Journal of Chromatography, 94 (1974) 53–64

CHROM. 7477

ISOTHERMAL ANALYSIS ON CAPILLARY COLUMNS WITHOUT STREAM SPLITTING

THE ROLE OF THE SOLVENT

K. GROB and K. GROB, JR.
GC-Laboratory, ETH Zürich, EAWAG, 8600 Dubendorf (Switzerland)
(Received March 8th, 1974)

SUMMARY

Injection on capillary columns without stream splitting has generally been understood to be a special application of cold trapping. As far as liquid samples are concerned, this is not so, as the method also works isothermally, *i.e.*, with injection and elution at the same temperature. In the first phase of the analysis, the large amount of solvent prevents migration of sample components, thus concentrating their vapour plugs to 1 % or less of their original length. The solvent should therefore be considered as an important parameter in the analysis, instead of being eliminated by means of complex additional equipment. In order to exert its full effect, the solvent has to be sufficiently retained by the column. The dependence of the effect on solvent volatility, column temperature, polarity and some instrumental conditions is discussed.

INTRODUCTION

Injection without stream splitting (direct injection) has been explained in detail in earlier papers[1–3]. The essential steps of the procedure are injection of several microlitres of dilute solution while the stream splitting system is closed, and re-establishing a split stream for thorough purging of the vapourizer chamber, the septum and the fittings when the vapourized sample has been transferred completely on to the column.

According to the basic theory, this procedure is supposed to give very poor results, as a simple example may show.

Littlewood[4] calculated that the largest tolerable volume ($V_{max.}$) of a vapourized sample that can be injected without detectable band broadening is $V_{max.} = V_R/2\sqrt{n}$ (V_R = retention volume of a given substance at the column temperature during injection; n = number of theoretical plates). For *n*-heptane, run on a 70 m × 0.32 mm Ucon LB 550 capillary column, yielding 250,000 theoretical plates at a hydrogen flow-rate of 4 ml/min at 25°, V_R is *ca.* 10 ml and hence $V_{max.}$ is 0.01 ml. A 2-μl sample of *n*-pentane, however, produces a vapour volume of 0.5 ml at the column exit. Dilution with carrier gas will increase this volume to *ca.* 1 ml, which means that the vapour plug of *n*-heptane (dissolved in *n*-pentane) becomes 100 times longer than is theoretically

allowed. Under more extreme conditions, the sample plug may become much longer than the capillary column itself.

Most probably because of the obvious discrepancies between the experimental results and theoretical interpretation, many workers did not consider our method to be reliable, while others were afraid of damaging their columns by heavy overloading with solvent, which in fact can happen with unsuitable columns. In other laboratories, the probable reason for unsatisfactory results was incorrect design of the injection components. Numerous workers, on the other hand, reported excellent results, *e.g.*, with steroids[5], natural flavours[6], cigarette smoke[7] and environmental samples[8]. In all of these laboratories the injection technique has been used routinely over long periods without adverse effects on the capillary columns.

The specific merits of the method have been discussed previously[1-3] and are not repeated here.

In our first paper we stated that injection without splitting had to be carried out at a column temperature at least 30–50° below the normal analysis temperature for a given sample. This statement still holds for certain solvents and, more important, for gaseous samples. The same statement, on the other hand, gave the impression that the principle of our method was similar to cold trapping, as reported by Rushneck[9], and it is the purpose of the present paper to correct this error.

