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Preparative Gas-Liquid Chromatography of Lipids

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Abstract

Applications of preparative gas-liquid chromatography of lipids are presented and discussed. Various designs of preparative units are reviewed, including those commercially available. A general discussion of preparative gas-liquid chromatography is presented with emphasis on the practical problems involved.

Introduction

THE ADVENT OF GAS-LIQUID chromatography (GLC) presented the field of analytical chemistry with a unique and valuable tool for the analysis of organic materials. The uniqueness of GLC lies in the fact that a highly efficient separation process and a highly sensitive detection process are combined into one system allowing the analysis of very small quantities of complex mixtures with relative ease and speed.

Although conceived as an analytical tool, GLC's function as a separation process naturally led to interest in it as a preparative tool in the purification of relatively large quantities of a mixture. The range of sample sizes, micrograms to milligrams, for analytical GLC can not be increased significantly on analytical size columns without an appreciable loss of resolution. This led to the scaling up of GLC systems by the use of columns of larger diameter with proportionately larger vaporizers. However, it was generally found that the high separating power of small diameter columns could not be obtained with large diameter columns. Despite the drawback of limited resolution, numerous examples of successful applications of large scale GLC have been published. Most of these reports deal with relatively volatile, stable materials, e.g. low molecular weight hydrocarbons and fluorocarbons. Relatively little has been published on the purification of lipids by preparative GLC, and a sizeable proprotion of the published work deals with the purification of small amounts of material on analytical size columns.

The collection of relatively small samples from analytical size columns can also be thought of as preparative GLC. This technique is often used in cases where only small quantities of a compound are required. There are no serious problems involved in such an application except in the collection system. The term "preparative GLC" is more commonly used to denote the purification of relatively large quantities of materials with columns of larger diameter than normally used in analytical work and as such, provides the basis for this paper.

Application of Preparative GLC to Lipids

Most of the published reports dealing with preparative GLC of lipids has been with relatively small scale systems with analytical columns. Kaneshiro and Marr (15) used small scale GLC with an unspecified instrument as one of their steps in isolating and identifying methyl esters derived from bacterial phospholipids. Methyl myristate, palmitate, palmitoleate, oleate, cis vaccenate, and lactobacillate were separated and collected from an 8 ft x ¼ in. column containing 25% diethylene glycol succinate (DEGS) on firebrick. Details of the collection procedure for the methyl esters were not presented nor was data included for the per cent recovery of the methyl esters by the procedure employed.

Chang and Sweeley (3) used preparative GLC as part of their procedure for isolating and identifying polyenoic acids from canine adrenal glands. Methyl

eicosatrienoate, eicosatetraenoate, and docosatetraenoate were separated and collected from an 8 ft x 3/8 in. (i.d.) column containing 30% ethylene glycol adipate (EGA) on 60/80 mesh Celite at 210C in a Barber-Colman Model 10 gas chromatograph. The effluent gas from the column was split with 10% going to the detector and 90% to a collection system which consisted of 3–4 ft of 1/16 in. (i.d.) Teflon capillary tubing. Eight 1 ml injections of a 25% solution (w/v) of the methyl esters in n-hexane were made. Each sample injection therefore amounted to approximately 0.25 g of esters. Only the middle half of each peak was collected by condensation in a tube at room temperature. The authors state that column efficiency was little affected by sample sizes up to 0.3 ml of hexane solution (~0.075 g esters). Analytical chromatograms of the substances obtained showed negligible amounts of impurities. Although relatively pure esters can be prepared by this procedure, recoveries are low.

In both of the above applications, stationary phase which bled from the column was found in the products. Contamination with "bleed off" is especially pronounced with the high concentrations of liquid phase that are needed for preparative work with small columns. However, in both of the cases cited, this was readily removed by the use of silicic acid column chromatography.

Pelick et al. (17) reported the separation and collection (according to carbon number) of milligram quantities of uniformly labeled C^{14} methyl esters. A 9 ft x 1/2 in. (o.d.) column containing 15% SE-30 (silicone gum rubber) was used. These authors stated that the small scale preparative GLC that they used was convenient since only milligram quantities of material were available.

Fales et al. (7) showed the feasibility of purifying milligram quantities of steroids with a 1/2 in. diameter column. Their studies were made with 9 ft x 12.4 mm (i.d.) column packed with 0.75% SE-30 on 100/140 mesh silanized GAS-CHROM P. This column was found to contain only 900 theoretical plates as compared to 4200 for a similar 4 mm (i.d.) column when the comparison was made below overload conditions. Best results were obtained with temperature programming. Preparations obtained from injecting five 50 μl portions of a dilute steroid solution over a 25 sec. period were comparable to those from a single injection of 50 μl of solution containing the same total amount of steroids.

Ackman et al. (1) described a preparative GLC unit which can house up to four 6 ft x 0.72 in. U-tubes connected in series with 0.25 in. diameter tubing. Using columns packed with 30% (w/w) Dow Corning high vacuum grease on 40/60 Chromosorb the following data were obtained: complete separation of 2 ml of a mixture of methyl caprate, laurate, and myristate at 230C; almost complete separations of 2 ml of a mixture of butyl acetate, propionate, and butyrate at 100C; and almost complete separations of 1 ml of a mixture of methyl laurate, myristate, palmitate and oleate. The column was grossly overloaded with samples of one gram or more, but this could be tolerated in order to effect practical separations.

Johns et al. (13) show the complete separation of 4 ml of a mixture of the C_6 to C_{14} even carbon number methyl esters. They employed the Beckman Megachrom with four 12 ft x 5/8 in. (i.d.) columns with Apiezon J as the liquid phase.

