

GAS-LIQUID PARTITION CHROMATOGRAPHY*

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ABSTRACT

This paper is concerned with the principles, apparatus, and applications of gas-liquid partition chromatography.

This relatively new technique has been found to be exceptionally versatile and effective for the resolution of volatile mixtures that are difficult to separate by other means. It consists essentially of moving the substances to be separated by means of a gas stream through a column packed with granules of a porous solid impregnated with a high-boiling liquid. In favorable cases each component moves through the column at a different rate and emerges from it at a different time in the effluent gas stream. Application of this principle to the qualitative and quantitative analysis of small amounts of volatile materials requires relatively simple and inexpensive apparatus. Separation of large quantities for investigation by other means can be accomplished by the use of larger columns.

Because of its simplicity and effectiveness, the use of gas-liquid partition chromatography is rapidly becoming widespread. It seems destined to become an important analytical method and possibly a means of purifying valuable chemicals.

INTRODUCTION

This paper is concerned with the principles, apparatus, and applications of gas-liquid partition chromatography.

The use of the gas-liquid partition method was first reported by James and Martin (16) in 1952, although it had been suggested by Martin several years earlier (22). In the relatively short period since this first paper appeared, the method has been applied to a variety of analytical problems by many workers (2-4, 6-28). The number of recent papers on the subject is an indication that use of this technique is rapidly becoming widespread.

The procedure consists essentially of moving the substance to be separated by means of a gas stream through a column packed with granules of a solid covered with a high-boiling liquid. Each sample component distributes itself between the gaseous and liquid phases and that portion in the gaseous phase

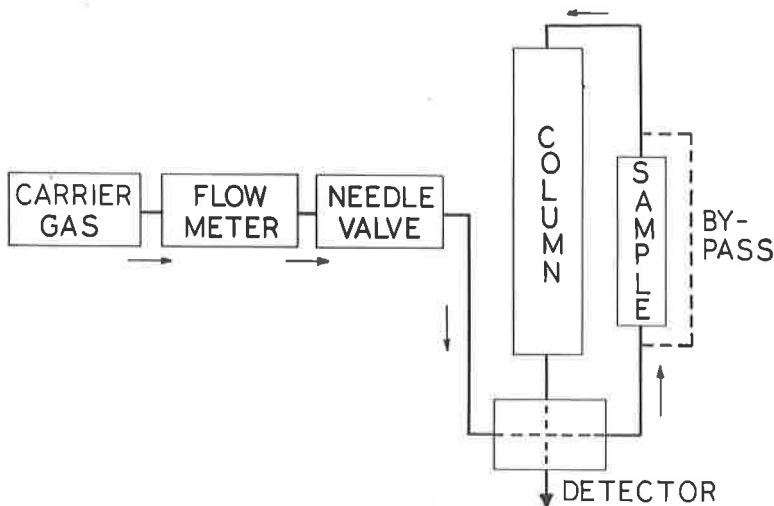
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at any time moves with the gas stream. In favorable cases each substance moves at a different rate and emerges from the column at a different time. A sensing device such as a thermal conductivity cell is used to monitor the gases leaving the column.

APPARATUS AND MATERIALS

The apparatus used for gas-liquid partition chromatography can be relatively simple and inexpensive. The literature contains descriptions of a number of units which differ primarily with regard to details of construction and are essentially the same in



1. Block Diagram of Gas Chromatographic Apparatus.

principle. At least four companies are offering a complete apparatus for sale. These companies are Burrell Corporation, Fisher Scientific Company, Perkin-Elmer Corporation, and Podbielniak, Incorporated.

The general arrangement of the apparatus used by the authors is shown by the block diagram in Fig. 1. The carrier gas, which is obtained from a cylinder, passes through the system continuously. The flow rate is indicated by a flow meter, of conventional design, that measures the pressure drop across a capillary. A needle valve capable of very fine adjustment enables control of the flow rate. Constant pressure is maintained at the input to the flow meter by allowing excess carrier gas to bubble through a 2-meter head of water. When the detector is a thermal conductivity cell (as is usually the case), the carrier gas passes

through the reference channel before entering the column and through the sample channel after leaving the column. When it is desirable, separated fractions are collected by condensation from the carrier stream.