THE BASIC SOLVENT EFFECT

What happens during and after injection without splitting is best shown by some typical examples (Fig. 1). Mixtures of equal amounts of *n*-hexane, *n*-heptane, *n*-octane and *n*-nonane were separated under identical conditions, except for the following sample conditions. For Fig. 1A, 2.0 μl of a 1:10,000 solution in *n*-hexane was injected, while for Fig. 1B the solvent was isopentane, and Fig. 1C was obtained by injecting 0.5 ml (*i.e.*, the same vapour volume as produced by the evaporation of 2 μl of solution, before mixing with carrier gas) of nitrogen containing approximately the same

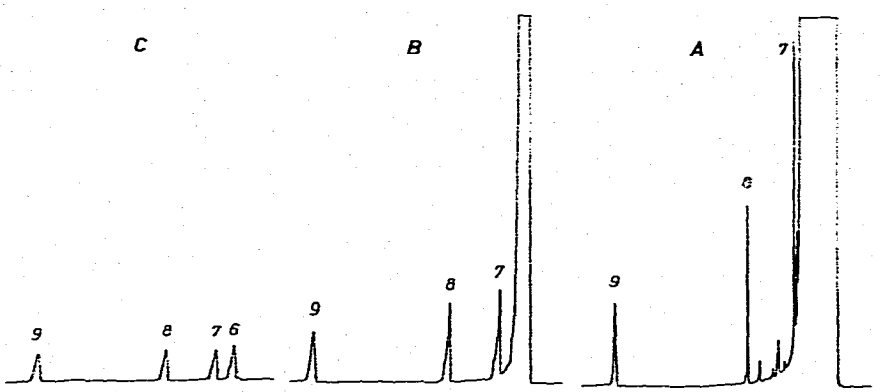

Fig. 1. Influence of solvent on band width of sample components (6 = *n*-hexane; 7 = *n*-heptane; 8 = *n*-octane; 9 = *n*-nonane) after injection without splitting at 25° and isothermal elution at 25°. Glass column, 50 m × 0.26 mm, squalane. Carlo Erba (Milan, Italy), Model GI; FID; carrier gas hydrogen, 2.5 ml/min. Sample for A and B, 2 μl, 1:10,000; sample for C, 0.5 ml of gas mixture. Solvent: A, *n*-hexane; B, isopentane; C, none (gaseous injection).

amounts of alkanes as 2 μl of 1:10,000 solution. Assuming a 1:1 dilution* with carrier gas, we had a total injection volume of 1 ml in all three instances. As the flow-rate of carrier gas (hydrogen) was 4 ml/min, transfer on to the column should be completed within 15 sec. Our actual injection time (time between injection and reopening the splitting system) was 25 sec, which resulted in *ca.* 90% transfer (for more details see the section on Influence of technique and equipment). The column temperature for all injections and separations was constant (25°).

It must be emphasized that the chromatogram in Fig. 1A represents an isothermal run obtained from a large liquid sample, injected without splitting. The separation in the first section of the chromatogram is even better than after injection with splitting. In contrast, the alkane peaks in Fig. 1C were obtained directly with an injection time of 25 sec, *i.e.*, no concentration of the very long vapour plugs occurred. When isopentane is substituted for *n*-hexane, the result is similar to the run without solvent. The reason is obviously that the very volatile isopentane is so weakly retained on the column at 25° that almost no solvent effect occurs.

The theoretical explanation of the large solvent effect, although not for our special application, was given by Harris[10] in terms of Raoult's law and activity coefficients. His statement about the effect of major sample components on closely following minor components can be simply repeated here, replacing Harris' major components by the very large amount of solvent.

The vapourized material is transferred on to the column essentially as a mixture. In the first stage of separation, the solvent shifts away from the sample components, leaving them on the back slope of its large peak. Thus, the moving vapour plugs of the sample components meet a liquid phase mixed with retained solvent, whereby the concentration of solvent increases rapidly in the direction of migration. Therefore, the front of every plug, in contact with stationary liquid containing more solvent, undergoes much stronger retention than the back of the plug. This effect causes the originally very broad bands of sample components to be condensed to a band width, which, under properly selected conditions, may become even smaller than that which can be obtained by injection with stream splitting.

The special nature of the solvent effect is due to the overwhelming amount of solvent present compared with the amounts of the sample components; in the first stage of the analysis, this amount becomes comparable with the amount of stationary phase in the inlet part of the column.