In addition to the previously mentioned preparative work with milligram quantities of methyl esters, Pelick et al. (17) also described large scale preparative GLC under practical conditions. The combination of analytical GLC and thin layer chromatography (TLC) together with preparative GLC and column chromatography were used to produce gram quantities of high purity methyl myristoleate. The charge for the preparative GLC contained approximately 90% methyl myristoleate. The impurities consisted of approximately equal quantities of methyl myristate and two unknown compounds. Analysis of the charge by GLC on an ethylene glycol succinate (EGS) column and an SE-30 column showed that neither stationary phase alone would separate all three impurities from the myristoleate. Both types of columns were required in the preparative work. The separation factors involved were of the order 1.2 and less. Manual repetitive injections of 100 μl samples of the charge on a 5 ft x 1 in. EGS column removed two of the impurities. Similar injections of the product on a 5 ft x 5/8 in. SE-30 column removed the third impurity. The product appeared pure by GLC analysis, but TLC analysis showed a number of minor impurities which were apparently decomposition products from the preparative GLC columns. These were removed by chromatography on a column of silicic acid. The final product was pure by both GLC and TLC analysis.

Column Designs for Preparative GLC

In the preceding section it was pointed out that resolution on large diameter columns is inferior to that obtained under analytical conditions. Frisone (8) succeeded in improving resolution on a 2 in. diameter column by use of doughnut-like restricting rings. He postulated that such rings alleviated the detrimental effect of radial band velocity gradients by causing a remixing of the various portions of the band. Sicilio et al. (20) utilized a different approach to improve the resolution with large samples by using a column with an expanded section. Comparison of a 12 ft x 0.24 in. (o.d.) column with a column which was identical except that the first 4.5 in. consisted of 0.375 in. (o.d.) tubing containing the same packing showed that appreciably narrower peaks were obtained with the latter. However, Verzele (23) claimed that he could not improve results with a 1.0 in. diameter column with either of the above designs but he was able to improve his results by temperature programming.

Several preparative chromatographs have been designed to improve heat transfer and to simulate small diameter column characteristics. These designs include the use of bundles of parallel small diameter columns, large diameter columns with internal fins, and large diameter columns of annular design.

The Beckman Megachrom uses 8 small diameter columns in parallel. The columns are connected to manifolds on each end, so that the sample is split among the columns at the inlets and remixed into one stream at the outlets. Column efficiencies equivalent to that of one column have been reported (13). This type of instrument requires careful matching of each column to insure equal retention times.

Dinelli et al. (6) described a rotating unit, consisting of 100 small diameter columns with an automatic sampling device. The columns (1.2 cm x 6 mm [i.d.] straight tubes) form the circumference of a rotating drum which moves between stationary

plates. The feed plate contains one hole under which each column passes. The collecting plate contains 100 holes to which traps are attached. Each column passes over all the collection traps. A detector is not used with this unit. Instead, the feed is first analyzed with a column identical to those in the preparative unit. Operating conditions, sample size per column, retention times and peak widths of the components of interest are determined from this preliminary analysis. The rotation speed of the drum is then set so that the sample will be completely eluted from each column in one revolution. A sample is injected into each column once per revolution and the various components continually collected in the appropriate traps. One component may collect in several traps but the same component from each column always collects in the same traps. From the retention times, peak widths, and drum rotation speed, the trap positions for the components of interest can be calculated. This design also requires that the columns be carefully matched and when this is done results equivalent to a single small diameter column are obtained. Taramasso and Dinelli (22) report that this instrument operated continuously for three months producing 99.9+% isoprene monomer at rates of 20 liters per week. There were no variations in band position and no problems were encountered. Results were also presented for a similar unit containing 36 U-shaped columns 2.4 meters long.

Wright (24) described a special design for large diameter columns which simulates parallel column designs. The column contains internal fins parallel to the column axis and extending inwards toward the center. Thus a number of parallel, interconnected channels are created. The efficiency of a 1¼ in. diameter finned column was reported to be 5 times that of a conventional 1 in. diameter column.

The Nester-Faust Prepkro II uses a biwall or annular column (⅝ in. [i.d.] and ¾ in. [o.d.]). It was designed for fast and accurate temperature programming, as heat is transferred at both the interior and exterior of the annulus.

Two other commercial preparative GLC units are the F & M Model 770 (16) and the Wilkens Autoprep Model A-700 (5). Both of these units have automatic injection, collection, and temperature programming systems. The F & M instrument is designed for several ¾ in. diameter U-shaped columns which can be connected either in parallel or in series whereas the Wilkens unit is designed for one 20 ft x ⅜ in. (o.d.) coiled column and relies on repetitive automatic operation for capacity.

The advantages of automatic operation are obvious regardless of the scale of equipment. However, the automatic system must be tested until it is highly reliable if the instrument is to be left on its own over a period of time. A single malfunction can spoil a large amount of product or waste a great deal of charge material.

Detailed comparisons of the various column designs are impossible to make from published data. Generally each design is shown accomplishing separations on particular systems, but it is difficult to find results on the same system for any two different units. In most cases, it is also difficult to determine from published results if column performance for a particular design is really good on an absolute basis or just an improvement over very poor results from some other design. The theory of GLC is not well enough developed to be useful for comparing results on various colums under the different conditions for which the data are available.

Although there has been some successful application of preparative GLC to the purification of lipids, the technique is difficult. The major factors which cause difficulty are: 1) product decomposition, 2) vaporization of the charge, and 3) equilibrium factors. These are in turn related to excessive overloading and band spreading as discussed in the lattter half of this paper.

Product Decomposition

High molecular weight materials of low volatility require high temperatures for vaporization and GLC. Substances may be sensitive to these high temperatures and some degree of decomposition may occur. Vaporizers for GLC are usually operated at higher temperatures that the column, and thermal decomposition here may be greater than in the column. The interior surfaces of preparative scale vaporizers are often found to be coated with carbon after repeated use with fatty acid methyl esters. However, product decomposition in the column can also be significant, for even though thermal decomposition rates are slower, the products spend more time in the column than in the vaporizer. If decomposition occurs to only a small degree, preparative GLC can still be highly useful. If relatively nonvolatile materials formed by decomposition contaminate the product from a GLC preparative column, they may not be detected by GLC analysis, but are often found by TLC analysis (17). In such cases the product can be purified by column chromatography.