Detectors

Although the use of several types of detectors has been reported, thermal conductivity cells are by far the most widely used. Cells specially designed for gas chromatographic applications are available from at least one company (Gow-Mac Instrument Company). The description of a cell employing thermistors rather than wire filaments was recently published (1).

Use of a device which continuously compares the density of gases leaving the column with that of the carrier gas by means of a thermoelectric flow detector has been reported by James and Martin (13, 14, 16). This detector is said to be extremely sensitive to sample components and insensitive to changes in flow rate of the carrier gas. However, there appears to be no detailed description of this device in the literature.

A novel means of detection was recently described by Scott (29). Hydrogen carrier gas or a mixture of hydrogen and an inert gas is burned as it escapes from a jet at the end of the column. A thermocouple in the vicinity of the flame makes it possible to monitor the temperature at that point. A temperature different from that resulting from combustion of the carrier gas alone indicates elution of a sample component.

Recorders

For most purposes the output of a thermal conductivity cell can be fed directly to a strip chart recorder without need for other amplification. Some means of changing the range of the recorder or providing stepwise attenuation of the output of the cell is desirable. When a properly designed cell is operating satisfactorily, the noise level should be imperceptible on the chart of a recorder with a full scale range of 1 mv.

In general the recorder is the most expensive part of the apparatus. It can be replaced by a suitable galvanometer, but this replacement results in a tedious and time-consuming job for the operator (5).

Columns

Columns used for analysis of small samples are usually 0.3 to 0.8 cm. in diameter and 60 cm. or more in length. The resolution of sample components increases with increasing length of the column, but there is a corresponding increase in the time required for a run and a decrease in sensitivity to trace components. Columns as long as 50 ft. have been used by some

workers. For general use, the authors have found columns 0.5 cm. in diameter by 110 cm. long to be quite satisfactory. The columns are commonly constructed of glass or metal tubing.

The amount of sample that can be handled on a column of given length is roughly proportional to its cross-sectional area. The flow rate of carrier gas should be increased by the same factor as the cross-sectional area. Although the efficiency of separation is not critically affected by a change in the column diameter, separations tend to be somewhat poorer with columns of larger diameter. As a rule of thumb, the length of the column should be increased by about 50 percent when the diameter is increased by a factor of five if the same degree of separation is to be maintained. The usual analytical column will handle not more than 0.1 ml. of sample. Columns that will separate as much as 6 ml. of sample have been built (9), and there is no apparent obstacle to the use of even larger columns.

Carrier Gases

The commonly used carrier gases are nitrogen, carbon dioxide, hydrogen, and helium. Of these helium is best for most applications. Hydrogen is equally good except for the explosion hazard and the possibility of reaction with sample components. The inferiority of nitrogen and carbon dioxide is related to their use with thermal conductivity detectors rather than the separations produced with these gases. With gases of low thermal conductivity the cell filaments operate at higher temperatures for the same current and this results in greater instability. Also, since the thermal conductivities of most of the substances to be detected are relatively low, the response of the thermal conductivity cells is poorer with carrier gases of similarly low thermal conductivity. The pressure drop across a given column is a function of the viscosity of the carrier gas. Again, hydrogen and helium are favored because of their desirably low viscosities.

