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Effect of Some Design and Operating Variables in Preparative Gas Chromatography

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Summary

The preparative gas-chromatography experiments described herein gave major variations in maximum production rates and maximum separable sample sizes as the result of changes in certain of the operating variables. The best results were realized by introducing the samples relatively slowly in the form of a rotary spray on the walls of an empty chamber vaporizer and by operating a packed inlet cone at least 60°C above the column temperature. Use of circulating air to thermostat the entire system gave much better results than when resistance wire windings were used to maintain the temperature of the column and associated equipment.

The preparative scale work described herein was done with common saturated fatty acid methyl esters with carbon numbers ranging from C_8 through C_{18}. In most cases, the experiments were carried out with binary mixtures of C_{12}, C_{14}, C_{16}, and C_{18}, utilizing a two-carbon-number difference in each sample. Work was also done with ternary mixtures of $C_8/C_9/C_{10}$ and $C_{16}/C_{17}/C_{18}$ and with a a four-component mixture of $C_{12}/C_{14}/C_{16}/C_{18}$. The separation factor of the above binary mixtures ranged from about 1.6 to 2.2 for the column temperature range studied. As expected, the separation factor for a particular binary sample decreased with an increase in column temperature. The conditions and method of operation described in the previous paragraph gave improved results for all the sample mixtures.

The standard columns used in these experiments were 1-in I.D.

TABLE 1

Preparative Chromatography Operating Conditions for Nichrome and Oven Heated Columns

Carrier gas	Helium flow of 1.5 liters/min for 1-in I.D. column used as a basis.
Vaporizer	Operating temperature 274–285°C (310°C for SE30 runs at column temperatures of 215–265°C).
Inlet cone	Externally heated by Nichrome wrappings and insulated with asbestos tape.
Column	All columns 3 ft in length. Packings: 17–22% egs on 80/100 Gas Chrom P, 17–22% SE30 on 80/100 Gas Chrom P. Base sample: C_{14}/C_{16} fatty acid methyl esters in 50/50 wt. fraction (also used C_{12}/C_{14}, C_{16}/C_{18}). Base temperature: 160°C for egs columns, 215°C for SE30 column.
Exit cone	Except in special cases was unpacked and run at column temperature. When the cone was unpacked, a porous screen covered with glass wool supported the column bed.
Detector	Thermal conductivity cell run at 275–285°C (310°C for SE30 runs at column temperature of 215–265°C).

by 3 ft long and packed with either 17 to 22% egs on 80/100 mesh Gas Chrom P, or 17 to 22% SE30 on the same support (Applied Science Laboratories, Inc., State College, Pa.). Table 1 summarizes operating conditions and related items. A few experiments done in 2-in. I.D. columns of the same length gave production rates within a few per cent of the value predicted from 1-in I.D. columns on a production-rate scale-up basis.

EXPERIMENTAL

A constant-temperature oven with forced circulating air was used for most experiments. Temperature variations over the column length did not exceed 2°C. In comparison, columns heated by externally wound Nichrome resistance wire and insulated with asbestos tape exhibited a minimum temperature variation of 5°C along the column length, even with extremely careful application of the heating and insulating tape.

Similar vaporizers and detectors were used with both methods of temperature control. The vaporizer was a 4-in O.D. cyclindrical carbon steel block 4½ in. long having a vaporization cavity 1¼ in. I.D. by 4 in. long. Steel shot of ¼ in. diameter was used to fill the vaporization cavity for some of the experiments. The detector was a 4-in. cube of carbon steel drilled to accept Gow-Mac w-9225 thermal conductivity filaments. Gow-Mac power-supply control units, Model 405, were used in sample detection. The detector took the full flow from the column through a ⅜-in.-diameter channel. The vaporizer and detector used in both systems were heated by Chromalox cartridge heaters embedded in the blocks.

All columns used were 1- or 2-in. I.D. standard 3 ft lengths of "Double Tough" Pyrex Pipeline fitted with commercial union flanges and Teflon gaskets. A standard helium carrier gas flow rate of 1.54 liters/min for 1-in. I.D. columns and 6.16 liters/min for 2-in. I.D. columns was used for all experiments, except where otherwise specified. The inlet and outlet cones used on these columns were attached by means of the union flanges and were made of stainless steel bored from an inside diameter of either 1 or 2 in. to an inside diameter of ⅜ in. over a cone length of 3¾ in. for 1- or 2-in. I.D. columns, respectively. The cones were heated by externally wrapped Nichrome resistance wire.

