PREPARATIVE SCALE GAS CHROMATOGRAPHY. III.

M. VERZELE, J. BOUCHE, A. DE BRUYNE AND M. VERSTAPPE
Laboratory of Organic Chemistry, State University of Ghent (Belgium)
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In two previous communications we investigated substance recovery[1] and choice[2] of carrier gas in preparative scale gas chromatography (PGC). The present paper is a further report on experimental work, using this technique.

THE CAPACITY OF PREPARATIVE SCALE GAS CHROMATOGRAPHY

In gas–liquid chromatography the plate number* should be derived from extrapolation to zero sample size and for a substance having a large partition factor. The number so obtained is then a measure of the analytical separation power of the column for that solute and for closely similar substances. With increasing sample size the relative band width increases and a graph of this, with relative band width expressed as "plate number", for a preparative scale gas-chromatographic column is shown in Fig. 1.

The smaller "plate number" for increased sample size reflects the separation power of the column for these large samples. This number could be named the "preparative scale plate number" and should be given with an indication of the sample size to which it refers.

In the case of mixtures which have to be separated, the sample size is determined by this preparative scale plate number and also by the relative retention of the substances. This relative retention, α, is equal to k_2/k_1 or K_2/K_1 or V'_{R2}/V'_{R1}; k, K and V'_R being the partition ratio, the partition coefficient and the adjusted retention volume[3], respectively. Large α values mean large samples of the mixtures and vice versa. The permissible sample size should obviously be as large as possible. This is the most important point in preparative scale gas–liquid chromatography.

In trying to increase the capacity of preparative scale gas–liquid chromatography, efforts have in the past been mainly directed to cancelling the adverse effect of increased sample size. This applies to the use of increased column diameters, parallel columns, automatic column operation repeating the same separation over and over again and also of continuous flow techniques.

The largest possible capacity could also be attained by finding the liquid phase which would give the largest relative retention, α, for the mixture in question. This,

* Plate numbers in this paper have been calculated with the equation $n = 16 \cdot (V_R \cdot V'_R)/Y^2$ in which V_R and V'_R are the retention volume (distance) and the adjusted retention volume (distance) and Y is the band width at the peak slope tangent intercepts with the base line. This equation is equivalent to $n = 16 \cdot (V_R/Y)^2/(1/k_2 + 1)$ and therefore partly takes into account the influence of the partition factor on plate numbers. For large partition factors it approximates to the equation usually employed, $n = 16 \cdot (V_R/Y)^2$.

however, leads to a large number of columns and to frequent column changes, which is an annoyingly time-consuming operation.

Still another approach, however, is to increase the separating power of the columns as much as possible and this is most simply obtained by increasing the column length of a relatively small bore column. Such a column will show an increased preparative scale plate number and will allow larger samples to be introduced.

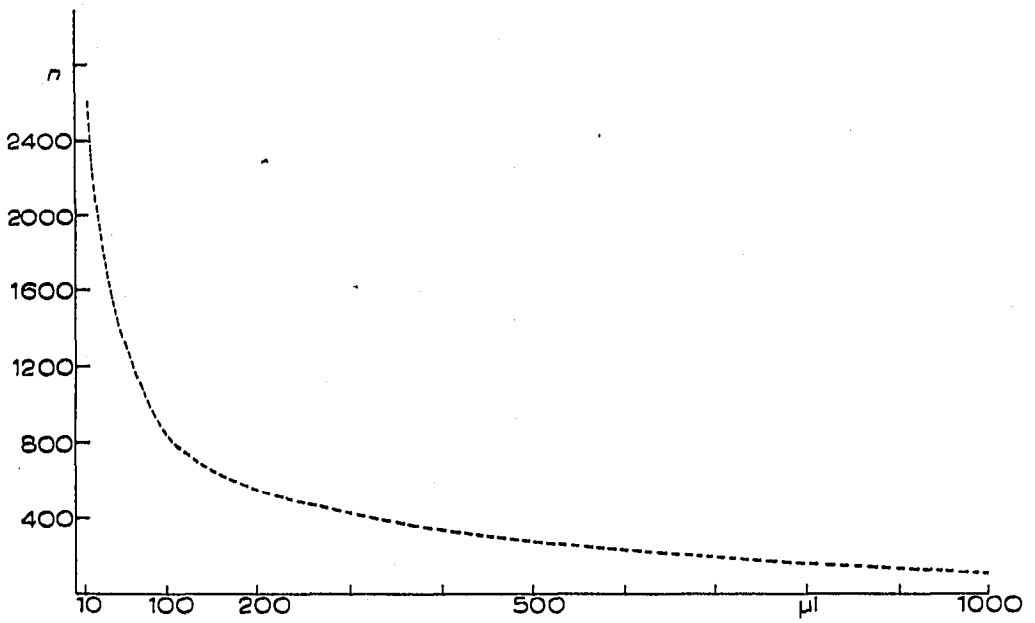


Fig. 1. Plate number, n, *vs.* sample size for a 6 m × 9 mm coiled glass Chromosorb P 30/60 mesh column coated with 35% SE. 30. 200 ml H_2/min and iso-octane as sample. The column was old and better results are obtainable.

It should also be possible to use only one or a very reduced number of such columns to solve most problems.

This paper discusses the experimental results of this approach, the parameters influencing the preparative scale plate number and the use of this number to find the permissible sample load.

The number of theoretical plates required for complete separation of two substances is given by the equation[3]:

$$N = 16\,[\alpha/(\alpha - 1)]^2\,[1/k_2 + 1]^2 \qquad (1)$$

This equation is, in principle, used to deduce the column length necessary for a given separation.

In preparative scale gas chromatography, eqn. (1) will not be used to find column length, but to find the maximum permissible sample size of a column. A sample calculation will serve to illustrate this. Consider the 6 m × 9 mm column of Fig. 1, a H_2 gas rate of 200 ml/min and a limiting duration of the separation < 2 h, which is equivalent to a partition factor in the range of 10 to 100. With such a partition factor the column will have its maximum separation power and the second term in eqn. (1) can be neglected. For an α value of 1.25 (this is the case for the *trans-cis*-decalin mixture at about 160°) eqn. (1) leads to 400 required plates. Fig. 1 shows that

he column gives that preparative scale plate number for sample sizes as large as ;50 μl. In 500 μl decalin mixture there is about 350 μl of the *trans*-isomer and 500 μl s therefore the maximum possible sample size of this mixture which will still give :omplete separation.

This is in practice found to be the case, and a graph of this separation has ılready been published in ref. 2.