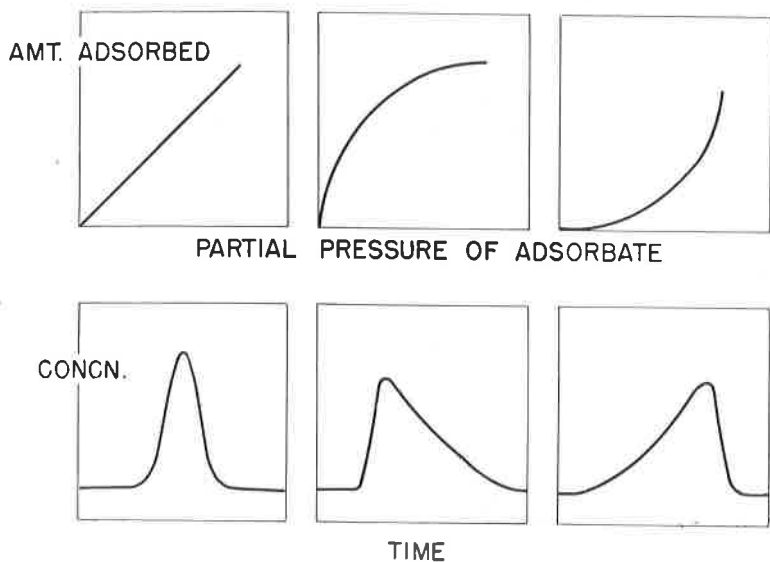
Column Packings

A wide variety of high-boiling liquids supported on sand, glass wool, glass beads, and kieselguhrs have been tried as column packings. Of these a finely divided kieselguhr (Johns-Manville Celite 545) is most commonly used. For some special applications involving the separation of very small amounts of materials that are relatively easily separated, sand or small glass beads have some advantages (4).

If it is used as received, Celite 545 produces excessively large pressure drops across the columns. A more suitable material can be prepared by screening the Celite and retaining for use only that part not passing through a 100-mesh sieve. An even

coarser material than this would be desirable for use in columns longer than 3 or 4 ft.

It appears that the most suitable high-boiling liquid must be chosen for the problem at hand. Because of the large number of variables involved and the paucity of theory and experimental data on which to base predictions, it is generally easier to prepare a column and test its chromatographic behavior than to evaluate it by other means. Nevertheless, a qualitative understanding of some relationships may be helpful in choosing materials to be tested.



2. Typical Isotherms and Corresponding Elution Curves.

Over the range of concentration encountered in elution chromatography, adsorption and solution isotherms approximate to the three types shown in Fig. 2. The elution curve corresponding to each type of isotherm is shown below it. The symmetrical elution peak, which is associated with a linear isotherm, is the most desirable for analytical purposes. Some asymmetry can be tolerated, but if it is excessive the components move in overlapping zones of low concentration.

Linear or nearly linear isotherms are much more common for solution than adsorption. For this reason, the gas-liquid partition method can be used to separate many substances which are not satisfactorily separated by adsorbents. Although nearly symmetrical peaks are quite common with the partition method,

peaks with sharp fronts on sharp tails are sometimes encountered. Hence, one consideration in the choice of the high-boiling liquid is its ability to produce symmetrical elution peaks for the sample components. Over the low concentration range involved this requirement is generally met if the solutions of the sample components in the high-boiling liquid obey Raoult's law or Henry's law. In practice few systems are found unsuitable because of unsymmetrical elution peaks, but this is sometimes a troublesome problem.

Specific interactions between sample components and the high-boiling liquid can often be used to advantage to separate substances of different molecular types. For example, aromatic compounds are preferentially detained by tricresyl phosphate, presumably because of mutual interaction of the aromatic groups, and alcohols are held rather strongly by glycerol or ethylene glycol because of hydrogen bonding.

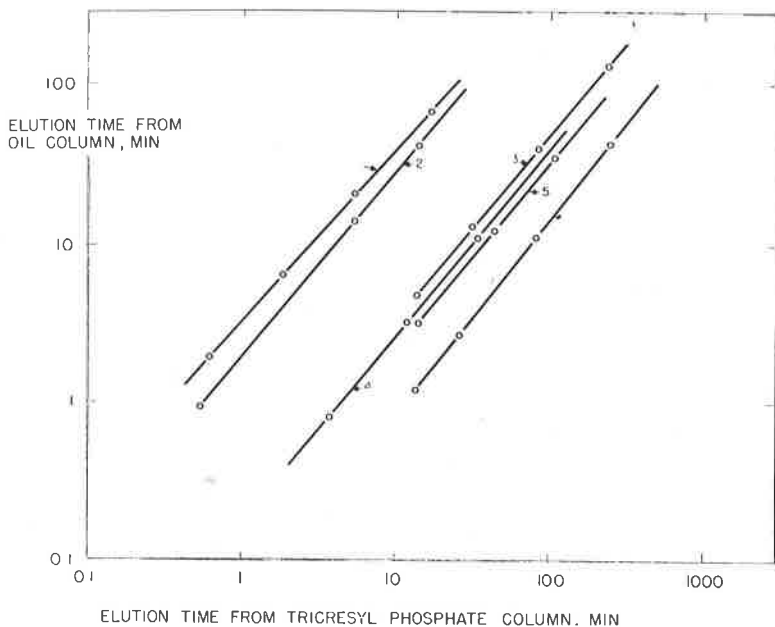
Ideally the volatility and viscosity of the high-boiling liquid should be as low as possible. Since these are generally contradictory requirements, some suitable compromise must be made. The boiling point usually takes precedence because it must be roughly 200 to 250°C. above the column temperature in order to maintain reasonably constant separatory characteristics over long periods of time. Fortunately, the viscosity is not critical.

