Preparative scale gas chromatography

VIII. Further experiments

In previous papers in this series[1–4] it was argued that a desirable sample size for organic laboratory scale preparative gas chromatography is between 0.1 and 10 ml. The columns to do this should possess separating power for these samples of 200–2000 theoretical plates (the N' value) and this can most easily be achieved with a rather long and thin column design (10 to 30 m long $\times$ 1–3 cm^2 section surface). However, it was found that, in practice, both small and large volume columns are desirable. A suitable choice for example for the larger volumes is around 7 l in a 20 m $\times$ 2 cm^2 column; for smaller volumes, around 1.5 l in a 10–20 m $\times$ 0.5–1 cm^2 column. The larger volume columns are best suited for rather easy separations which are carried out with sample sizes in the ml range (examples in Table I), while the smaller volume columns are indicated for complex mixtures (see Fig. 2) or when only a small volume of the sample is available.

In every separation, be it of one or two components from impurities or a multicomponent mixture, there is a pair of peaks that are closest together or with a minimum α value (ratio of corrected retention times). On a given column the sample size that can just be separated (Maximum Allowable Sample Size or MASS)[4] is then determined by the lowest α value of the mixture.

This α value must be deduced from an analytical gas chromatogram. With this α value the number of theoretical plates (N) necessary for the separation can be calculated by means of eqn. (1):

$$N = 16 \left\{\frac{\alpha}{\alpha - 1}\right\}^2 \qquad (1)$$

From the analytical run the percentage (x) of the largest component of the two, used to determine α, can also be deduced. Thus:

$$\text{MASS} = 100 \cdot SS(N')/x \qquad (2)$$

where $SS(N')$ is the sample size of a simple substance giving the calculated N value on the preparative scale column to be used (see for example such values in Table II).

Table I shows a series of separations illustrating the influence of α and gives the general conditions for larger volume columns.

The *cis* and *trans meta*-dioxanes in this series were needed for P.M.R. studies[5].

Use of glass columns and glass bead supports

Some separations were unsuccessful on the columns of Table I, because of decomposition of the substances. This decomposition can be caused by the support material, by the stationary phase[4] or by the column material (certain halogenated compounds in aluminum columns). An improvement in this situation is obtained by using glass beads, glass wool or quartz wool as support material and this change allows the chromatography of some delicate mixtures which are otherwise decomposed.

In gas chromatography the amount of stationary phase on the support is usually

TABLE I

PREPARATIVE SCALE SEPARATIONS OF *meta*-DIOXANES SHOWING THE INFLUENCE OF α ON MASS

The second column had indeed an extreme ovalisation of 1.5 × 4.0 cm, but this proved not superior over the more normal ovalisation ratio.

No.	*Mixture*	α (*min*)	*MASS* (*ml*)	*Total amount separated* (*ml*)	*Aluminum column dimensions*	*t* (°C)	*Support mesh 20–30*	*Stationary phase* (*g*/*ml*)	*Gas flow rate* H_2
	meta-dioxanes *cis*/*trans*				21 m × 1.5–2.5 cm		Chromosorb A	Carbowax 20 M 5/100	
1	*meta*-Dioxane/benzene	—	10	150		100			700
2	4-Methyl-6-isopropyl	1.36	8	25		140			600
3	4,6-Di-*n*-propyl	1.25	6	30		140			600
4	4-Methyl-6-*tert.*-butyl	1.30	6	20		140			600
5	4,6-Dimethyl	1.35	8	8	15 m × 1.5–4.0 cm	100	Chromosorb G	SE. 30 5/100	1000
6	4,5-Tetramethylene	2	2	15		100			600
	+ 1,3-diolacetate	1.32							
	+ 2 other peaks	1.22							
7	4,6-Di-isobutyl	1.1	0.2	0.2		170			700
8	4,5-Pentamethylene	1.11	0.5	8	21 m × 1.5–2.5 cm	170	Chromosorb G	Carbowax 20 M 5/100	600
9	4,6-Diisopropyl	1.25	4	28		150			700
10	4-Methyl-6-*n*-butyl	1.3	5.5	12		150			700
11	4,6-Diethyl	1.3	2.5	12		170			600
12	4-Methyl-6-isobutyl	1.31	6	15		170			600
13	4-Methyl-6-ethyl	1.38	10.5	10.5		130			600
14	4-Methyl-6-*n*-propyl	1.36	17.5	21		135			700
15	4-Methyl-6-*sec.*-butyl	1.36	5	42		165			700
16	4,6-Di-*n*-butyl	1.14	2	18.5		180			700
17	4,6-Di-*sec.*-butyl	1.12	0.5	19		180			700
18	5-Ethyl-5,5-diethyl	2.3	3.8	3.8		150			700