Column chromatography can also be used to remove stationary phase from the product (3,15). Although all stationary phases bleed to some extent, this usually does not cause problems in analytical GLC. In preparative GLC, however, this factor must always be taken into account.

In cases where decomposition within the vaporizer is the main problem, vaporizer temperatures can be minimized by distributing the sample evenly over a large surface area. This can be accomplished by filling the vaporizer with glass beads or stainless steel balls.

Vaporization of the Charge

To obtain sharp separations in preparative GLC, the charge should be vaporized rapidly to form a narrow solute band at the column inlet. The vapor pressures of many lipids are low. The rate of vaporization of a component into a gas stream is limited by the vapor pressure of that component. A material with a low vapor pressure will usually not vaporize rapidly, even in a vaporizer designed for rapid heat transfer, if the carrier gas becomes saturated before vaporization is complete. This occurs with the relatively large samples used in preparative GLC. When rapid, complete vaporization does occur under such conditions, a supersaturated gas is formed, and partial condensation and revaporization may occur in the connecting conduit before the sample enters the column. This can produce a detrimental increase in the solute band width at the column inlet. Partial condensation of the sample may also occur before entering the column when the sample gas phase concentration is at or below the saturation point and if a large temperature drop exists between the vaporizer and the column.

The detrimental effect of slow vaporization can be

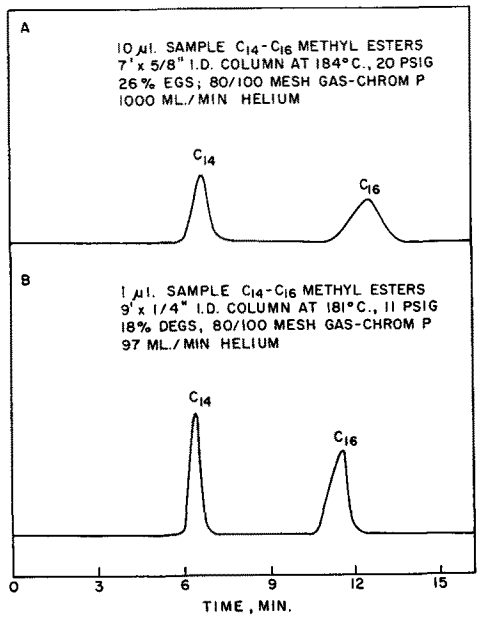


FIG. 1. Chromatograms showing comparison of a 5/8 in. (i.d.) column and a 1/4 in (i.d.) column under slightly overloaded conditions.

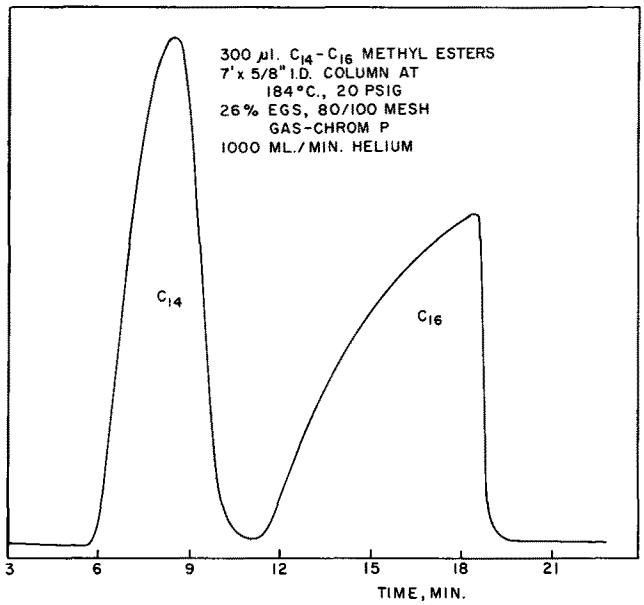


FIG. 2. Chromatogram of a 5/8 in. (i.d.) column under overloaded conditions.

counteracted if the column is operated under conditions which cause the sample to greatly compress at the column inlet. These conditions, discussed later, are not always practical, however.

Equilibrium Factors

The excellent separation often achieved in analytical GLC is mainly due to very favorable equilibrium separation factors that exist at the low concentrations characteristic of analytical GLC. In preparative GLC, high concentrations are desirable to maximize productivity, but the high concentrations sometimes have major effects on the equilibrium. In some cases, unfavorable equilibrium factors in preparatve GLC cause excessive band spreading and far outweigh the effects of slow vaporization. The ease of separating two or more components when high solute concentrations are encountered is determined by the equilibrium at both low and high concentrations. The detrimental effect of relatively small separation factors (ratio of the retention volumes or retention times of two components) at low concentrations and unfavorable equilibrium factors at high concentrations are exemplified by the purification of methyl myristoleate on a preparative scale EGS column (17).

Ideally for preparative GLC, separation factors should be large and concentration dependence of the partition coefficients should be small. However, these conditions cannot always be found. Many methyl ester separations of practical interest can only be made on polyester columns. Unfortunately, the equilibrium factors for such systems are relatively unfavorable at high solute concentrations. Furthermore even the separation factors at the lower concentrations involved in practical separations are often not large.

Column Overloading

A column is overloaded if symmetrical peaks similar to Gaussian curves are not obtained. Overloading is a result of unfavorable equilibrium factors at high solute concentrations and of slow sample vaporization with large samples and is often detrimental to component separation.