On a weight basis, vapors are generally more soluble in liquids of lower molecular weights. This fact can be used to advantage for the separation of slightly soluble substances such as the gaseous hydrocarbons. Again a compromise must be made between molecular weight and boiling point of the high-boiling liquid.

APPLICATIONS

Since the gas chromatographic method is relatively new and is developing at a rapid rate, its ultimate scope cannot be reliably assessed from published information. At present it appears that the separation of mixtures containing very volatile materials, such as hydrogen, carbon monoxide, carbon dioxide, nitrogen, and methane, requires the use of adsorbents. Several of the gaseous hydrocarbons can be separated by either adsorption or partition columns. For hydrocarbons above C₅ and most other types of compounds, the partition packings are definitely preferable. With the proper partition columns and conditions it is possible to separate mixtures as volatile as the gaseous hydrocarbons. At the other extreme the limitation is determined largely by the thermal stability of the sample components and column packing. Satisfactory separation of com-

pounds boiling as high as 250°C. has been reported (6). In general the column temperature should not be more than 70 to 100°C. below the boiling point of the least-volatile component. Separation of compounds with a wide range of volatilities on a given column can be improved by continually increasing the temperature of the column during a run (24, 10, 4). Because of the difficulties concerned with reproducing the heating rate, this technique is seldom used in practice.