VOLATILITY OF SOLVENTS AND THE SOLVENT EFFECT

It is obvious that the most important parameter governing the efficiency of a solvent in concentrating a large vapour plug of a sample component is volatility, which can be expressed in terms of boiling points. For any given analysis, a solvent with optimum volatility can be found. Too high a volatility will cause broadened peaks, while a solvent with too low a volatility will obscure the earliest peaks without causing further improvement of a separation. In order to show this relationship, we selected five alkane solvents with different boiling points, and five solvents with different structures, as listed in Table I.

Figs. 2 and 3 show chromatograms obtained under identical conditions (con-

* This dilution may easily become 5–10 fold.

TABLE I

SELECTED SOLVENTS WITH ABBREVIATIONS AND BOILING POINTS

Alkane solvents	*Abbrev.*	*B.p. (°C)*	*Other solvents*	*Abbrev.*	*B.p. (°C)*
Isopentane	IP	27	Diethyl ether	Eth	35
n-Pentane	P	36	Methylene chloride	CH_2Cl_2	40
2,2-Dimethylbutane	22D	50	Carbon disulphide	CS_2	46
2,3-Dimethylbutane	23D	58	Ethyl formate	Form	53
n-Hexane	H	69	Acetone	Ac	56

stant column temperature 25°), but with different solvents. In the alkane series a progressively impaired separation from *n*-hexane to isopentane was observed. Concentration from long to short vapour plugs is more difficult for more volatile sample components and therefore the earliest peaks are most affected by small differences in the efficiency of the solvent effect. 2,3-Dimethylbutane, for instance, is significantly less effective in concentrating the *n*-heptane and *n*-octane peaks compared with *n*-hexane as a solvent.

In order to study the solvent effect more quantitatively, we selected the ratio of the peak height to the integrated peak area as a measure of the concentration efficiency of a solvent. We call this ratio the relative peak height. In Fig. 4, relative peak

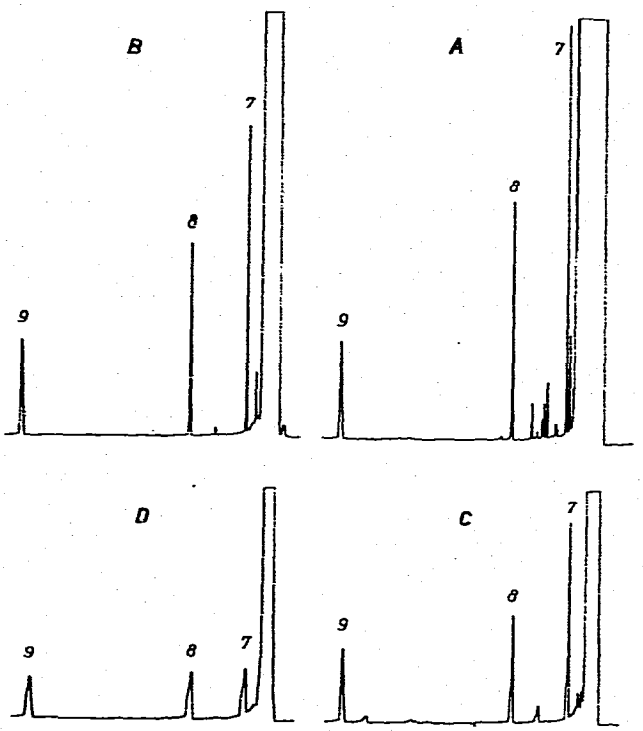

Fig. 2. Isothermal analysis without splitting using various alkane solvents. Glass column, 80 m × 0.32 mm, Ucon LB 550. Carrier gas hydrogen, 6 ml/min. Technique and equipment as in Fig. 1. Solvent: A, *n*-hexane; B, 2,3-dimethylbutane; C, *n*-pentane; D, isopentane.