The desire for maximum productivity in GLC has always caused a tendency to overload even the large diameter columns. There is nothing wrong with this as long as the desired results are obtained in preparative work. However, in the evaluation of such columns comparison of an overloaded large diameter column with an analytical column which is not overloaded is rather unfair to the former, and misleading conclusions can be drawn. This fact has been emphasized by Sawyer and Purnell (18) and Henly et al. (11).

Figures 1–3 exemplify the fact that poor results obtained with an overloaded large diameter column can also occur with an overloaded analytical small diameter column. Also, the figures show that good results can be obtained on both columns when they are not overloaded. Figure 1A shows an experimental chromatogram for a 10 µl sample of a methyl myristate-palmitate mixture run on a 7 ft x 5/8 in. (i.d.) ethylene glycol succinate (EGS) column. Figure 1B shows the results for 1 µl of the same mixture run on a 9 ft x 1/4 in. (i.d.) diethylene glycol succinate (DEGS) column. Both columns are slightly overloaded, but both chromatograms have the same general appearance—that of an analytical scale chromatogram. Figures 2 and 3 show experimental chromatograms, respectively, of a 300 µl sample of the methyl myristate-palmitate mixture run on the 5/8 in. (i.d.) column and a 25 µl sample of the same mixture on the 1/4 in. (i.d.) column. In this case, both columns are extremely overloaded, and again both chromatograms have similar appearances. Results are not as good with a 5 µl sample of the myristate-palmitate mixture run on the 1/4 in. (i.d.) column (Fig. 4) as with a 10 µl sample on the 5/8 in. (i.d.) column.

Columns are often evaluated in terms of theoretical plates or height equivalent to a theoretical plate (HETP). Theoretical plates and HETP's calculated in the conventional manner from the methyl myristate peaks in Figures 1–4, are listed in Table I. On this basis, if run 1 is compared with runs 4 and 5 or if run 2 is compared with run 4, it appears that the 5/8 in. (i.d.) column is better than the 1/4 in. (i.d.) column.

In analytical work, a 1 µl sample size generally gives good results on 1/4 in. (i.d.) columns. The cor-

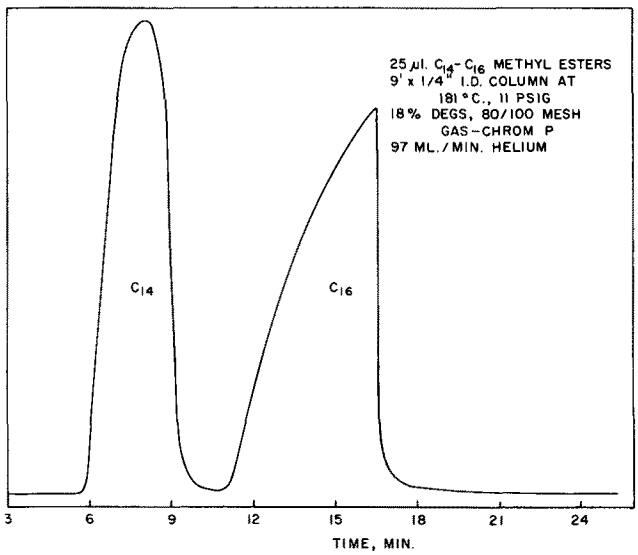


FIG. 3. Chromatogram of a ¼ in. column under overloaded conditions.

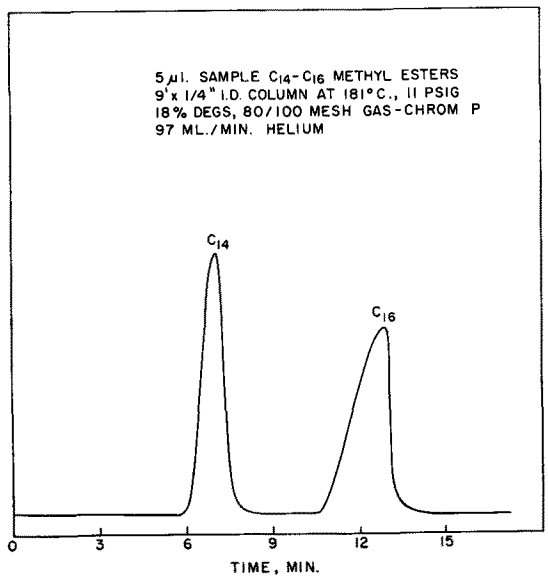


FIG. 4. Chromatogram showing the results of a 5 µl sample on a ¼ in. column.

responding sample size for a 1 in. diameter column is only 16 µl. This would, on the average, be expected to give results similar to ¼ in. (i.d.) columns, since the sample size is scaled up in proportion to column cross-sectional area or the square of column diameter. On the same scale-up basis, the average sample size for a 2 in. (i.d.) column could be only 64 µl. To scale up to a sample size of 1 ml on this basis would require a column 7.6 in. in diameter. All these scaled up figures assume that the large diameter columns have the same operating characteristics as the ¼ in. columns.

Considering the theoretical factors involved in column overloading, Sawyer and Purnell (18) estimate 28 µl as an average value of sample size for a 1 in. diameter column containing 100 theoretical plates and operating under non-overloaded conditions. The corresponding average for a 1 in. diameter column containing 5000 plates is 4 µl. Sawyer and Purnell state that in preparative GLC much larger samples are commonly used for 1 in. diameter columns and this can account for the poor efficiencies frequently observed.

It has been shown here that there is not an extreme difference in characteristics between the ⅝ in. (i.d.) and the ¼ in. (i.d.) columns, and that in order to properly compare a large diameter column with an analytical size column, the sample size for the former should be scaled up only in proportion to ration of column cross-sectional areas. If the two columns give equivalent results under these conditions, then the limitation on the maximum useable sample size is due to the GLC process itself and not to a difference in the characteristics of the two columns. The scale up of analytical GLC to practical preparative GLC often involves operating large diameter columns under overloaded conditions which are detrimental to the component separations.