expressed on a percentage weight/weight basis. On glass beads this percentage is normally below 1 % and this might be thought to be much too low to permit preparative scale separations. The density of glass beads is however high (~ 2.0) compared to, for example, Chromosorb W (0.22). This means that comparable column lengths, one filled with glass beads with 1 % stationary phase and another with Chromosorb and 10 % stationary phase, will contain about the same amount of stationary phase. For this reason it is preferable to express the amount of coating on a weight/volume basis. For example 2 g stationary phase/100 ml Chromosorb W is about 10 % w/w while 2 g stationary phase/100 ml glass beads is only about 1 % w/w. Glass beads when coated with this last amount of an oily stationary phase are rather sticky and very difficult to fill into a column (although certainly not impossible). Stationary phases which are solid at room temperature present no great difficulties and Carbowax 20 M on glass beads gives thus a satisfactory column filling.

Different glass bead sizes were tested. The finer the beads the greater the separation power of the column, but also the greater the pressure drop. A satisfactory compromise turned out to be glass beads with a diameter of 0.8 mm (Ballotini shot lead glass beads No. 11, obtainable from Jencons, Hemel Hempstead, England).

Similar experiments were carried out with glass wool. This was cut in a mixer until the longest strands were about 4–5 mm. The resulting material was purified by frequent decantation and by washing with strong acid, soap, water and chloroform. Coating with stationary phase was carried out as usual. The filling of the columns with this coated glass wool is more difficult than with coated glass beads. The separation power of the glass wool columns is better than of those filled with glass beads, but the pressure drop is excessive. Therefore it was decided to use, exclusively, glass beads of 0.8 mm diameter.

A 20 × 2 cm coiled glass column was filled with about 6 l of such glass beads coated with 2 g/100 ml Carbowax 20 M. The glass column was made from 1.5 m glass tubes with a 2.0 cm opening as described in more detail before[2] for columns of 75 × 9 mm. With these large diameter glass tubes the coiled column has an ovalised section, but this is a favourable factor, as has been shown before[4].

To facilitate connection to and removal of this column from the gas inlet and

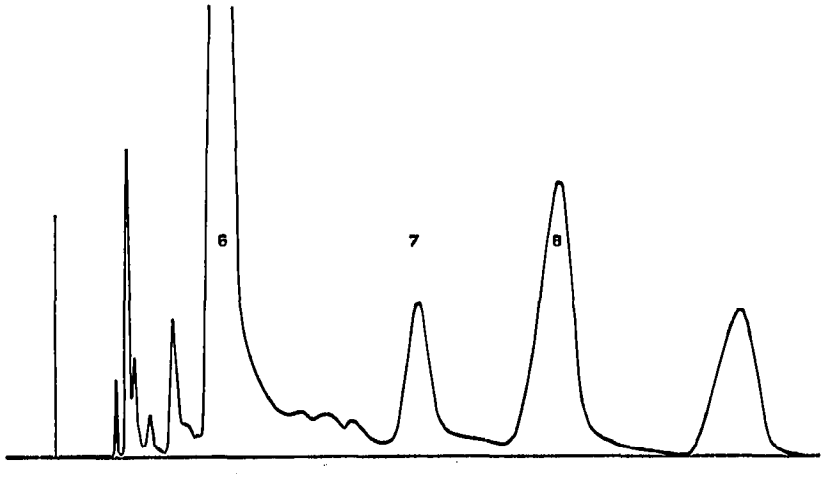

Fig. 1. 2 ml reaction mixture, isothermal at 150°. 600 ml H_2/min. Coiled glass column 20 m × 1.2–2.4 cm filled with 0.8 mm glass beads coated with 2 g Carbowax 20 M/100 ml support.