?ERCENTAGE OF STATIONARY PHASE

The percentage of stationary liquid phase could have an influence in preparative ;cale gas chromatography. In order to verify that this parameter is critical, a 6 m ×) mm glass column filled with Gas-Chrom P (Applied Science) as support was tested ;uccessively with 5, 10, 15, 20, 25, 30 and 35 % SE. 30 silicone gum as stationary phase in an Autoprep 700 instrument. The substances tested were iso-octane and :yclohexane. While there is a maximum in the plate number around 20–30 % coating with analytical size samples, the preparative scale plate number is still increasing slightly at 35 % coating with the larger samples (as shown for one case in Fig. 2).

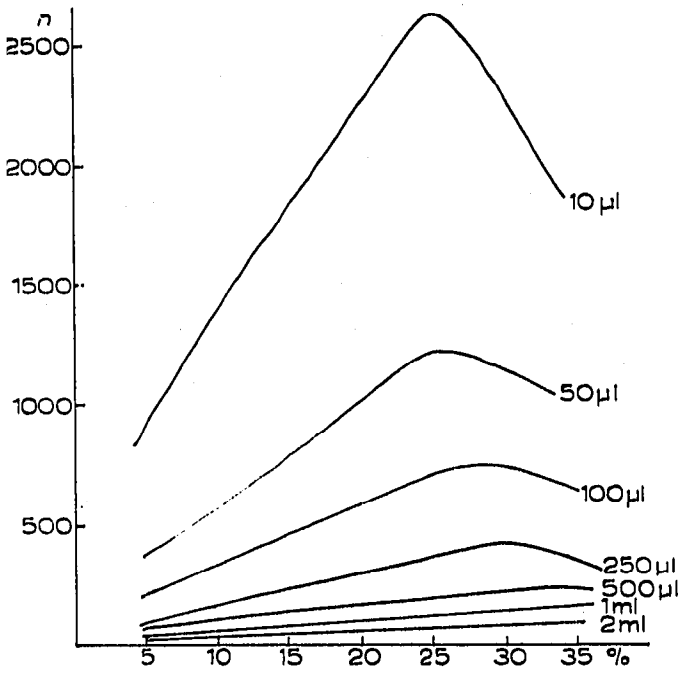


Fig.2. Plate number, n, as a function of liquid coating percentage and sample size.

It could be concluded that liquid loadings should be further increased, but this is not so. Larger liquid loadings increase the separation time and especially increase the working temperature. This is often detrimental to the capacity of a column, since relative retention generally diminishes with higher temperatures. In fact, since Fig. 2 shows that the difference is quite small between 10 and 35 % coating, the lower percentage coatings can be used with advantage.

Fig. 2 was obtained with a low-boiling substance. Experimental results with the high-boiling decalin mixture confirm these results. Separation with 10 and 25 %

coating were remarkably similar and, even at 5 % coating, preparative scale work is possible (*cf.* Table I). The 500 μl decalin sample is nearly completely separated with the 5 % coating.

TABLE I

PLATE NUMBERS FOR A 20 m COLUMN FILLED WITH A LABORATORY-MADE COARSE GRAIN SUPPORT COATED WITH SE. 30 IN THE PERCENTAGES GIVEN

Decalin sample size		*Plate numbers*		
		Liquid coating (5 %)	*Liquid coating (10 %)*	*Liquid coating (25 %)*
10 μl	*trans-*	—	—	1553
	cis-	—	—	1908
100 μl	*trans-*	954	1323	1351
	cis-	2833	2919	1778
200 μl	*trans-*	631	833	958
	cis-	2303	2459	1631
500 μl	*trans-*	292	427	618
	cis-	1507	1745	1541
1 ml	*trans*	211	307	354
	cis-	1087	1404	1210

COLUMN DIAMETER AND PACKING METHOD

We have also tried to increase the capacity of gas chromatography by increasing the column diameter. The influence of this increase on the separation power (plate number) must be considered, and very variable claims for the plate number of increased diameter columns can be found in the literature. Some workers find a big drop in the plate number, others find no significant difference and this is often attributed to some special way of packing the column.

We have always found a large drop in the analytical plate number when the column diameter was increased. For 6 mm and 30 mm diameter columns of 2 m length, for example, and with a small sample load proportional to the section surface, the plate number showed a ten-fold difference under the most favourable circumstances. Preparative scale plate numbers per unit length are, however, more similar and with very large samples are of course even better on large bore columns.

The methods which we used for packing the columns varied from simply pouring in the material with or without lateral or lengthwise tapping or vibrating, to tamping the column with a glass rod as in the method used for packing liquid–liquid partition chromatography columns. Vacuum packing and adding the material in small portions or suspended in a liquid were also tested. The method which produces the largest density with celite (vertical tapping as hard as the metal column will stand) is not the most efficient for Chromosorb W (lateral vibrating).

This density is indeed a factor which markedly influences the column plate number for large bore columns. For "hard" supports like Chromosorb P and W and

Gas-Chrom P the packing density cannot be varied very much. Extreme values which we found with Chromosorb W 60/80 mesh and a 2 m × 30 mm column were between 0.37 to 0.415 g/ml with a corresponding plate number increase of 20 % (about 150 plates per metre for the highest density and for 150 μl samples).

With more sticky supports like the very fine mesh Chromosorb W or with Celite 545 (Johns Manville) screened by flotation to eliminate all but the coarsest 10 %, variations are much bigger. The extremes in density in this case are 0.24 to 0.46 g/ml for the celite coated with 20 % SE. 30, and the plate number is more than double for the denser packing. In fact there is an almost linear relationship, as shown in Fig. 3.

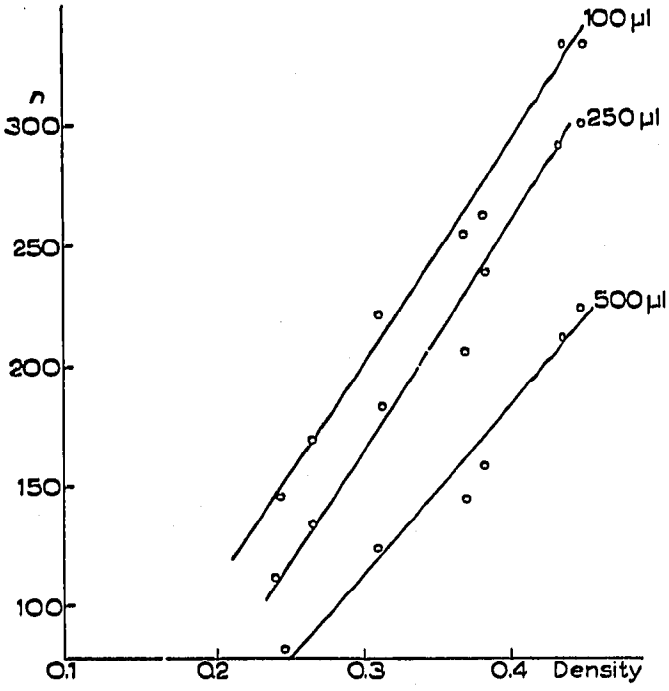


Fig. 3. Plate number, n, as a function of packing density and sample size.