The columns were packed in a standard manner adopted in this laboratory. This consisted of vibrating the column in a vertical, as well as a horizontal, manner while the packing was poured into the column through a funnel at a rate of about 10 g/min. Vibration in the vertical direction was achieved by placing the column on a Syntron Model v-4 electric vibrator; horizontal vibration was achieved by a Burgess Model v-73 Vibro-Graver. The packing density obtained was normally 0.30 to 0.32 g/cm^3 for both packings.

The maximum efficiency achieved with 1-in. I.D. columns was 500 apparent theoretical plates per foot for small samples (1 to 16 μl) of the binary methyl ester mixtures. However, the average apparent theoretical-plates-per-foot values for the sample sizes just mentioned ranged from 330 for egs columns operating at 125°C, to 150 for the same columns operating at 200°C. Essentially the same results were recorded for SE30 columns operating between 175 and 260°C. In both cases, plates-per-foot values decreased continuously with increased column temperature for the same sample mixture.

Definition of Maximum Separable Sample Size and Production Rate

A convenient means of comparing a series of preparative chromatography experiments done under a variety of conditions is the maximum separable sample size and the production rate.

The broad definition of maximum separable sample size is the largest sample size which still results in essentially complete separation of the desired components in the sample. To be more specific, for this work it is defined as the largest sample size which gives collected components of 99+% purity if the components are trapped in their entirety and if the cut point is made at the minimum points in the valleys between peaks. It was found experimentally for the methyl esters used here that a 97% return to base line (based on the maximum height of the preceding peak) between two peaks resulted in a minimum component purity of 99+% if the cuts are made as specified above.

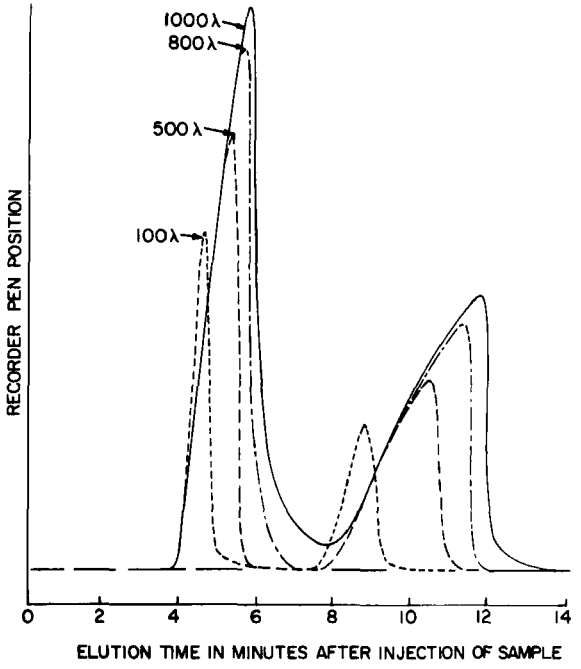

FIG. 1. Typical poor preparative chromatography results. 50/50 wt. C_{14}/C_{16} methyl ester injection, 1-in. I.D. × 3-ft-long column, 20% SE30 column at 218°C.

Definition of the maximum separable sample size is aided by referring to Fig. 1, which gives tracings for a series of oven column tests in which operating conditions were kept as nearly constant as possible and sample size alone was varied. In this case, all the samples injected were equal-weight mixtures of high-purity methyl myristate and methyl palmitate. The 0.10-ml sample in Fig. 1 was recorded at an attenuation of ×2; all others were recorded at an attenuation of ×4. The tracings show that all samples up to and including the 0.80-ml sample were separated completely, while binary samples larger than about 0.80 ml could not be separated into their essentially pure individual component parts. Therefore, the 0.80-ml injection is referred to as the maximum separable sample size under these particular operating conditions.

With proper operating conditions, maximum separable sample sizes as large as 28 ml were obtained in the 2-in. I.D. columns. The objectives of this work did not, however, include obtaining a startlingly large maximum separable sample size. The values were used to compare one set of operating conditions with another, and to calculate values of production rate. More complex criteria for comparison, such as calculation of the number of stages and analysis of the exact composition of separated fractions, were not used because they add greatly to the complexity of the apparatus and procedures.