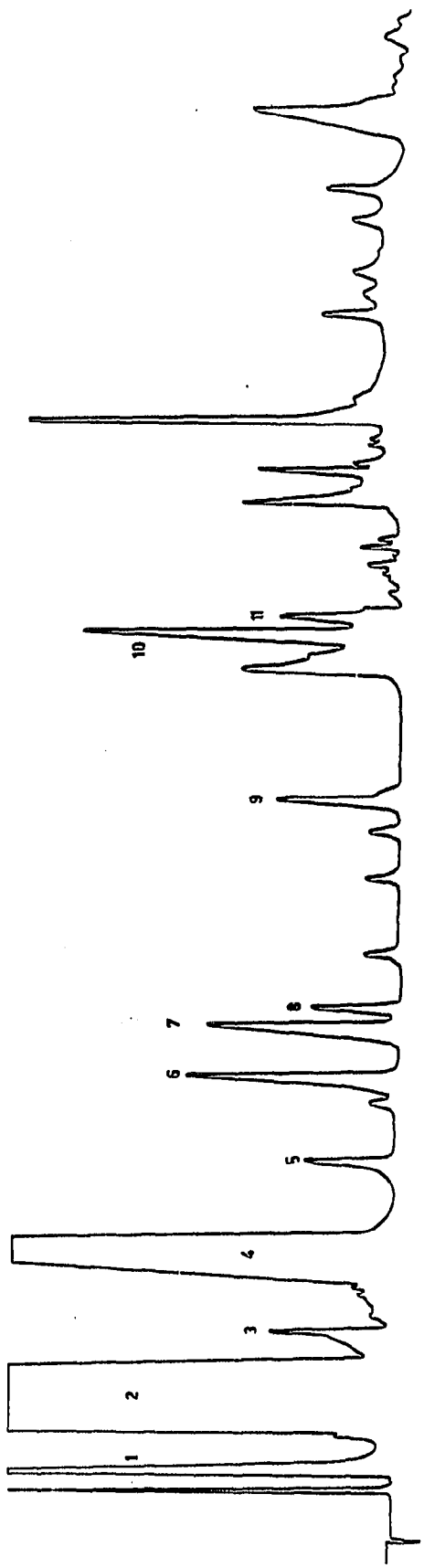

Fig. 2. 4 ml of volatile hops aroma components on 20 m × 9 mm coiled glass column (Chromosorb W, 30–60 mesh, coated with 5 g/100 ml of polytetrahydrofuran). 250 ml H_2/min. Programmed at 1°/min from 50° to 200°, isothermal afterwards. Modified Autoprep 700. Peak 2 is ether solvent, peak 10 is myrcene. Most other peaks were collected in a sufficient amount to be identified by normal NMR and other spectrometric techniques.

outlet, a 30 cm piece of stainless steel capillary with 1.5 mm opening was fixed to its ends. The permeability of this column was good, giving 500 ml H_2/min with 1250 mm mercury inlet pressure at temperatures up to 200°. The column was mounted in the oven described in ref. 2 and connected to an Autoprep 700 (Varian Aerograph— formerly Wilkens Instruments). The resolving power of this column for small samples is much below that obtainable with conventional supports. For large samples it compares well however with the earlier columns developed in this laboratory for large scale preparative scale gas chromatography (see Table II).