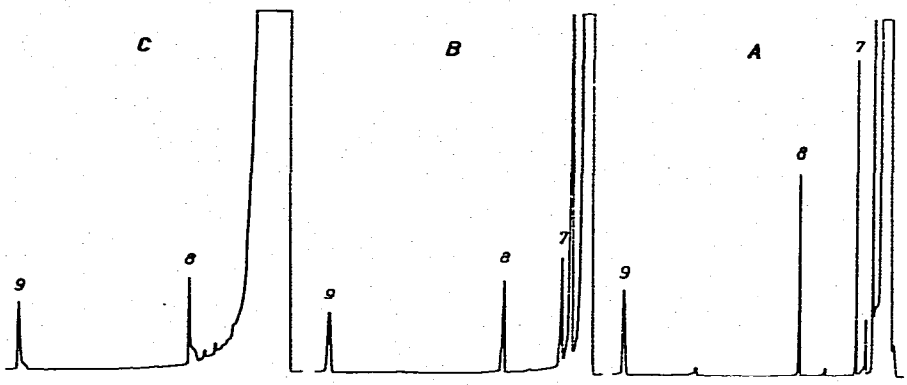

Fig. 3. As Fig. 2 with solvents with different structures. Solvent: A, carbon disulphide; B, diethyl ether; C, acetone.

heights are plotted for different sample components and for the selected solvents. The more effective is a solvent, the more different are the relative peak heights for early and late peaks. The expected relationship becomes even more clear when the relative peak height is plotted against the boiling points of the solvents (Fig. 5).

COLUMN TEMPERATURE AND THE SOLVENT EFFECT

The second parameter governing the solvent effect is column temperature. For any solvent–stationary phase pair, an optimum column temperature should exist, allowing sufficient condensation of solvent vapour in the liquid phase without causing excessive retention of the solvent. This logical deduction from the interpretation of the solvent effect is qualitatively confirmed by the measurements shown in Fig. 4 (unfor-

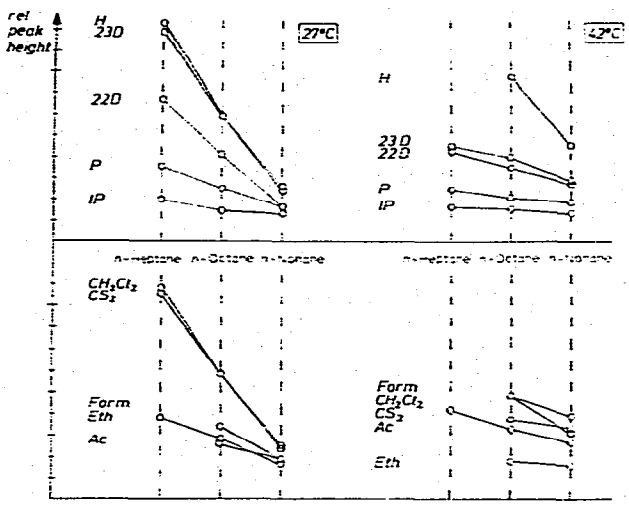

Fig. 4. Relative peak heights (peak height:peak area) for three sample components and ten solvents (for abbreviations, see Table I) at two column temperatures. Sample: 2 μl of 1:10,000 solution. Column and procedure as in Fig. 1.

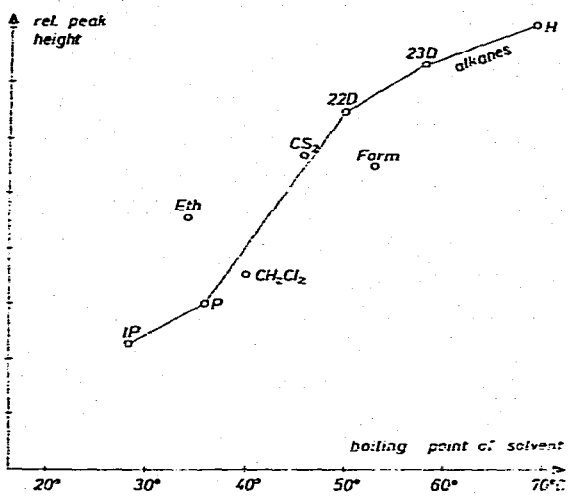

Fig. 5. Relative peak height of the sample component *n*-heptane *versus* boiling point of the solvent Column as in Figs. 2 and 3. Room temperature.