The second parameter used in measuring column performance in these experiments was the maximum rate at which a sample, such as that in Fig. 1, could be separated into its essentially pure component parts. The production rate as defined here has the units milliliters per hour and is the sample size in milliliters divided by the time elapsed between injection of the sample and complete passage through the detector of the last component in the sample. The production-rate calculation for the 0.5-ml sample in Fig. 1 is as follows:

$$\frac{0.50 \text{ ml}}{11.8 \text{ min}} \times 60 \frac{\text{min}}{\text{hr}} = 2.6 \frac{\text{ml}}{\text{hr}}$$

It has been found, as will be illustrated later, that the maximum production rate for a specific set of conditions was always obtained with the maximum separable sample size. Therefore, the maximum production rate is defined as the production rate for the maximum separable sample size. The maximum production rate from Fig. 1

(0.8-ml sample) is

$$\frac{0.80 \text{ ml}}{12.0 \text{ min}} \times 60 \frac{\text{min}}{\text{hr}} = 4.0 \frac{\text{ml}}{\text{hr}}$$

It will be shown in subsequent paragraphs that the maximum production rate has more significance than the maximum separable sample size for evaluation of a column.

RESULTS

Inlet-Cone Effects

Typical preparative chromatographic data obtained for C_{14}/C_{16} injections in an oven heated SE30 column with nonpacked inlet and outlet cones (both of which were maintained at the column temperature) are shown in Fig. 1. In these initial data runs, the vaporizer was filled with steel shot, and sample injections were made directly onto the vaporizer bed through a standard 22 gage hypodermic syringe needle. As was noted previously, the maximum separable sample size was about 0.80 ml and the maximum production rate was 4.0 ml/hr. However, more favorable operating conditions were realized when the inlet cone was heated to about 60°C above the column bed temperature and the inlet cone was filled with column packing, while all other operating conditions were kept as nearly constant as possible, including the use of the same vaporization technique. Figure 2 illustrates such improved results obtained with a hot, packed inlet cone which gave a maximum separable sample of about 3.0 ml and a maximum production rate of 12.0 ml/hr. The hot, packed inlet cone had two major effects on the separation of the binary sample. First, the width of the component peaks and the sample tailing characteristics were both reduced. Second, a displacement of the second peak occurred as the sample size was increased. The hot, packed inlet cone is thought to affect mainly the solute-stationary phase equilibrium because of advantageous concentration changes in the column, thereby allowing better separation.

An increase in maximum separable sample size and maximum production rate for columns operated with a hot, packed inlet cone has been noted for all tests in this laboratory in which successful runs could be made in a typical chromatographic manner. The advantage of the altered inlet cone appears to be quite general for

methyl ester injections, carbon numbers ranging from C_8 to C_{20}, into either polar (egs) or nonpolar (SE30) columns over the complete range of operating temperatures investigated.

Peak Displacement

Peak displacement often has an important effect on the results obtained in preparative work. Figure 2 illustrates the relatively small, but important, peak-displacement effect noted in SE30 columns in which relatively short sample elution times are encountered. Samples having longer elution times, such as those injected into egs columns, show the peak-displacement effect to a greater extent. Figures 3 and 4 illustrate the dramatic displacement effect which occurred with egs columns for C_{12}/C_{14} and C_{14}/C_{16} methyl ester injections, respectively. In Fig. 3 the initial elution time for the second peak was displaced from about 8 min for a 0.10-ml in-

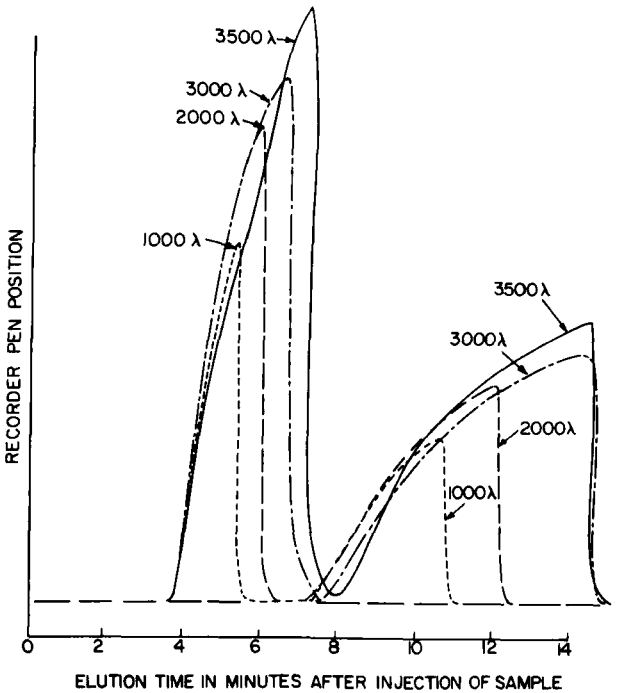

FIG. 2. Effect of hot packed inlet-cone msss increased from 0.8 to 3.0 ml, production rate from 4.0 to 12 ml/hour, 20% SE30 column at 215°C, 1-in. I.D. column, C_{14}/C_{16} injection.