Possibilities and Limitations of Preparative GLC

The preceding sections of this paper have indicated that successful large-scale preparative GLC of lipids is limited by thermal decomposition and column overloading. The latter is related to the limitations imposed by vaporization of large samples and by equilibrium relations at high solute liquid-phase concentrations.

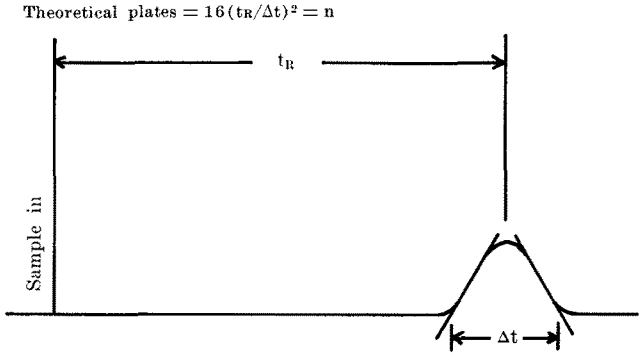
The remainder of this paper attempts to integrate the various factors that affect results in preparative GLC by discussion of band spreading. Theoretical considerations are greatly simplified but the simplified theory serves as a starting point for discussion. The main oversimplification of the theory of analytical GLC is the assumption that the following conditions are true: 1) the solute partition isotherms are linear, i.e., the equilibrium partition coefficients and separation factors do not change as concentration increases; 2) the volumes of the gas and stationary phases at any point are not changed by the solute; 3) the sample is introduced into the first plate only. These simplified situations are valid only at solute concentrations approaching infinite dilution in the stationary phase, i. e., with infinitely small samples. This is exactly the opposite of the actual situation with preparative GLC, where success requires proportionately larger samples per unit column cross-sectional area than are used in analytical GLC. The use of larger samples not only destroys the usefulness of the simplified theory, but the larger samples and higher concentrations also cause excessive band spreading.

TABLE I

Theoretical Plates and HETP's based on Methyl Myristate Peaks in Figures 1–4

Run	Figure	Sample size, µl	Column	Theoretical plates [a]	HETP, mm [b]
1	1A	10	7 ft x ⅜ in. (I.D.)	635	3.35
2	2	300		75	28.4
3	1B	1	9 ft x ¼ in. (I.D.)	1340	2.05
4	3	25		87	31.6
5	4	5		340	8.08

Theoretical plates $= 16(t_R/\Delta t)^2 = n$



[a] Based on C_{14} peak.
[b] HETP = L/n, where L = column length.

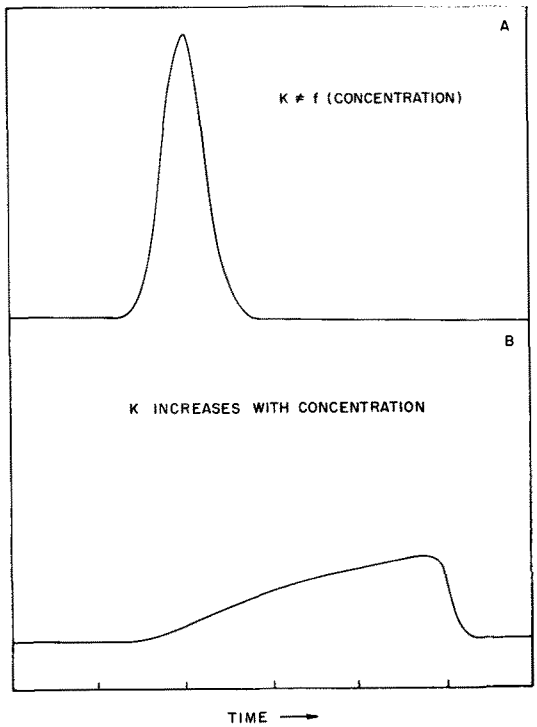


FIG. 5. Schematic representation of band spreading due to a concentration dependence of the partition coefficient (K).

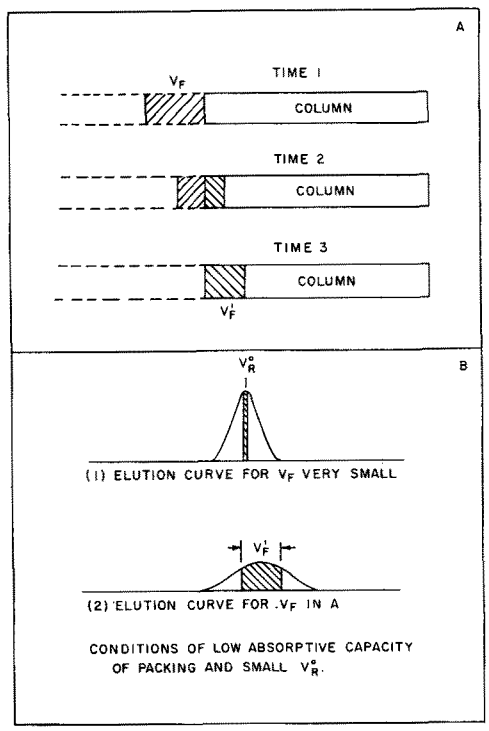


FIG. 6. Schematic representation of the effect of a large gas feed volume (V_F) on peak width when a column is operated under conditions resulting in a small V^0_R. Partition coefficient assumed to be independent of concentration.

The main concern in preparative GLC is limitation of band spreading. In the sharp separations of good analytical GLC, the bands containing two components spread lengthwise due to axial diffusion and resistance to mass transfer (related to solution and revaporization of solute in the stationary phase). The spreading due to these processes is limited so that the difference in rate of band movement causes the two bands to become completely separated before they reach the end of the column. Poor separation occurs when band spreading is great enough to prevent complete separation of the two bands before they reach the end of the column.