It seems that, for these large bore columns, the denser the packing, the larger is the column plate number. In smaller bore columns the packing density effect seems relatively unimportant. For a 6 m × 9 mm glass column (Autoprep 700 Wilkens Instrument) filled with Chromosorb W 60/80 mesh and coated with 30 % SE. 30 we could vary the packing weight between 68 and 81 g, the column still being completely filled for the lower value. With these packing densities and for *trans–cis*-decalin, the preparative scale plate numbers at 160° and with 200 ml/min H_2 as gas rate were remarkably similar, and so were in fact the analytical plate number values. There was, however, a big difference in retention time because of the difference in inlet to outlet pressure ratio (compressibility factor). The loosely packed column is nearly twice as "fast" as the most densely packed column. It seems therefore that, for smaller bore preparative scale gas-chromatographic columns, the aim should not be maximum packing density but that on the contrary lightly packed columns should be used because of the reduction of analysis time.

Comparing a 2 m × 30 mm diameter column with a 6 m × 9 mm diameter column, filled with Chromosorb W 30/60 mesh and coated with 20 % SE. 30, the

preparative scale plate number per unit length is larger for the bigger column. For 500 μl samples the plate number is about the same for the two columns; thus the preparative scale HETP for this sample size using the wider column is about three times smaller than that obtained when using the longer column. Their separation power for these sample sizes is the same. The longer small-bore column contains, however, only about a quarter of the volume of stationary support of the wider bore column, and consequently requires much less carrier gas for the same separation. An important factor also is the higher concentrations in the effluent gas using the smaller column, with consequent improved recovery.

To quadruple the sample size, the long column should be made about four times longer. This is indeed experimentally confirmed, as will be discussed later. To quadruple the sample size by column diameter increase of the shorter and wider column would lead in principle to a 60 mm diameter column. It still remains to be seen if such a wide column would show the desired plate number. The volume ratio for the enlarged columns is again about four. The volume of support material for the wider-bore column becomes excessively large for a research laboratory. In conclusion, we think long small-bore columns are to be preferred to shorter wide-bore columns for preparative scale gas chromatography.

HIGH PRESSURE CHROMATOGRAPHY

Columns for preparative scale gas chromatography have normally a large percentage of liquid stationary phase (low β) and are therefore so-called liquid-controlled[3]. Although the influence of pressure in the column would therefore be expected to be negligible we have carried out experiments to confirm this, using a conventional Autoprep 700 unit. With the usual 6 m $\times$ 9 mm columns filled with some suitable support the inlet to outlet pressure ratio was *ca.* 3.

By attaching a "restrictor" (a small piece of very fine metal or glass capillary) to the instrument outlet, the inlet pressure had to be raised to 5–7 kg/cm^2 to obtain a gas rate of 200 ml/min at atmospheric pressure. The effect on the capacity of the instrument was indeed negligible.

The differences from comparable "normal" chromatograms were: increased analysis time, a diminished katharometer detector response and peak asymmetry reversal from tailing to leading. Where a "tailing" peak was obtained in normal circumstances, use of a restrictor resulted in a markedly "leading" asymmetric peak shape. This is for example the case for iso-octane at 110° and will be discussed in more detail further. These differences can all be explained easily. The increased analysis time is due to the lower linear gas velocity in the pressurised column, the lower detector sensitivity is caused by the lower concentration of the solute at high pressure, and the peak asymmetry reversal from tailing to leading (only for large PGC samples) is due to a fall in the gas rate while the vapor is forced through the restrictor.

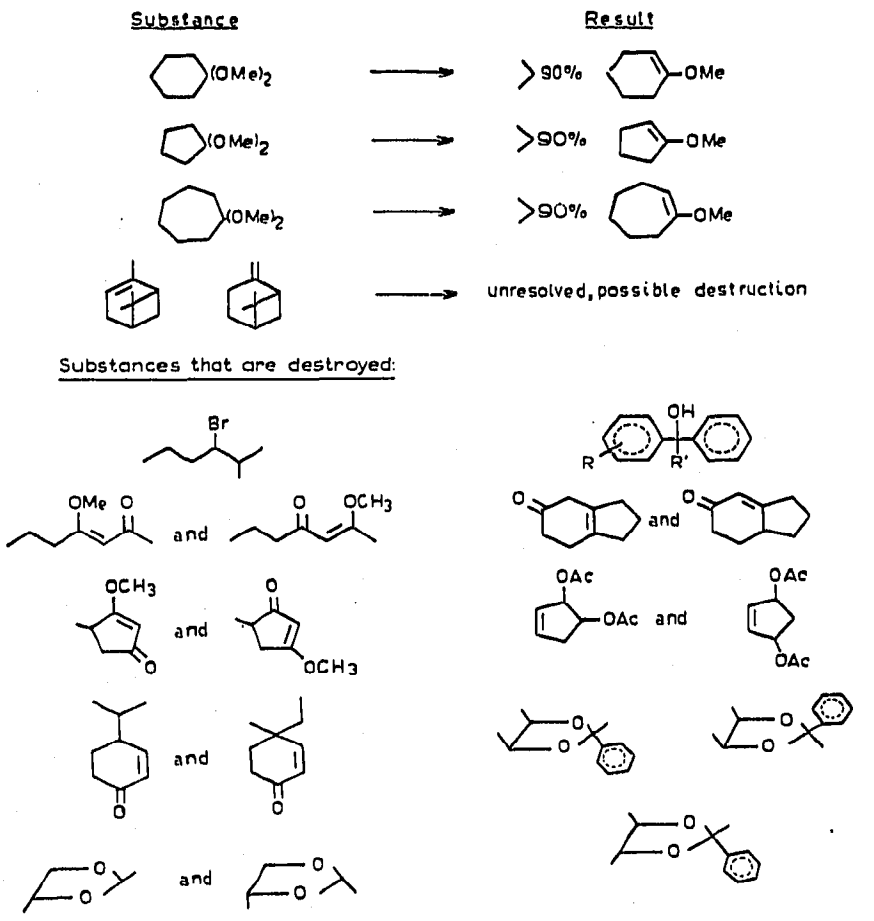
For 200 μl *trans*-decalin on the above column, for example, the measured gas rate fell gradually from 200 to 160 ml/min at peak maximum while returning to 200 ml/min when the base line was once again reached. These gas rate changes must result in a leading peak shape. With the restrictor between the column and the detector the sensitivity was restored. With a cold trap condensor placed ahead of

the restrictor no significant difference was found but we do not know if any condensation had occurred in the trap. Because of the negative result with regard to capacity, we did not press this point any further.