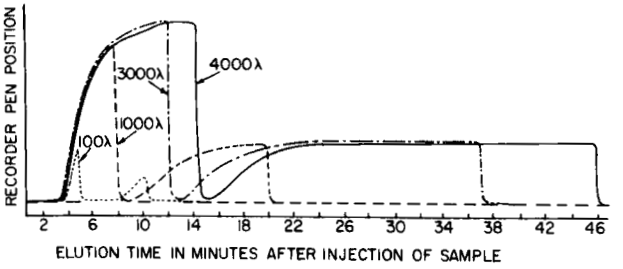

FIG. 3. Peak behavior and widening characteristics with increasing sample size. 17% egs column at 143°C, 1-in. I.D. column, C_{12}/C_{14} injection.

jection to about 15 min for the maximum separable sample size of 4.0 ml. If it were not for this peak displacement, possibly only a 1.0-ml sample of the binary mixture would have been separated in this case. Also, the first peak began to elute slightly earlier as the sample size was increased in this sample range; however, the magnitude of the first peak displacement was comparatively small. The peak-displacement effect has also been important in separating large samples of three- and four-component mixtures.

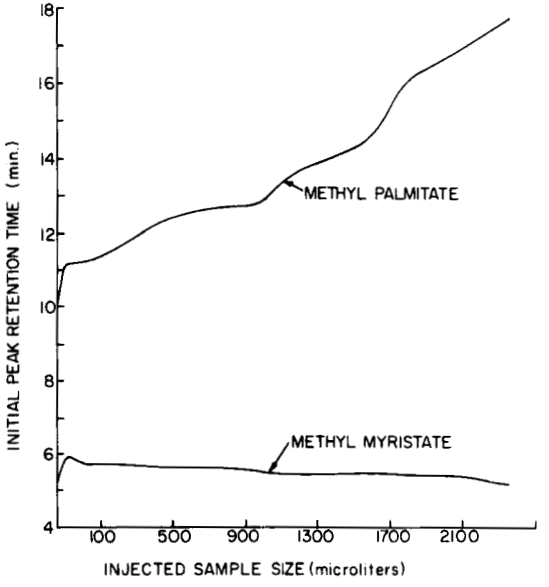

FIG. 4. Peak displacement occurring for the individual components in a 50/50 wt. binary mixture; 22% egs column at 160°C.

Column Heating and Operation

An investigation of the effects of uneven column heating on the maximum separable sample size was begun by comparing the results obtained in a Nichrome heated column with the results obtained in the same column which had several "hot spots" along the column axis produced by wrapping additional insulation on the column exterior at these positions. The unevenly heated Nichrome column had axial temperature variations up to 15°C, compared to 5°C axial temperature variations in the evenly heated column. Table 2 gives the data obtained, from which it is evident that a detrimental effect is produced by the uneven column heating. Also, it is seen in both cases of column heating that as the inlet-cone temperature was increased, the maximum separable sample size also increased.

TABLE 2

Variations in Maximum Separable Sample Size with Axial Temperature Variations and Inlet-Cone Temperatures[a]

Inlet cone,[b] °C	Column wrapping	Approx. Separable sample size, ml
140	Uneven	0.090
140	Even	0.175
160	Uneven	0.225
160	Even	0.300
225	Uneven	0.325
225	Even	0.450

[a] Evenly Nichrome wrapped column, 17% egs, 1-in. I D. × 3-ft-long column, column temp. 160°C, injection 50/50 wt. C_{14}/C_{16} fatty acid methyl ester mixture.
[b] Inlet and outlet cones filled with glass wool and screen used for support.