An example of a separation on the glass bead column is shown in Fig. 1. The mixture was obtained by alkaline treatment of dichloromethyl isobutyl ketone. Only peaks 6, 7 and 8 were identified, being $(CH_3)_2CH\text{-}CH{=}CHCOOMe$, $(CH_3)_2CHCH_2\text{-}COCHCl_2$ and $(CH_3)_2CHCH(CH_2Cl)COOMe$ respectively. Similar mixtures obtained from other dichloromethyl alkyl ketones were also separated on this column. Purification of *n*-butyl iodide and *n*-butyl bromide by preparative scale gas chromatography was also only possible on glass beads as support material. A series of deuterated *n*-butyl halides was thus isolated from the reaction mixtures, using a 1.5 m × 9 mm column and SE.30 as stationary phase.

TABLE II

SEPARATION POWER EXPRESSED AS RELATIVE BAND WIDTH (N')

All results were obtained with cumene as sample at 145° ($k \sim 5$) and with 500–700 ml H_2/min. Column 1: 0.8 mm glass beads coated with 2 g Carbowax 20 M/100 ml support in 20 m × 1.2–2.4 cm coiled glass column; volume 6.2 l. Column 2: 15 m × 1.5–2.5 cm, filled with Chromosorb A 20–30 mesh coated with 5 g SE. 30/100 ml support; volume 5.1 l. Column 3: 21 m × 1.5–2.5 cm filled with Chromosorb A 20–30 mesh coated with 5 g Carbowax 20 M/100 ml support; volume 7.7 l.

Sample size	*Glass beads*	*Chromosorb A*	
	Column 1	*Column 2*	*Column 3*
100 µl	508	2626	3075
200 µl	542	1913	3301
500 µl	541	1803	2584
1 ml	456	1093	1731
2 ml	314	673	976
5 ml	174	270	373
10 ml	99	110	154

Complex mixtures

For mixtures containing a large number of components (10 or more) the columns listed in Table I are less useful. In this case α values below 1.1 are unavoidable and the column will need an exceptionally high separating power. In this case the boiling range will also be rather large and programming is indicated. A characteristic of preparative scale separations is that they take longer than analytical separations. Therefore the programming rate must be slower and will normally be in the range of 0.5 to 2°/min. Suitable columns for this sort of problem have the following characteristics: 20 m × 9 mm filled with Chromosorb W, 30–60 mesh and coated with 5 g stationary phase/100 ml. Such a column with Apiezon L as stationary phase produced 20,000 plates with 10 µl samples. An example of such a separation is shown in Fig. 2. Polytetrahydrofuran is used because it is a "universal" phase, giving equally good results for substances having very different polarity.

Technical remarks

On injection of a large volume of sample, volatilisation occurs suddenly, creating a large pressure increase. Back flash is normally avoided by installing an automatic back flash valve. However in our experience even the best back flash valves tend to become dirty and thus fail after some time. Therefore, columns under the back flash valves are better replaced by a needle valve. This is closed just before injection and reopened about 1 min later. The effect of this operation can hardly even be seen on the chromatograms obtained with the long and large volume columns under discussion.

Another point is that stable stationary phases can be prematurely destroyed by some mixtures. Versamid, normally used without trouble up to 300°, is such an example. After a series of high boiling amides had been separated successfully on a versamid column (21 m × 1.5–2.5 cm; Chromosorb A, 20–30 mesh), the column was put aside. A few months later, when it was needed again, excessive bleeding occurred even at 200–250° showing that something was wrong. The bleeding did not stop and the column was useless for further work.

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1 M. VERZELE, J. BOUCHE, A. DE BRUYNE AND M. VERSTAPPE, *J. Chromatog.*, 18 (1965) 253.
2 M. VERZELE AND M. VERSTAPPE, *J. Chromatog.*, 19 (1965) 504.
3 M. VERZELE, *J. Gas Chromatog.*, 3 (1965) 186.
4 M. VERZELE, *J. Gas Chromatog.*, 4 (1966) 180.
5 M. ANTEUNIS, D. TAVERNIER AND F. BORREMANS, *Bull. Soc. Chim. Belges*, 75 (1966) 396, and unpublished results.

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