The cause of excessive band spreading in preparative GLC can be divided into two main categories. These are factors due to overloading and factors due to the column.

Band Spreading due to Overloading

When large samples are used none of the conditions listed in the simplified theory are attained, and each factor contributes to poor separation of components.

When high solute concentrations exist due to large samples, nonlinear isotherms cause asymmetrical band shapes and excessive band spreading. An example of this is shown in Figure 5. There is also interaction among solutes at high concentrations. Each solute increases the non-linear behavior of other solutes. Thus, the shape and width of the solute band, and elution time, of any particular solute are affected by the other solutes present. Calculations for hypothetical but typical mixtures of various kinds (11) indicate that in some cases this interaction is detrimental to separation, but in other cases it is beneficial. This latter gives some hope of finding new stationary phases that will improve preparative GLC separations.

It has also been shown (11) that the least amount of band distortion and spreading due to equilibrium factors should occur for many solute-stationary phase systems with negative deviations from Raoult's Law. Such systems would be those where the solutes and the stationary phase are chemically similar. The nonideality is caused by the large difference between molar volumes of the solutes and the stationary phase. A good example is the separation of low molecular weight hydrocarbons on a high molecular weight hydrocarbon packing. With such systems, the partition isotherms will remain nearly linear at higher concentrations than with systems with positive deviations.

Extreme band distortion and spreading of the type shown in Figure 5B occur for nonideal systems with large positive deviations. These effects are observed when methyl esters of fatty acids are run on highly polar polyester stationary phases such as DEGS and EGS (17). These stationary phases give good separation factors between saturated and unsaturated esters at the low concentrations of analytical GLC, but much of this good separation effect is lost in preparative GLC.

When a column is overloaded, the high concentration of solute causes the volume of the liquid phase to increase and thus the volume of the gas phase decreases. This amounts to an increase in adsorptive capacity of the stationary phase in the regions of high solute concentrations. Therefore, even if the partition coefficient were independent of concentration, the solute band in regions of high concentration moves slower than in regions of low concentrations. The results of this are band asymmetry and excessive band spreading.

It is practically impossible to introduce an entire sample into the first plate of a column when large samples are used. Some insight into the resulting effects can be obtained from simplified theory of the effect of large sample sizes. When highly nonlinear solute equilibrium relations occur, the simplified theory is not applicable but in such cases the effect of the inability to load into the first plate is overshad-

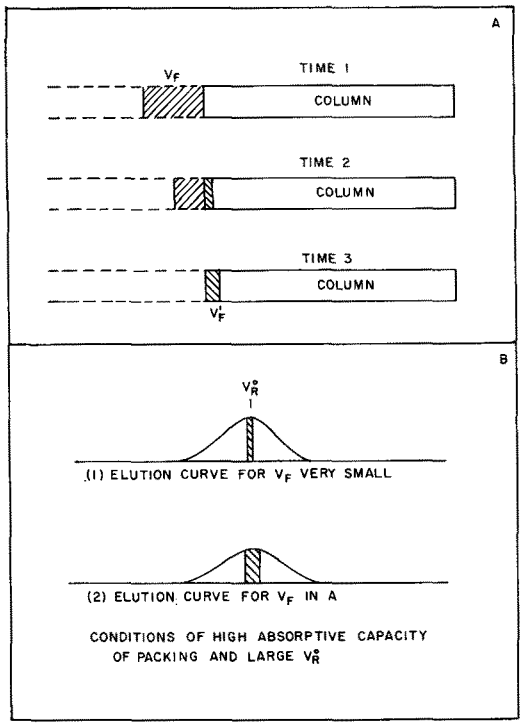


FIG. 7. Schematic representation of the effect of a large gas feed volume (V_F) on peak width when a column is operated under conditions resulting in a large V^0_R. Partition coefficient is assumed to be independent of concentration.

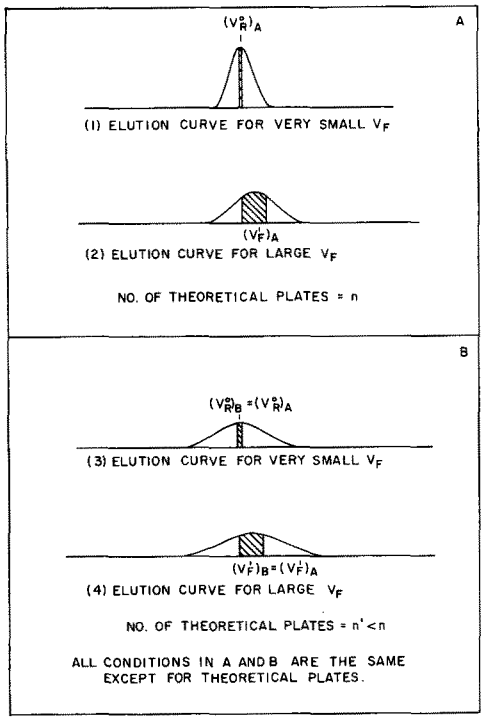


FIG. 8. Schematic representation showing the effect of a large gas feed volume for the case of (A) a relatively large number and (B) a relatively small number of theoretical plates.

owed by the non-linear equilibrium at high concentrations. The simplified feed volume theory is still worth careful review because in any case the feed volume has some effect, even when overshadowed by the equilibrium effect. In addition, when a column is operated at a high enough temperature to cause small retention times, then liquid phase solute concentrations are relatively small. Over such a relatively small concentration range nonlinear effects are likely to be limited, but large gas feed volume will have a sizeable effect. Van Deemter et al. (4) have shown that for linear isotherms the width in terms of volume, ΔV, of a solute peak is a function of the gas feed volume, V_F. The gas feed volume is the volume of carrier gas which initially contains the solute vapor at any concentration. For liquid samples it is the volume of gas into which the sample is vaporized. As V_F increases the solute peak remains symmetrical, but the peak width increases. For very large values of V_F, the peak maximum becomes flat. It has also been shown (4) the effect of V_F on ΔV is negligible when:

$$V_F < 0.5V^0_R / \sqrt{n} \qquad [1]$$

where V^0_R is retention volume as measured when V_F is very small, and n is the number of theoretical plates in the column. The latter is calculated from a solute peak (small sample) on a chromatogram using the formula at the bottom of Table I. Thus larger gas feed volumes can be handled by increasing retention volumes.