Table 3 gives further data for an evenly heated Nichrome heated column in which inlet- and outlet-cone temperatures were varied while the cones were either "unpacked" or "packed" with column packing. Again, an inlet cone filled with packing and maintained approximately 60°C above the column temperature gave the best results. It is seen that the influence of the outlet cone on the over-all results was not substantial. Similar results have also been obtained on oven heated columns. Therefore, the outlet cone was unpacked

TABLE 3

Effects of Cone Packing and Inlet Gas Temperature on Solute Separation[a]

Inlet cone, °C	Outlet cone, °C	Approx. max. separable sample size, ml
160, unpacked[b]	160, unpacked	0.30
160, packed[b]	160, unpacked	0.30
225, unpacked	160, unpacked	0.50
225, packed	160, unpacked	1.00
160, packed	160, packed	0.60
225, packed	160, packed	1.00
225, packed	225, packed	1.10

[a] Evenly Nichrome wrapped column, 17% egs, 1-in. I.D. × 3-ft-long column, column temp. 160°C, injection 50/50 wt. C_{14}/C_{16}, fatty acid methyl ester mixture.
[b] Packed, filled with column packing; unpacked, filled with glass wool and screen for support.

and maintained at the column temperature for all routine experiments with Nichrome and oven heated laboratory columns.

Column-performance results for egs and SE30 columns placed in an efficient constant-temperature oven are given in Table 4. By comparing the data for the egs column in Table 4 to the correspond-

TABLE 4

Effects of Inlet Cone Packing and Temperature for egs and SE30 Oven Heated Columns[a]

	20% egs column[b]		20% SE30 column[c]	
Inlet cone	Max. separable sample size, ml	Max. production rate, ml/hr	Max. separable sample size, ml	Max. production rate, ml/hr
Unpacked, at column temp.	0.250	0.968	0.80	3.99
Unpacked, 60°C above column temp.	0.400	1.74	2.20	9.42
Packed, at column temp.	1.20	2.75	2.20	10.0
Packed, 60° above column temp.	3.50	3.48	3.50	12.8

[a] Oven heated columns 1-in. I.D. × 3 ft; injection 50/50 wt. C_{14}/C_{16} fatty acid methyl ester mixture.
[b] Column and outlet-cone temperature 160°C.
[c] Column and outlet-cone temperature 216°C.

ing data in Table 3, the advantages of oven heating are quite apparent. The SE30 column in Table 4 was run at 216°C so that elution of the first peak would begin in the same length of time as for the first peak eluting from the egs column operating at 160°C. Higher maximum production rates of the methyl esters were obtained for the SE30 column than for the egs column in all cases, although under these conditions the maximum separable sample sizes were the same for both columns. The higher maximum production rate occured because a shorter time was needed for complete sample elution in the SE30 column, owing to less band spreading. For a general comparison of the over-all sample elution time, see Figs. 2 and 3.

Sample Injection and Vaporization Studies

An initial attempt, which was not successful, to scale-up the preparative process from a 1-in. I.D. to a 2-in. I.D. egs column initiated an investigation of injection and vaporization studies. In Table 5 entries 1 and 4 show data for normal sample injections

TABLE 5

Comparison of Injection and Vaporization Technique for 1- and 2-in. I.D. egs Oven Column[a]

Column diameter, in.	Vaporizer cavity	Injection technique	Max. separable sample size		Max. production rate	
			Achieved, ml	2-in. I.D. scale-up,[b] ml	Achieved, ml/hr	2-in. I.D. scale-up,[b] ml/hr
1	Filled	Normal	3.00		4.44	
1	Empty	Normal	2.60		4.52	
1	Empty	Turned-sprayed	4.00		5.00	
2	Filled	Normal	3.20		11.3	
2	Empty	Normal	4.50		8.20	
2	Empty	Turned-sprayed	13.0	16.0	19.5	20.0

[a] 20–22% egs column at 160°C; column length 3 ft; carrier gas flow rates: 1-in. I.D. column 1.54 liters/min, 2-in. I.D. column 6.16 liters/min.

[b] Scale-up based on column cross-sectional area and data of entry 3.

using a hypodermic needle which directed the sample downward onto the vaporizer bed consisting of ¼-in.-diameter steel shot. The sample injections were made as rapidly as possible, with both samples being injected in about 5 sec. With this technique and vaporizer design, the maximum separable sample size increased slightly from 3.00 to 3.20 ml and the maximum production rate increased from 4.44 to 11.3 ml/hr in going from a 1-in. to a 2-in. I.D. column. The 2-in. I.D. column data were characterized by peak tailing, which was believed to have been caused by improper sample entry into the column, thereby initiating further studies in both 1-in. and 2-in. columns.