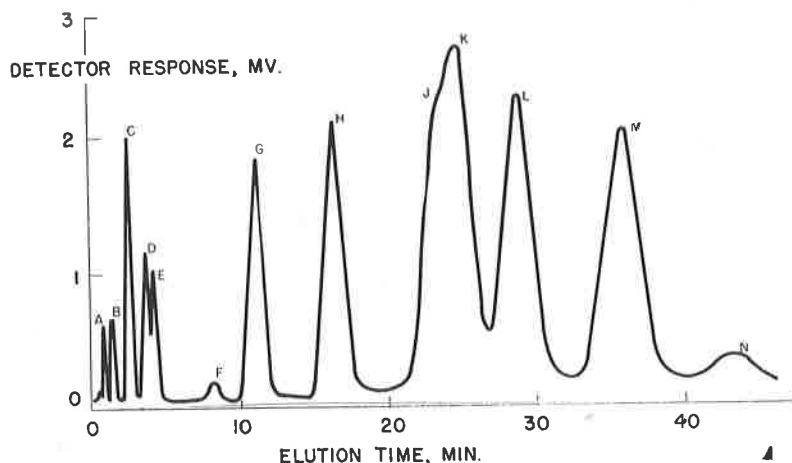


3. Logarithmic Relation of Elution Times: 1, Alkanes; 2, Cycloalkanes; 3, Esters; 4, Aldehydes; 5, Ketones; 6, Alcohols.

Qualitative Analysis

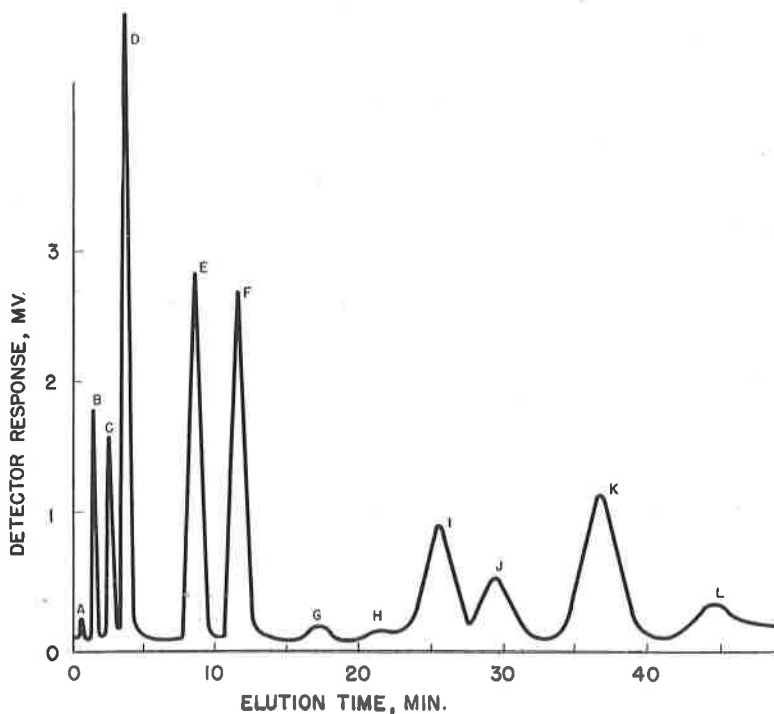
In general the identity of a separated component is based on the time required for it to pass through the chromatographic column. However, this is sometimes insufficient information for characterization. This is particularly true of fractions from very complex mixtures which may contain a variety of molecular types. Although members of a homologous series are generally separated with ease, the characteristic times for members of different series may not be sufficiently different to avoid confusion.

Condensation of separated fractions for identification by some other method has been reported by a number of workers. Because of the small amount of sample required, the mass spectrometer is particularly useful for this purpose (3, 4, 23). Ultraviolet absorption spectra are quite helpful when the spectra of the compounds involved are sufficiently characteristic (23). The amount of sample required for recording the infrared spectrum is generally greater than that present in a fraction from the usual analytical column. However, this method can be used, particularly in conjunction with a large gas chromatographic column (9, 23).



4. Elution of Hydrocarbons From Celite 545 Impregnated With Hexadecane: A, Ethane; B, Propane; C, Isobutane; D, Butane; E, 2,2-Dimethylpropane; F, 2-Methylbutane; G, Pentane; H, 2,2-Dimethylbutane; I, Cyclopentane; J, 2,3-Dimethylbutane; K, 2-Methylpentane; L, 3-Methylpentane; M, Hexane; N, Methylcyclopentane.

The uncertainties associated with identification by the gas chromatographic method alone can be considerably reduced by the use of two columns with different separatory characteristics (18). The complex mixture is put through one column, and fractions corresponding to separate peaks are collected and put through the second column. Comparison of these two elution times for a substance with those of reference compounds makes possible differentiation of many compounds for which elution times from a single column would not be conclusive. This comparison is facilitated by plotting the data for reference compounds on logarithmic paper as shown in Fig. 3. On this type of plot, which is somewhat analogous to a two-dimensional paper chromatogram, points for compounds of a homologous



5. Elution of Straight-Run Gasoline From Celite 545 Impregnated With Hexadecane: A, Ethane; B, Propane; C, Isobutane; D, Butane; E, 2-Methylbutane; F, Pentane; G, 2,2-Dimethylbutane; H, Cyclopentane; I, 2,3-Dimethylbutane and 2-Methylpentane; J, 3-Methylpentane; K, Hexane; L, Methylcyclopentane.

series generally fall on a straight line with approximately equal spacing between adjacent members.

