and liquid chromatography and typically provide 4–25 effective plates per second (GC) as compared with 0.1–4 for packed columns and 30–100 for open (capillary) columns. They have highly ordered interfaces leading to unusually high mass transfer, so that separations in columns packed with brushes are about as fast as those with open tubes. They have low sublimation pressure (i.e., bond decomposition, which in this case corresponds to "bleed") and can be employed at temperatures up to 145°C with normal tank N_2 or above 200°C with dried nitrogen. HETP for these columns is independent of temperature, retention time or sample type and load.

Still another new class of stationary phases contains those substances described by Annino and McCrea (*6*) which undergo reversible temperature-dependent compositional changes to produce large variations in selectivity for various classes of solutes. By combining substances differing in melting point and solute selectivity (e.g., stearic acid and 1,9-nonanedioic acid), a mixture is produced which can be used to prepare a column exhibiting varying degrees of specificity through choice of an appropriate column temperature. Positive relative retention shifts of the order of 100% can be achieved with a 20° increase in temperature, facilitating solute identification and resolution.