Best results were obtained for both 1-in. and 2-in. I.D. columns in which a slow, rotary-sprayed sample injection was made on the walls of a vaporizer which had been essentially emptied of steel shot, with the exception of a single layer covering the exit vaporizer port. The sample injection was made by a hypodermic needle which had been bent and then crimped on the end to produce a spray issuing against the vaporizer wall. Sumantri (3) used an alternative form of vaporization in 1961 in which the samples were sprayed into a vaporization chamber in a manner analogous to a diesel-engine injection system. In comparing entries 3 and 6 of Table 5 (using the altered injection and vaporization technique), it is seen that scale-up was essentially obtained in regard to maximum production rate, although the maximum separable sample size was smaller than the direct scale-up value. Figure 5 is a comparison of entries 5 and 6 in Table 5 and illustrates the advantage of proper sample introduction into an empty vaporizer. Both samples shown

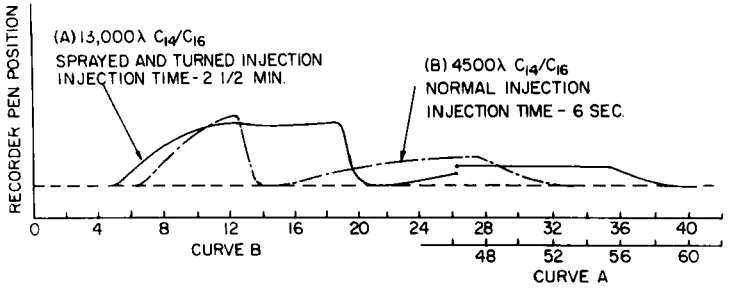

FIG. 5. Effect of the injection technique on the maximum separable sample size in a 2-in. I.D. column, 22% egs column at 160°C.

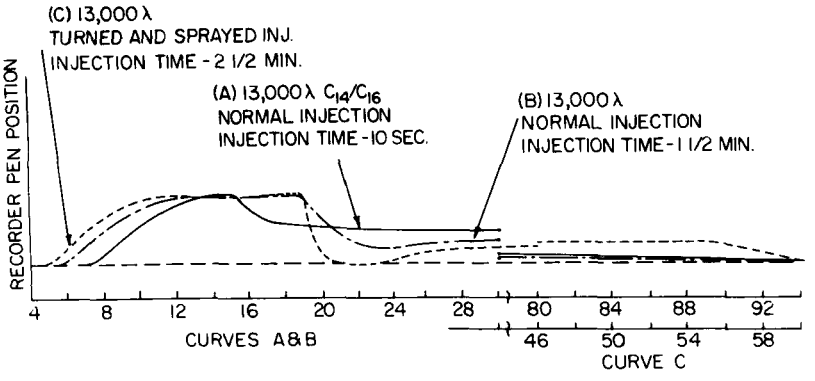

FIG. 6. Effect of injection rate and technique on maximum separable sample in a 2-in. I.D. column, 22% egs column at 160°C.

in Fig. 5 are maximum separable sample sizes for that type of injection. Figure 6 illustrates with curves A and B that it is actually injurious to inject preparative scale samples in too rapid a manner for normal injection techniques. Detrimental results also occur for a too rapid, rotary-sprayed injection. In addition, as Fig. 7 shows, an overly lengthy injection time is also injurious to sample separation. Sumantri predicted from digital-computer calculations in 1963 that the sample elution band width should not be extremely sensitive to the sample vaporization time when isotherms are nonlinear (*4*). Gordon et al. (*1*) in 1964 called attention to somewhat similar results by Haarhoff (*2*).

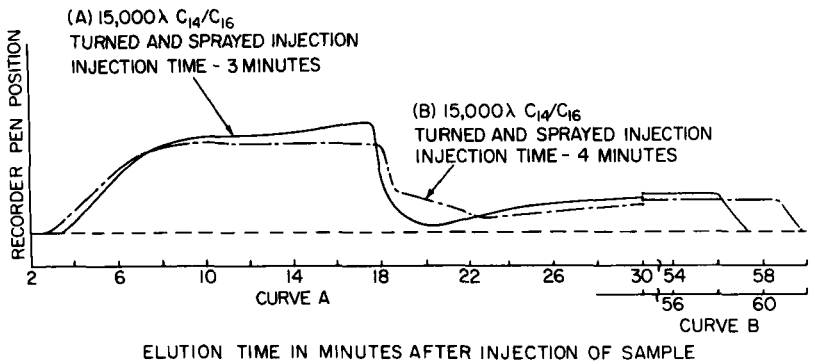

FIG. 7. Effect of injection rate on separation in a 2-in. I.D. column, 22% egs column at 160°C.