INFLUENCE OF THE STATIONARY SUPPORT

It is important that the stationary support material should be as inert as possible. For analytical gas chromatography of pesticides and steroids, for example, this has been generally recognised and has resulted in the introduction of glass columns and the exclusive use of deactivated white diatomaceous supports. For preparative scale gas chromatography this point is just as important. We have indeed encountered many separations which could not be carried out on metal columns with pink supports, but which were generally possible using glass columns and white supports. Because of this we are now working exclusively with the latter materials although the pink supports (Chromosorb P) give much more plates per unit length even for increased sample sizes. A number of examples of mixtures or pure substances which are destroyed or badly resolved through some support interaction on metal columns with pink supports are given in Table II.

TABLE II



Some supports are found to have a very high destructive power, *e.g.*, a laboratory-made support destroyed even SE. 30, which was depolymerised and blown off the column at temperatures as low as 150°. The support was made by backing Celite 545 with 3 % sodium carbonate at 1000°, crushing, screening, boiling with nitric acid and hydrogen chloride, washing, boiling with methanolic sodium hydroxide and thorough washing. The maximum allowable working temperatures (MAOT) for liquid phases must depend much more on the support than is generally believed, since SE. 30 can normally be used even at 350°.

Obviously the stationary supporting material can also affect the capacity of preparative scale gas chromatography. We have pointed out already in this paper that higher plate numbers, which go with better supports, mean greater capacity. But the support material giving the highest plate number for analytical scale work does not show necessarily the same superiority in preparative scale gas chromatography. This is the case for the seemingly very similar white diatomaceous supports Chromosorb W (Johns Manville) and Gas-Chrom P (Applied Science). Under identical circumstances, using the same 6 m × 9 mm coiled glass column filled with 30/60 mesh material, coated with 30 % SE. 30, at 160° and with 200 ml/min H_2 as carrier gas, the plate numbers shown in Table III were found for the *trans–cis*-decalin mixture.

TABLE III

PLATE NUMBERS FOR A 6 m × 9 mm COILED GLASS COLUMN

Chromosorb W/Gas-Chrom P mesh material coated with 30 % SE. 30. Isotherm at 160° with 200 ml H_2/min.

Decalin sample size (*μl*)	*Plate numbers*	
	Chromosorb W	*Gas-Chrom P*
10 *trans-*	2340	3240
cis-	2850	5080
50 *trans-*	1570	1130
cis-	3260	3770
100 *trans-*	720	540
cis-	2380	2150
200 *trans-*	420	300
cis-	1520	1470

These results show that while Gas-Chrom P is better for analytical work, Chromosorb W is superior for preparative scale gas chromatography. On truly analytical gas-chromatographic columns with openings of 4 and 2 mm we have also found consistently that Gas-Chrom P gives more plates per unit length than the similar Chromosorb W or Anakrom ABS (Analabs Inc.) in 10 mesh cuts.

RESULTS WITH COLUMNS OF INCREASED LENGTH

Plate numbers measured on the *trans*-decalin peak for increasing sample loads of *trans–cis*-decalin at 180° on 1.5 m, 6 m, 12 m and 20 m columns, filled with Chromosorb W 30/60 mesh with 25 % SE. 30 are shown in Fig. 4.

The results summarised in Fig. 4 show that increasing the column length indeed produces a linear increase of the plate number and improves the capacity of the column. The plate numbers are somewhat lower than can be obtained on these columns because of the high working temperature and the corresponding low partition factors.

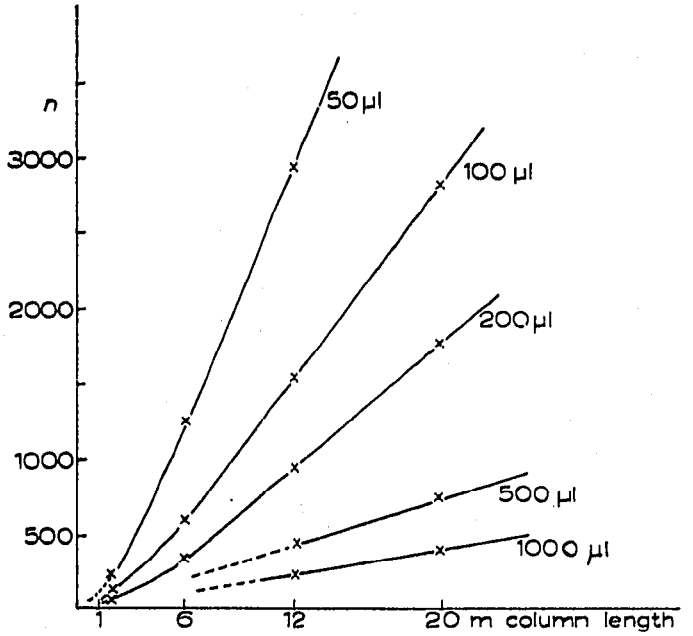
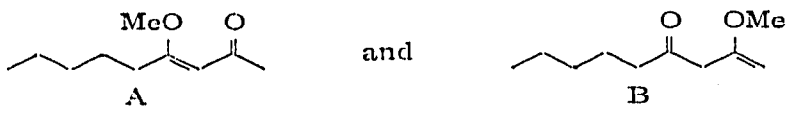


Fig. 4. Plate number, n, for increasing column lengths and sample sizes.

To fit the 12 m and 20 m columns to the Autoprep 700 unit, an insulated "hat" of sufficient size was adapted to the column oven. For the largest column the oven temperature was not very uniform although an additional fan was provided. Comparison with experiments in an oven in which the oven temperature *was* the same at all points showed, however, that this had no measurable effect. Fig. 4 can be used to find the permissible maximum sample size for a given problem and column. The sample sizes of Fig. 4 refer to the decalin mixture which has roughly about 75 % *trans*–25 % *cis*-composition. The plate numbers correspond therefore with only 75 % of the given sample size.