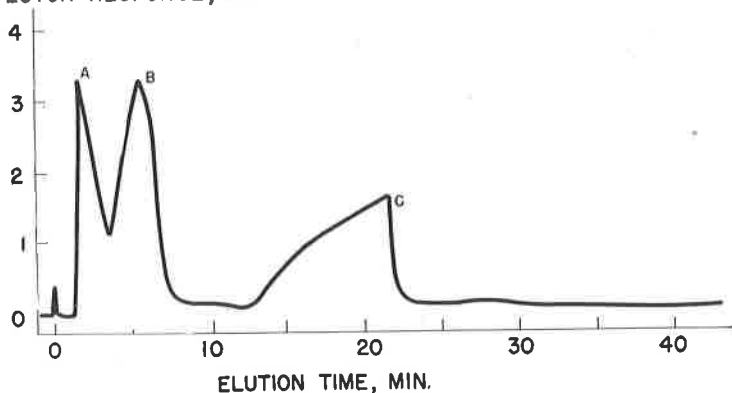
It is sometimes possible to gain information concerning the identity of the compound responsible for a given peak on the elution curve by determining if it is removed from the carrier stream by a chemical reagent (18, 23). For example, a short column (about 0.5 cm. in diameter by 5 to 10 cm. long) filled with Celite impregnated with sulfuric acid will remove basic compounds, most unsaturated hydrocarbons, and organic compounds containing oxygen, sulfur, or nitrogen. A similar column containing Ascarite removes water, acidic compounds, and aldehydes, and detains alcohols considerably.

Quantitative Analysis

Both the height of an elution peak and the area under the peak are generally proportional to the amount of the substance that produced it. The peak height is influenced by the tem-

perature of the column or the loss of high-boiling liquid from the column. It is not changed markedly by variations in flow rate. The area under the peak is much less affected by changes in column temperature or amount of high-boiling liquid, but it is approximately inversely proportional to the flow rate of carrier gas. In general, measurement of the area gives better precision unless the area to be measured is so small that the precision of this measurement is a limiting factor. The product of the peak height and its width at one-half the maximum height is often used as an approximation of the area (5). Quantitative results based on this method are usually not inferior to those based on planimeted areas. Incorporation of an internal standard in the sample improves the precision when the peak height alone is used as a measure of quantity (6, 28).

DETECTOR RESPONSE, MV.



6. Elution of Alcohols From Celite 545 Impregnated With Hexadecane: A, Methanol; B, Ethanol; C, Propanol.

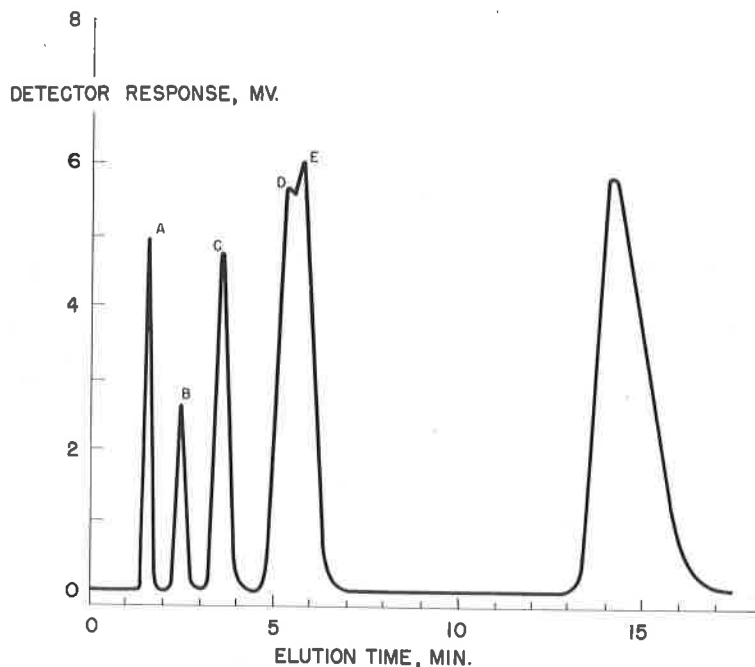
Illustrative Elution Curves

A few elution curves are presented to illustrate the effectiveness of the method. All of the curves shown were obtained with columns 0.5 cm. in diameter by 110 cm. long. These were packed with Celite 545 impregnated with various high-boiling liquids. The columns were operated at 25°C. Helium was used as the carrier gas with a flow rate of 40 ml. per min.