The important factor is the initial solute band width inside the column relative to the column length and relative to the width of the band as it emerges from the column. Large retention volumes are produced by high absorptive capacities of the packing. This causes the solute feed band to compress as it enters the column. This phenomenon and the effects of it are shown schematically in Figures 6 and 7. Figure 6 represents the case of low absorptive capacity of the packing which results in low retention volumes. In Figure 6A, the gas feed volume, V_F, is shown entering the column where it is compressed only slightly into V'_F. (If there were no absorption of the solute in the packing, there would be no compression.) Chromatogram 1 in Figure 6B represents the elution curve for the solute when V_F is very small. In this case band spreading is due only to the normal column processes of axial diffusion and resistance to mass transfer. Chromatogram 2 represents the elution curve for the solute when the sample enters as in Figure 6A. The eluting solute band is appreciably wider because V'_F represents an appreciable portion of the spreading process. As the solute moves through the column V'_F does not compress and band spreading still occurs due to the normal column processes.

Figure 7 is similar to Figure 6 but represents the case of high absorptive capacity of the packing which results in large retention volumes. In this case V_F is greatly compressed, and V'_F is much smaller, while band spreading due to normal processes is greater. Therefore V'_F represents only a small portion of the spreading process and its effect on band width is small.

It should not be concluded that the inequality expressed by relation 1 indicates that better results can be obtained with large gas feed volumes by using less theoretical plates. This obviously is not true. What relation 1 does show is that the results from a column with a large number of theoretical plates degrade more rapidly with increasing gas feed volume than the results from a column with a small number of plates. This is represented schematically in Figure 8. In Figure 8A (large number of plates), comparison of curve 1 (small V_F) and curve 2 (large V_F) shows that the increase in the band width due to the large V_F is appreciable. In Figure 8B (small number of plates), comparison of curve 3 (small V_F)

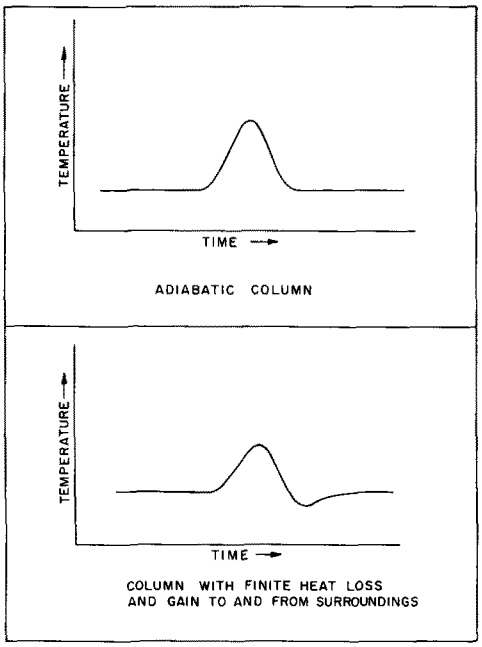


FIG. 9. Schematic representation of transient temperature changes in a column due to passage of a solute.

and curve 4 (large V_F) shows that the increase in band width due to the large V_F is small. However in the latter case, comparison is made of two poor results. Comparison of curves 2 and 4 in Figure 8, both for the case of a large V_F, shows that better results are obtained with the greater number of plates.

Relation 1 can be expressed in units of time as:

$$t_F < 0.5t^0_R / \sqrt{n} \qquad [2]$$

where: t_F = time required for gas feed volume to enter the column

t^0_R = solute retention time when t_F is very small.

If a liquid sample is injected into a vaporizer, t_F is the time required to vaporize the sample. The gas feed volume can be minimized by concentrating the sample vapor as much as possible upon vaporization. However even a plug of pure solute vapor occupies a finite volume and this volume becomes appreciable at large sample sizes.

Some data are available on the way large samples vaporize. Sumantri (21) measured methyl linolenate peak shapes leaving a preparative scale vaporizer for a liquid sample size range of 50 to 1000 μl. The vaporizing chamber was filled with ¼ in. steel ball bearings. Increasing sample size by a factor of 20 increased the vaporization time by a factor of approximately three. The vaporization profiles exhibited tailing. For a 1 ml sample, about 80% of the sample was vaporized in 1 minute, the remainder tailing off over a period of 2 more minutes. If the absorptive capacity of a column is low, a tailing vaporization profile could be associated with tailing of the elution band. However, under conditions of high column absorptive capacity where the gas feed volume is greatly compressed inside the column, the shape of the vaporization profile should not significantly affect the shape of the elution band.

High adsorptive capacity of a packing, required to lessen the adverse effect of large gas feed volumes, can be obtained by the use of high liquid-phase loadings and low column temperatures. High liquid-phase loadings also lessen the adverse effect of nonlinear isotherms, because the solute liquid-phase concentrations are decreased. However, the use of low column temperatures result in high solute-liquid-phase concentrations, and nonlinear isotherms become significant. For any two components which emerge from the column adjacent to each other, there is an optimum temperature which minimizes the adverse effects of both large gas feed volumes and nonlinear isotherms. For this reason, temperature programming is advantageous when a sample contains three or more components with a wide boiling point range. Each adjacent pair of components essentially moves through the column at an optimum mean temperature.