As already inferred, the approximate composition of the sample should be kept in mind; this is shown in the following example.

On methylating 2,4-nonanedione, a 50:50 mixture of the enol ethers A and B is produced:



This mixture has $\alpha = 1.17$ on 35 % butanediol–succinate polyester on Chromosorb W 60–80 mesh at 138°. About 760 plates are necessary according to eqn. (1) and Fig. 4 shows that this corresponds to a 100 µl sample on a 6 m column. The results of these experiments are found in Fig. 5.

Fig. 5 gives about 1400 plates for the two substances; this, as can be inferred from the chromatogram, shows that the sample size could have been doubled. The 100 μl of 50:50 sample contains indeed only 50 μl of each component. It is thus found that the preparative scale plate number is about the same for polyester and SE. 30. The α difference in this case between the two liquid phases just mentioned is, however,

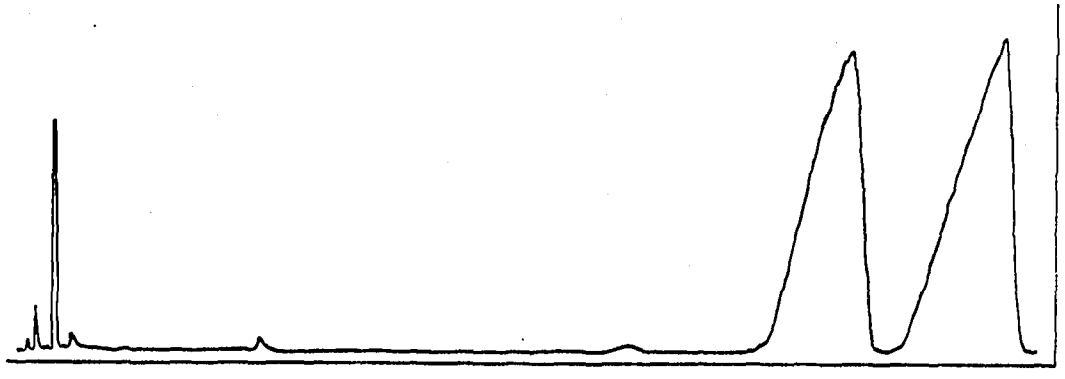


Fig. 5. Separation of isomeric enol ether ketones.

considerable. The separation of Fig. 5 is impossible on a metal column with a pink support (firebrick — see Table II).

DEVIATIONS FROM NORMAL CHROMATOGRAPHIC BEHAVIOUR DUE TO INCREASED SAMPLE SIZE

The approach described in the preceding paragraph works well as long as the sample size remains relatively small. With larger samples, deviations from normal chromatographic behaviour become more and more pronounced. There are typical changes in retention times and in peak asymmetry.

It is well known that the retention time increases with sample size and this aspect is, for example, briefly mentioned by DAL NOGARE AND JUVET[3]. This increase is attributed to peak width increase only in the direction of increasing carrier volume and the peak front is said to appear at the same retention volume regardless of sample size.

This is only exceptionally the case. In practice the first peak front of mixtures shows an increased or reduced retention time according to circumstances. For the second peak of mixtures, large increases in the peak front position are often found. This is exemplified in the results of Table IV obtained for decalin on a 12 m $\times$ 9 mm coiled glass column.

Decreasing values of peak front retention volume as a function of sample load are thus found when the gas rate is low, for substances with low partition factors, and/or with coarse supports. Increasing the gas rate considerably, without changing other conditions, again produces increasing peak front retention volumes.

An intermediate value of the gas rate can be found at which this peak front retention distance remains constant. This is shown in the results of Table V obtained on a 6 m $\times$ 9 mm coiled glass column filled with Chromosorb W 30/60 mesh, isooctane as sample, at 110° and with hydrogen as carrier gas (Autoprep 700 instrument).

Constant peak front retention times are found at a gas rate of 200 ml/min. Below this value there is a decrease, above this gas rate there is an increase.

TABLE IV

DECALIN ON 12 m × 9 mm COLUMN

Chromosorb W 30/60 mesh coated with 30% SE. 30. Isotherm at 180° with 200 ml H_2/min. Modified Autoprep 700 instrument.

Decalin sample size (μl)	*Peak front in mm on chart*	
	First peak, trans-decalin	*Second peak, cis-decalin*
10	92	111
50	95	115
100	96	117
200	97	120
500	98	127
1000	99	141

The decreasing values can be explained as the result of a displacement effect by the high maximum peak concentration. The tailing asymmetry of the peaks indicates a type I isotherm. With lower gas rates and with increased sample size the influence of exponential sample introduction is also stronger and can also lead to this decrease.

We suggest that the increase in the peak front distance for larger gas rates and on longer columns can be explained by the increase in the resistance to gas flow with larger samples. This is reflected, even when the flow rate change (because of the sample introduction) measured at outlet pressure is negligible, in a larger p_1/p_0 ratio with resulting longer retention times. Large samples have, however, an influence on

TABLE V

DEPENDENCE OF PEAK FRONT RETENTION ON GAS RATE FOR A 6 m × 9 mm COLUMN FILLED WITH CHROMOSORB W 30/60 COATED WITH 30% SE. 30

Iso-octane sample size (μl)	*Peak front retention in mm for different H_2 rates*				
	50 ml/min	*100 ml/min*	*200 ml/min*	*400 ml/min*	*500 ml/min*
10	132	68	47	31	28
50	130	69	47	31	29
100	126	68	47	31	29
200	115	64	47	32	30
500	109	64	47	32	30
1000	105	63	46	33	31
2000	102	62	46	35	32

the gas rate measured at the outlet. For the isothermal experiment of Fig. 8, for example, the gas rate goes through a minimum which is 10% below the initial 200 ml/min and then increases again, even before elution of the mixture has started. For smaller samples the effect is less pronounced. This is probably the reason for the

increase in the peak front distance of the first peak of Table IV and also partly explains the displacement of the second peak. Additional confirmation is given by the fact that shorter columns show no increase of the peak front retention time with sample increase. The effect is much less pronounced with nitrogen as carrier gas. This could be expected, since the difference in resistance to gas flow between sample and carrier gas is smaller for nitrogen than for hydrogen.