Fig. 4 shows the separation of 0.01 ml. of a synthetic mixture of hydrocarbons, using hexadecane as the high-boiling liquid. This separation is considerably better than is obtained using the same weight of paraffin oil or lubricating oil instead of the hexadecane. The lower molecular weight and viscosity of hexadecane are believed to be responsible for the improvement.

A similar curve for 0.01 ml. of straight-run gasoline is shown in Fig. 5. As an example of some highly unsymmetrical peaks, Fig. 6 shows the elution curve for some alcohols from the hexadecane. The marked difference in the shapes of elution peaks for the hydrocarbons, which form nearly ideal solutions in hexadecane, and the alcohols, whose solutions deviate considerably from ideality, is apparent.

Some very unusual separatory properties of a column containing ethylene glycol saturated with silver nitrate were first reported by Bradford, Harvey, and Chalkley (3). Separation of a mixture of C_4 hydrocarbons with a 220-cm. column of this type is shown in Fig. 7.



7. Elution of C_4 Hydrocarbons From a Column Containing Ethylene Glycol Saturated With Silver Nitrate: A, Air; B, trans-2-Butene; C, 2-Methylpropene; D, cis-2-Butene; E, 1-Butene; F, 1,3-Butadiene.

SUMMARY

Although gas-liquid partition chromatography is relatively new, it is already an important analytical tool. Some of its outstanding features include:

1. The remarkable speed and efficiency with which it enables separation of materials which are difficult to separate by other means.

2. The relatively inexpensive equipment required to determine the identity and quantity of separated components.
3. The versatility which may be had by the use of various column packings and conditions.
4. The very small samples required for an analysis.
5. The nondestructive separation that results in relatively pure components which can be used for further studies.

Because of the great amount of work now being done in this field, it seems almost inevitable that a flood of papers will appear on the subject. These will undoubtedly reveal new applications of the method and a better understanding of its principles. In addition to analytical applications, use of this very efficient separatory device on a relatively large scale may prove economically feasible for separation and purification of expensive chemicals.

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NEWS OF TENNESSEE SCIENCE

(Continued from Page 184)

Department of Biology, Vanderbilt University

Staff members added:

Dr. R. B. Channell, presently of the Gray Herbarium, Harvard, has accepted a position as Assistant Professor in the Biology Department.

Dr. Relis B. Brown, of Lawrence College, has been appointed Visiting Associate Professor in the Biology Department for the year 1957-58.

Alan I. Gebben, Calvin College, Michigan, will be Instructor in Botany for the year 1957-58.

Ph. D. Graduates places elsewhere:

Neal Buffaloe will be Professor and Head of the Department of Biology at Arkansas State Teachers College, Conway, Arkansas.

Francis Trainor will be Instructor in the Biology Department and Research Biologist at Noank Marine Biological Station, University of Connecticut, Storrs, Connecticut.

Gina Arce goes to The Biology Department at Fresno State College, Fresno California, as Instructor.

Elsie Quarterman was elected Chairman of the Southeastern Section of the Botanical Society of America at the ASB meeting in Athens.

University of Tennessee Medical School

The University of Tennessee Medical Units has completed a \$5,000,000 building program.

The addition of a seventh and eighth floors to the Institute of Pathology Building was completed at a cost of \$600,000, and will permit the expansion of research and clinical facilities for the College of Medicine's Divisions of Medicine and Surgery.

The Pharmacy Building at 874 Union Avenue has been remodeled at a cost of \$400,000, and a fifth floor added. This building houses the Division of Pharmacology and the School of Pharmacy. Space also is provided for a laboratory for the United States Public Health Service.

A study by Dr. T. P. Nash Jr., dean of the School of Biological Sciences, indicates that medical students admitted during the last decade are better prepared than those during the decade ending 10 years earlier. The study shows that fewer failures are occurring during the first year of the medical course. It is during the first year that the majority of students drop out. For the 10 year period from 1936 to 1946, 1,104 students were admitted to the College of Medicine with an average pre-medical grade for all students

(Continued on Page 207)