In actuality, a proper injection time for best results with a specified sample can be chosen. Representative data for such a study are shown in Fig. 8, which illustrates the production rates obtained for various injection times with selected sample sizes of an equal-weight C_{14}/C_{16} mixture. The study was made on a 1-in. I.D. SE30 oven column with a carrier gas flow rate of 2.00 liters/min. All sample injections were sprayed onto the wall of an empty vaporizer cavity in a rotary manner. The objective of this study was to determine the range of sample injection times for which essentially complete sample resolution could be attained. The complete range of injection times could not be investigated for the 3.0- and 4.5-ml samples because the injection technique used in this study did not permit sample injections in less than 5 sec for these sample sizes, although it was apparent that a more rapid sample injection was necessary to determine the injection rate limit. Also, the longest practical injection time for the 3.0-ml sample was about 80 sec, which should be well below the longest possible injection time for which sample resolution would still occur for that sample size. Several observations can be made from Fig. 8. First of all, the pro-

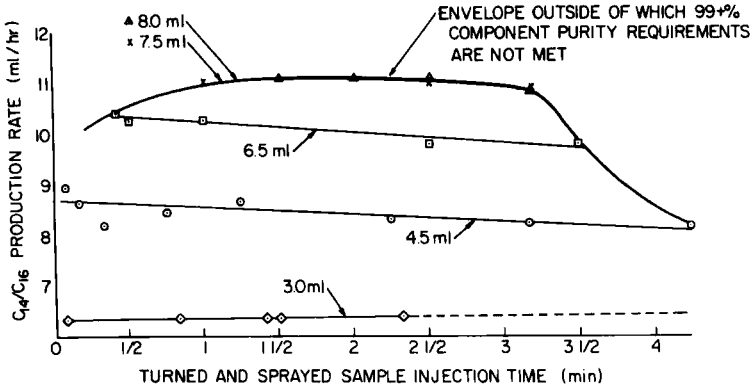

FIG. 8. Effect of sample injection time and sample size on column production rate, 1-in. I.D. × 3-ft-long column, 20% SE30 column at 195°C, C_{14}/C_{16} methyl ester injection, flow rate 2.00 liters/min.

duction rate for a given sample size was not very sensitive to the injection time. The production rate was essentially constant or decreased slightly as the injection time was increased within the range of injection times for which individual component peak purity requirements of at least 99+% were met. Second, the pro-

duction rate was found to be very dependent on the sample size, except near the region of the maximum separable sample size. As the sample size was increased, the production rate increased rapidly until the sample size approached the maximum separable sample size, after which the production rate remained essentially constant until the maximum separable sample size was attained; e.g., compare the 7.5- and 8.0-ml sample production rates. Finally, although it is necessary to choose a proper sample injection time for a given sample size in which the injection is made neither too rapidly nor too slowly, there exists a range of possible injection times for that sample size over which acceptable sample resolution still results. This range of injection time narrows as the maximum separable sample size is reached; however, a sufficient latitude of choice in the injection time exists at the maximum separable sample, so that it is not experimentally difficult to describe and achieve the maximum separable sample size and the maximum production rate for a given situation. For the operating conditions described above, a maximum production rate of about 11.1 ml/hr of the binary sample was attained with a maximum separable sample size of 8.0 ml.