The main factor determining the greater retention time increase of the front of the second peak of Table IV must, however, be due to something else. It is possible that with the larger sample sizes the substances no longer partition individually but that there is some other interaction than displacement. Temperature effects due to evaporation and condensation could also play a part here. At the front of the peak, condensation will cause a temperature increase while the rear boundary will have a lower temperature because of evaporation. This will retard second peaks and is related to the heat conductivity of the column material. In our glass columns the effect should be greater than in metal columns and the first experiments in this direction show that it is so. The same temperature effects could partly explain why our peaks show such a pronounced leading asymmetry. These explanations are rather unsatisfactory, but we can advance no other and the effect is important, since, as will be explained further, it increases the capacity of preparative scale gas–liquid chromatography columns. Even in conditions in which the first peak front is not markedly displaced (coarse supports, low liquid loading, relative low partition factors) 10 to 35 % increases for second peak front retention are found for larger samples. It occurs also with simple aromatic hydrocarbons on SE. 30 and with decalin on Carbowax 20M. We intend to investigate whether this effect is general.

The peak asymmetry changes mentioned above must be related to the peak front retention time changes. Peak asymmetry is generally discussed in terms of leading (—) or tailing (+). Tailing is the result of exponential sample introduction (large samples) with substances having a small partition factor. In preparative gas–liquid chromatography, leading is, however, more frequent and is normally encountered when dealing with higher concentrations and higher partition factors. The influence of the activity coefficient, γ, is predominant here and for dissimilar solute–solvent combinations, which is very often the case, γ is large, and leading skewness of the peaks results. This is adequately discussed by DAL NOGARE AND JUVET[3]. With preparative scale samples, peak asymmetry is very pronounced and leading and tailing for close homologues can be found on the same chromatogram. An example is shown in Fig. 6. On calculating the plate number for the different peaks a maximum value is found for the symmetrical peak.

Peak asymmetry can, however, be changed from this "normal" pattern by trivial factors. The effect of pressure has already been briefly mentioned. Indeed, iso-octane for example gives tailing peaks with all except the lowest sample sizes on a 6 m × 9 mm column filled with Chromosorb P 30/60 mesh, 30 % SE. 30 and at 110°. When a restrictor is placed on the column the peaks become leading at all except again the lowest sample sizes.

The gas rate, as could be expected, has also an influence on peak asymmetry. On a 6 m × 9 mm Chromosorb W 30/60 mesh column with 30 % SE. 30 at 110° and with iso-octane as sample, tailing is observed for all sample sizes from 10 μl onwards with a gas rate of 50 ml H_2/min. Under the same conditions, except that a gas rate

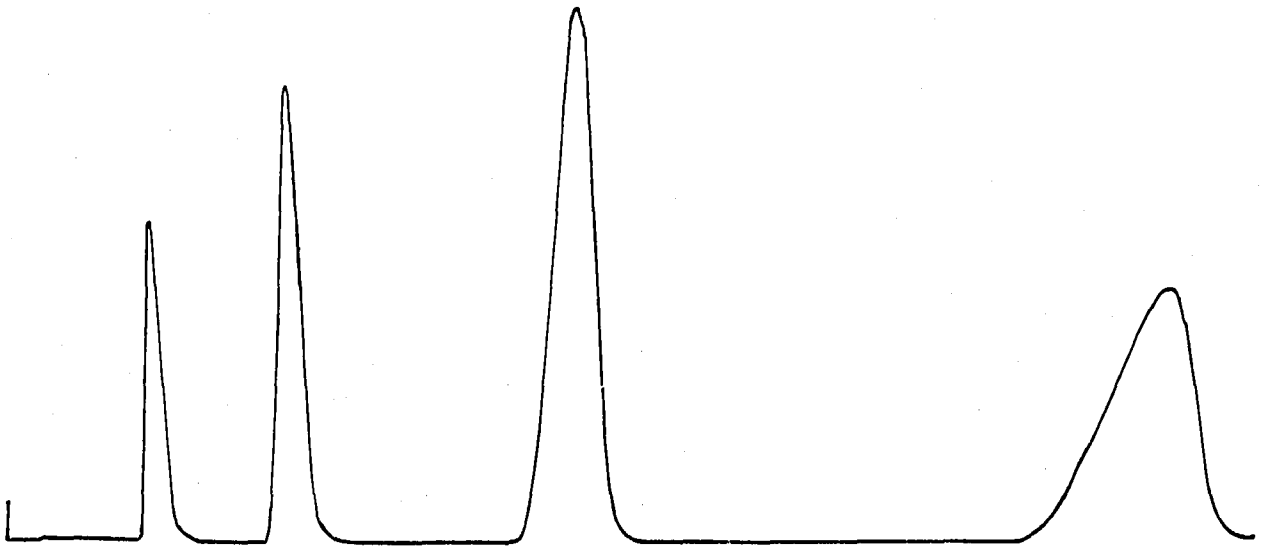


Fig. 6. 400 μl of the straight-chain hydrocarbons in C_6, C_7, C_8 and C_9 on a 12 m × 9 mm coiled glass column filled with sterchamol 0.5–1 mm grain size coated with 25 % octylphthalate. 200 ml H_2/min at 120°. Note the transition of tailing to leading.

of 500 ml H_2/min is used, the peaks show leading, especially for large sample sizes, while symmetrical peaks are obtained with intermediate gas rates of 200–300 ml H_2/min. Using nitrogen as carrier gas, leading is observed even at 50 ml/min for the larger samples, while helium has an effect intermediate between those of hydrogen and nitrogen.

These results could be expected from exponential sample introduction and concentration effects (γ) but, as far as we know, have not been reported before.

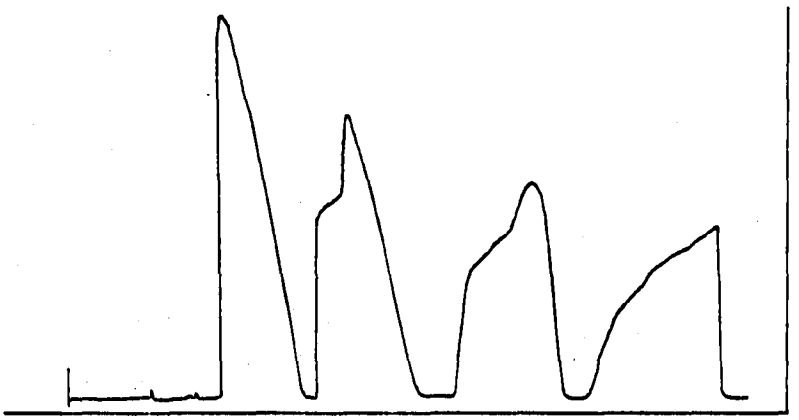


Fig. 7. 4 ml of benzene, toluene, ethylbenzene and cumene on 20 m × 9 mm coiled glass column filled with Chromosorb W 30/60 coated with 25% SE. 30. 200 ml H_2/min at 140°. Note high capacity which could still be increased. For peak shape irregularities, see text.