Band Spreading Within the Column

There is some loss of separation upon increasing column diameter even when columns are not overloaded. This appears to be due to factors other than the normal column processes of axial diffusion and resistance to mass transfer. These factors have been discussed by Giddings (10), but will be reviewed briefly here.

Frisone (8) has presented data indicating the existence of a radial solute band velocity gradient in a 2-inch diameter column. The solute moves faster near the wall than along the axis of the column. If a band moves at different rates in different parts of the column cross-section, the results are equivalent to excessive band spreading.

Radial band gradients can be caused by a number of factors. These are: 1) radial gas velocity gradients, 2) nonuniform stationary phase gradient, and 3) radial temperature gradients. The published data on these factors and their effects are sparse and not conclusive. Huyten et al. (12) measured point gas velocities from the center to the wall in a 3-inch diameter column. They found that the gas velocity increased from the center to the wall. This is attributed to the particle size variation of the packing from the center to the wall and the effect of the wall itself. Giddings and Fuller (9) made a study of particle size distribution in a column and found the larger particles tended to accumulate near the wall. Huyten et al. found that the gas velocity gradient and the column HETP varied with mode of packing. However, according to their results the packing methods that gave the lowest HETP (2 mm) also gave the most extreme gas velocity gradient (velocity near the wall was 1.5 times as fast as at the center). Their HETP data were obtained with 1 ml samples of n-pentane.

Bayer et al. (2) also found that the HETP of large diameter columns varied with the mode of packing the column. They obtained their best and most consistent results by filling their columns while mounted on a specially designed rocking table. They obtained HETP's of 2 mm for a 1 cm diameter column and 3 mm for a 10 cm diameter column. Their HETP's were determined from 140 mg/cm^2 cross-sectional area samples of n-pentane.

If a column contains bends, a gas velocity gradient will exist across the column from the inside of the bend to the outside. This gradient becomes worse as the column diameter is increased (10). Therefore the use of straight columns appears to be advantageous.

Non-uniform stationary phase gradients arise if a column cross-section contains a nonuniform distribution of particle sizes. A difference in packing density is expected. This causes the amount of stationary phase to vary over the column cross-section. If

a support is not coated uniformly, the particles with more stationary phase may accumulate in a particular part of the column due to the increased weight. A non-uniform distribution of the stationary phase causes a non-uniform solute band velocity.

The rate of heat transfer to the center of a column decreases with larger column diameters. A temperature gradient between the center and the wall creates a solute band velocity gradient. Both gas velocity and solute partition coefficient vary with temperature. The two effects oppose, but not necessarily cancel, each other. A radial temperature gradient occurs when a large diameter column is temperature-programmed. With isothermal conditions, an axial temperature gradient along a column will produce a radial temperature gradient. If the carrier gas enters the column at a temperature above or below the column temperature, a radial temperature gradient will be set up in the first part of the column.

Another factor which is important in large diameter columns and with the use of large sample sizes is transient temperature changes caused by the solutes themselves passing through the column. Such temperature changes are due to latent heats of vaporization of the solutes. As a solute band enters a plate or section of a column, a net condensation process occurs accompanied by the evolution of heat. After the band maximim passes, the net process is that of vaporization, and heat is absorbed by the solute. If a column were adiabatic with no heat loss to or gain from the surroundings, heat would be transferred to the packing as the solute band condensed in a section, and the packing temperature would rise. As the solute band evaporates from the section, the same amount of heat is transferred from the packing and its temperature returns to the base level. Assuming a symmetrical solute band, a temperature curve of the type shown in Figure 9A is expected. In an actual column, with finite rates of heat loss and gain, as the temperature rises, some of the heat evolved upon solute condensation is lost to the carrier gas and to the surroundings. As the solute is revaporized from the packing, all of the heat originally evolved is not available in the packing and the temperature falls below its base value before returning to steady state as shown in Figure 9B. Scott (19) has calculated, from theory, temperature curves of the types shown in Figure 9 and has experimentally measured temperature changes of the order of one degree in an analytical column. The experimental curves were of the type shown in Figure 9B.

These temperature changes due to the solutes themselves cause the band maximum to move faster than the rear of the band, thereby producing a tailing effect and band spreading.

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Isolation and Characterization of Insect Attractant Lipids

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Abstract

Isolation and characterization of the naturally occurring sex attractants of the silkworm moth (*Bombyx mori*), gypsy moth (*Porthetria dispar*), cotton leafworm (*Prodenia litura*), American cockroach (*Periplaneta americana*), an introduced pine sawfly (*Diprion similis*), and mating attractants of the honey bee (*Apis mellifera*) and bumblebee (*Bombus terrestris*) are discussed.

MOST INVESTIGATIONS of insect attractant lipids have been concerned with insect sex attractants. Other insect attractant lipids will therefore be treated in this presentation in only a minor way. The sex attractants of only two insect species have thus far been identified, namely those of the silkworm moth and gypsy moth. The sex attractant of the American cockroach has not as yet been prepared synthetically.

The Sex Attractants

Silkworm Moth (Bombyx mori)

Isolation. Isolation of the silkworm moth sex attractant was begun by Butenandt (7) in 1941 and completed 19 years later (18,9). The early work was done with a benzene extract of the last two abdominal segments from 7,000 virgin female moths; the 1.5 g of extract obtained was freed of acidic and basic substances, esterified with succinic acid, and the resulting esters saponified. Sublimation at 60–70C of the neutral product obtained gave 100 mg of an impure, waxy, crystalline substance reported to be a diol of approx composition $C_{16}H_{30}O_2$.

In 1956, Makino et al. (30) described a purification procedure, including column and paper chromatography, that gave a substance designated as "bombixin." An ethanolic extract of the abdomens of 60,000 females that had previously mated was evaporated to a small volume, treated with sodium hydroxide at below 40C, freed of solvent, extracted with