Figures 9 through 11 illustrate selected datum points taken from the injection-time study discussed in the preceding paragraph. Figures 9 and 11 illustrate improper injection times for the 7.5-ml binary injection, in which the injection time was respectively too rapid (11 sec) and then too slow (4 min). Neither injection time resulted in a sample separation that met the sample resolution requirements as defined in this work. The 2½ min injection time

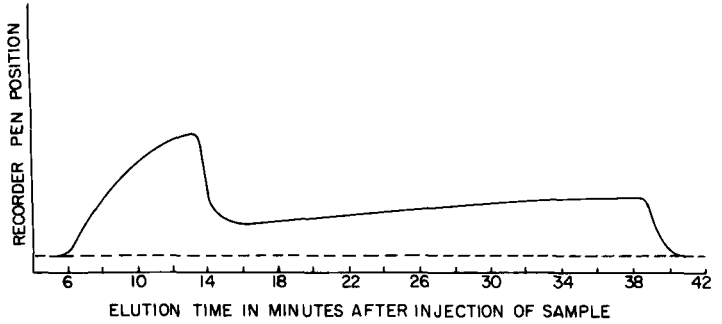

FIG. 9. Turned and sprayed injection-time studies, 7.5 ml C_{14}/C_{16} mixture injected in 11 sec, 1-in. I.D., 20% SE30 column at 195°C.

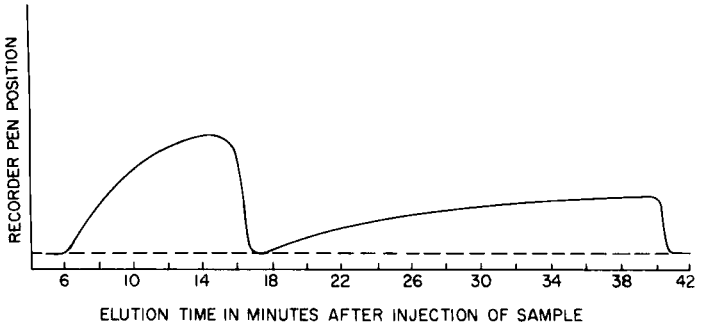

FIG. 10. Turned and sprayed injection-time studies, 7.5 ml C_{14}/C_{16} mixture injected in 2½ min, 1-in. I.D., 20% SE30 column at 195°C.

for the 7.5-ml sample as shown in Fig. 10 does result in an acceptable sample resolution. It should be noted that the peak characteristics of Figs. 9 and 11 are markedly different from one another, thereby allowing the experimeter to correct the injection time for succeeding sample injections. Observation of the peak characteristics has been found to be an invaluable aid in determining proper operating conditions in the preparative work discussed here.

DISCUSSION

For the column arrangement used in this work, consisting simply of a glass tube and inlet and outlet cones, best results have been obtained when the columns were oven-heated and when the inlet cone was filled with column packing and operated about 60°C above the column temperature. This manner of operation has been

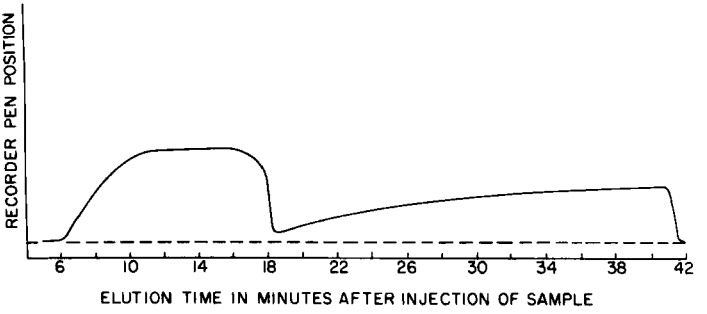

FIG. 11. Turned and sprayed injection-time studies, 7.5 ml C_{14}/C_{16} mixture injected in 4 min, 1-in. I.D., 20% SE30 column at 195°C.

quite successful over a relatively wide range of column temperatures for fatty acid methyl ester injections in both egs and SE30 columns. The effects of outlet-cone variations on column performance have also been investigated. The outlet cone apparently has only a slight effect on the column performance when compared to inlet-cone effects.

Sample introduction and vaporization techniques have been found to be an important factor in achieving successful column operation; especially for 2-in. I.D. column operation. A proper rate of solute introduction into the system is necessary for best results. This rate must be neither too fast nor too slow and is apparently most dependent on the sample size relative to the over-all retention time of the sample within the column. It may be most important in preparative gas chromatography, as it is in liquid-liquid extraction, for example, to try to avoid regions of high solute concentration, in which the solute separation factor decreases significantly with increasing concentration. Introduction of the sample at a relatively slow rate should have the effect of producing wider-than-normal concentration bands in the inlet section of the column, where sample concentrations tend to be highest, thereby giving a more suitable separation factor. However, sample introduction must not be too slow or peak overlapping may occur because the sample volume occupies too large a proportion of the column. A balance between the two extremes is necessary and has not been difficult to achieve in practice.

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