Yet another change in peak shape is encountered with large samples and at relatively low column temperatures. An indentation appears in the leading part of the curve. This is shown in the separation of Fig. 7 and is most evident here in the middle peaks. The same phenomenon is also seen to a lesser extent in the graph of Fig. 10. We do not know the reason for this.

TEMPERATURE GRADIENTS

The temperature gradients to be considered are of the chromathermography type where a temperature gradient is applied along the column, and of the so-called "temperature-programmed" type where the temperature is gradually and generally linearly increased in the oven as a whole.

Temperature programmation in preparative scale gas chromatography chiefly results in a narrowing of the peaks with corresponding higher concentration of solute in the outflowing carrier gas. This has a beneficial effect on the recovery of substances as we have pointed out before[1,4].

Chromathermography has also a peak-narrowing effect.

Application of the two gradients together is easily achieved experimentally. A 20 m × 9 mm coiled glass column is installed in a long narrow oven (1 m × 20 cm). The heater and a blower are placed at one end of the oven. Fresh air is introduced at the same end and is blown out through a small opening in the oven at the other end. Variable chromathermographic gradients are easily obtained in this way. Temperature programmation, but not uniform over the column length, is also easy. Isothermal operation is also possible with a relatively large fresh air flow. Results obtained with such an instrumental set up are shown in Fig. 8.

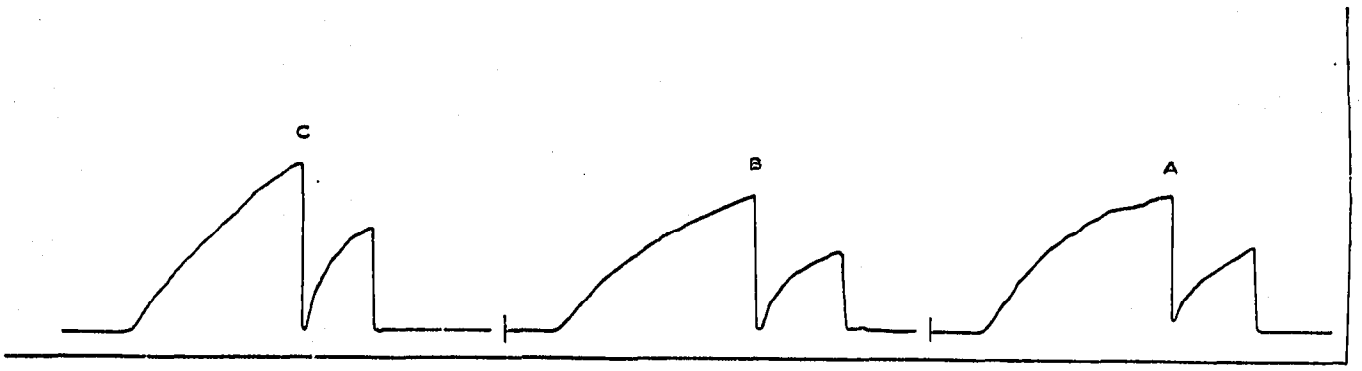


Fig. 8. Separation of 2 ml decalin samples on 20 m × 9 mm glass column (see text). Only part of each chromatogram is shown. The separation time was 80 min.

The separations of Fig. 8 were obtained on a 20 m × 9 mm coiled glass column filled with Chromosorb W 30/60 mesh coated with 25 % SE. 30. The sample size was 2 ml decalin and the carrier gas was hydrogen. The oven was as described above and was fitted with an injection port and a detector oven which was connected to an Autoprep 700 instrument.

Graph A is obtained with isothermal operation at 180° and with a carrier gas rate of 200 ml/min. The separation is not nearly complete. Graph B is obtained with a chromathermographic gradient from 185 to 145° and with a carrier gas rate of 200 ml/min. The separation is complete. The band width is only slightly larger, although the elution temperature is much lower than for graph A. Graph C then is obtained with programmation starting from 50°. At the end of the separation there is a chromathermographic gradient along the column from 205° to 158° (programmed chromathermography). The separation is complete and the peaks are obviously more narrow.

The three experiments were carried out in such a way as to obtain approximately equal retention times. The temperatures in the oven were measured with six permanently installed thermocouples.

For graph C the gas rate was 400 ml/min at the start and had fallen to 200 ml/min at the end of the experiment. Increased resistance to gas flow with a rise in temperature is a well known phenomenon. This is a desirable side effect, giving high speed for the initial separation and the necessary slow gas rate for recovery of the substances[2]. This change in gas rate does not adversely affect the separation. Whereas the plate number is fairly strongly dependent on the gas rate for small samples, this is not the case for large sample sizes. (*cf.* Fig. 9 for hydrogen. Similar results were obtained for helium and for nitrogen.)

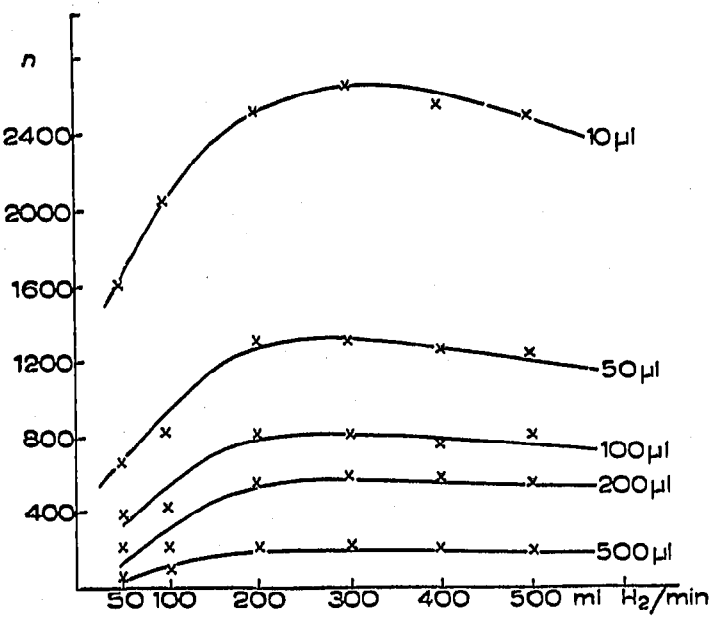


Fig. 9. Plate number, *n*, *vs.* gas rate and sample size showing that gas rate is less critical for preparative scale samples.