tunately, the relative peak height at 42° for *n*-heptane, when injected in *n*-hexane solution, is missing, as the solvent peak obscures that of *n*-heptane). For every solvent, there seems to be a critical and relatively narrow temperature range above which the solvent rapidly loses its efficiency. The column temperature of 25° is clearly too high for *n*-pentane and isopentane, but is in the critical range for 2,2-dimethylbutane. In contrast, 2,3-dimethylbutane and *n*-hexane both remain outside the critical range and therefore show perfect and almost identical solvent effects. The column temperature of 42°, however, lies above the critical temperature for 2,3-dimethylbutane, but below that for *n*-hexane. This causes the efficiency of the former solvent to fall to the level

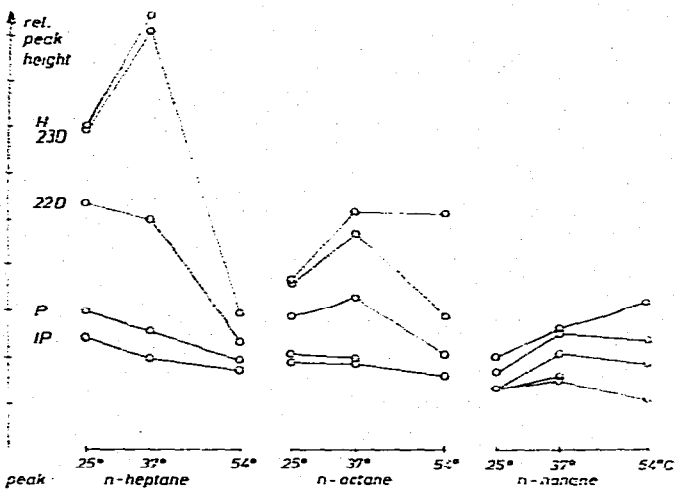

Fig. 6. Influence of column temperature on solvent effect, shown for three sample components and five alkane solvents. Column and procedure as in Fig. 1.

of its 2,2-isomer, while the efficiency of the latter even increases. This increase is not connected with the solvent effect but merely reflects the sharper peak as it is automatically obtained at a higher temperature (shorter retention). A constant relative peak height with increasing column temperature indicates a decreasing solvent effect.

A summarized presentation of the same relationship is given in Fig. 6, which shows relative peak heights for three sample components (*n*-heptane, *n*-octane and *n*-nonane) using five different alkane solvents and three different column temperatures. Without discussing the details, it can be concluded from Fig. 6 that for the routine application of injection without splitting it is worth while optimizing experimentally the solvent and column temperature for a given stationary phase.

ISOTHERMAL ANALYSIS WITH INJECTION WITHOUT SPLITTING AT ELEVATED TEMPERATURE

If our interpretation of the solvent effect is valid, isothermal analysis without splitting must be feasible at any temperature (with identical injection and elution temperatures). The column temperature and solvent have to be selected for a given column type. In order to demonstrate this, we chose an OV-1 column, on which the C_{18}–C_{20} alkanes were suitably eluted at a column temperature of 170°. Fig. 7 shows