According to Fig. 4, the 20 m column should barely be capable of accepting a sample load of 1 ml decalin mixture. Fig. 8, however, obtained with the same column, shows that 2 ml can be separated on it. This is ascribed to the use of temperature gradients and to the special front peak retention increase of the second peak, already mentioned in this paper, which also occurs with the temperature gradients. In the specific case of the conditions of Fig. 8, B and C, the peak front retention of the first peak was independent of sample size but the peak front retention of the second peak showed a 10 to 20 % increase.

GRAIN SIZE OF THE SUPPORT

An objection to the use of longer columns is the increased resistance to gas flow and the larger pressure drop. We have therefore investigated the effect of a coarse support material. White diatomaceous supports of mesh size below 30/60 are commercially unavailable. We have, however, made our own supports as described

under Influence of the stationary support. Additional alkaline washings made it sufficiently inactive for use. Its mesh size was 15/20. Sterchamol of mesh sizes 10/15 and 15/30 was also tried. With analytical size samples the plate number is, as expected, very much higher for small grain size supports. The preparative scale plate number is, however, practically the same for all the supports and is therefore independent of grain size. The drop in plate number with increasing sample size is indeed not so pronounced for the coarse grain supports. This can be seen with the column given in Table I (25 % coating), which was obtained with the laboratory-made support of mesh size 15/20. The experimental results for separations on coarse support columns confirm the above statements. Decalin mixture samples (1 ml) are completely separated in 40 min at 165° on a 20 m × 9 mm column filled with a support of mesh size 15/20 and coated with either 10 or 25 % SE. 30. Samples (2 ml) in isothermal operations give only slightly inferior results to graph A of Fig. 8. We have not yet tried programmed chromathermography with a column of this type, but intend to do so. A major advantage of the coarse support column is the speed due to the negligible pressure drop and the possibility of reducing the working temperature. This can be deduced by comparing the figures just mentioned with those of Fig. 8. Support material of mesh sizes below 30 should also make it possible to use even longer small-bore columns. The pressure drop would be negligible and the separation power of the column for preparative scale samples would be high. The use of nitrogen as carrier gas should also be more easily possible because of the slight pressure drop. As we have shown before, the preparative scale plate number is independent of the nature of the carrier gas[2].

We are building a 75 m column preparative scale gas chromatograph.

SPEED AND LONGER COLUMNS

Instead of using longer columns in order to increase sample size, they can also be used with advantage to shorten the separation time and to increase recovery percentages. This may seem paradoxical but is exemplified by the following facts, illustrated by Fig. 10. A 0.5 ml decalin sample can just be separated on a 6 m × 9 mm

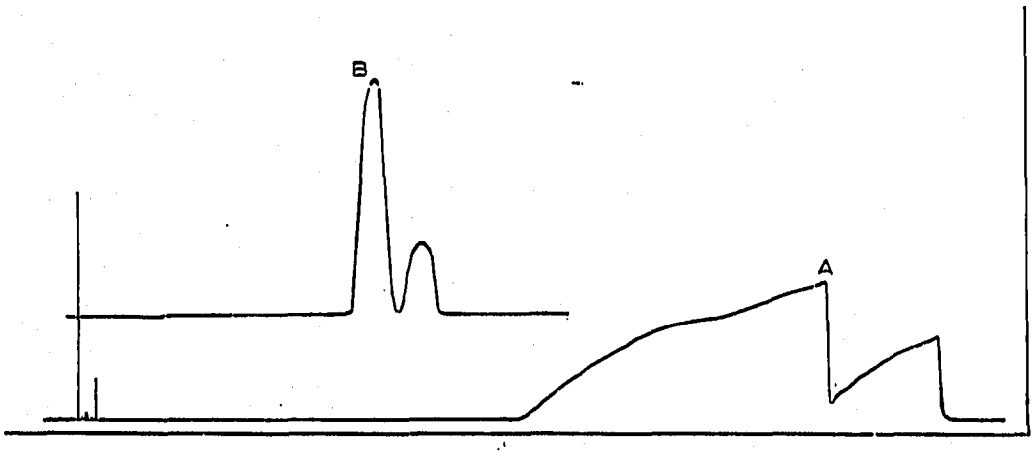


Fig. 10. Separation of 0.5 ml decalin mixture; same time scale and gas rate; (A) at 120° on a 6 m × 9 mm column; (B) at 220° on a 20 m × 9 mm column. The long column is more than twice as fast as the shorter column.

column filled with Chromosorb W 30/60 mesh coated with 30 % SE. 30. The maximum separation power of the column must be applied by using sufficiently low temperatures to ensure long developing times. In this case the separation is achieved at 120° and lasts over an hour. On the 20 m × 9 mm column used for the experiments of Fig. 8 a sample of 0.5 ml decalin is very easily separated and even when the temperature is increased to 220°, separation is still complete. The analysis time, using the same gas flow rate in the two comparative experiments, has, however, been reduced for the longer column to 25 min.

The concentration of the substances in the outflowing gas mixture is much greater for the longer column so that recovery problems should also be much simplified by working in this way. A point which was mentioned in our study of recovery in gas chromatography[2] but which was perhaps not sufficiently stressed is that the collection bottles must be filled as completely as possible with glass wool. The first drops of recovered substance form a liquid layer on the glass wool and this acts as a stripping gas-chromatographic column.

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SUMMARY

Methods of improving preparative scale gas chromatography were investigated. The best results as regards separation power, sample size, recovery, economy of operation etc. should be obtainable on long narrow-bore columns filled with a loosely packed very coarse support, coated with 10 % liquid phase and with "programmed chromathermography".

Attention is drawn to the necessity of using glass columns and inert supports. It should be possible to replace a large number of columns with different liquid phases by a restricted number of columns of much increased length with general purpose liquid phases *e.g.* SE. 30, or Carbowax. The influence of preparative scale sample size on peak shape and retention is discussed. The most remarkable feature here is the big increase in peak front retention with increased sample size for the second peaks of decalin mixtures. There are indications that this could be a general effect. For not unduly large samples (shorter columns) and not unduly small α values, the maximum allowable sample load can be derived fairly accurately from the "preparative scale plate number". Where the conditions are different, the maximum sample load is best found experimentally and will be greater than could be expected from the preparative scale plate number. This is due to the retention effect on the second peaks.

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