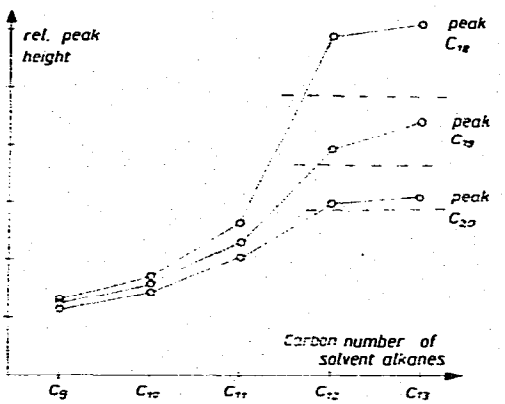

Fig. 7. Solutions of C_{18}–C_{20} alkanes in different alkane solvents (1:50,000), injected without splitting and eluted at 170°. Column, 25 m × 0.30 mm, OV-1.

the dependence of relative peak heights of the three sample alkanes on the solvent (C_9–C_{13} alkanes), and indicates that the solvents *n*-nonane, *n*-decane and *n*-undecane are too volatile at 170° and consequently produce low and broadened peaks (*i.e.*, low relative peak heights) for the sample components. The critical volatility of the solvent at 170° lies between that of *n*-undecane and *n*-dodecane and therefore *n*-dodecane and *n*-tridecane show perfect and very similar solvent effects. The ideal solvent, under these conditions, is *n*-dodecane.

The broken lines in Fig. 7 indicate the level of relative peak height for the corresponding sample alkane obtained by regular injection with stream splitting. The results are lower than those obtained with injection without splitting, provided that

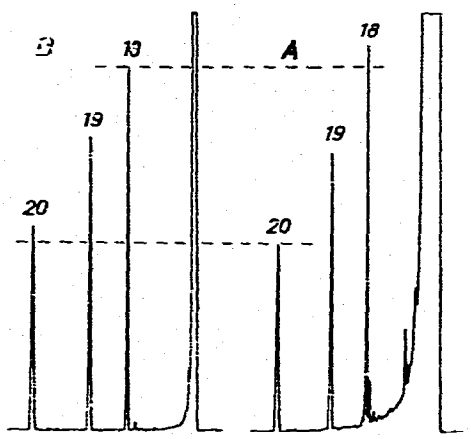

Fig. 8. Comparison between regular injection with stream splitting and injection without splitting. Solvent *n*-dodecane, 170°. Column and conditions as in Fig. 7. A, 2.0 μl of 1:50,000 solution without splitting; B, 0.2 μl of 1:200 solution with splitting.

the solvent gives a perfect solvent effect. For too volatile solvents, of course, injection with splitting is superior.

Fig. 8 shows a direct comparison of both injection methods, using an effective solvent (*n*-dodecane). Comparison of the C_{18} and C_{20} peak heights shows that injection without splitting produces narrower bands for the sample components than does injection with splitting.

POLARITY AND THE SOLVENT EFFECT

The influence of polarity on the solvent effect is complex, as interactions of three variable polarities (of the stationary phase, solvent and sample components) have to be considered. Without going into detail, we mention below some examples showing the importance of polarity.

(1) It is easy to understand that particularly large differences will be observed when the efficiencies of non-polar and polarizable solvents on a strongly polar stationary phase are compared. Fig. 9 shows the effects of the solvents *n*-pentane and carbon disulphide, which differ by only 10° in their boiling points, on Carbowax 600. It is important to note that the sample components are completely non-polar, which makes the concentration of their vapour plugs on a polar phase especially difficult.

(2) As mentioned above, in Fig. 2 the solvent effects of *n*-hexane and 2,3-dimethylbutane differ significantly, while in Fig. 4 (25°) they are almost identical. The reason for this apparent discrepancy is that the former results relate to the moderately polar phase Ucon LB 550, while the latter results were obtained on squalane. As alkanes are less retained on a Ucon column, 2,3-dimethylbutane shows a reduced efficiency at 25°, while on squalane it retains maximum efficiency.

(3) Fig. 5 shows that the relationship between the solvent effect and the boiling point of the solvent is much more complex for polar and polarizable solvents than for non-polar solvents.

(4) The peak distortion in the chromatograms in Fig. 2C and 2D (alkane

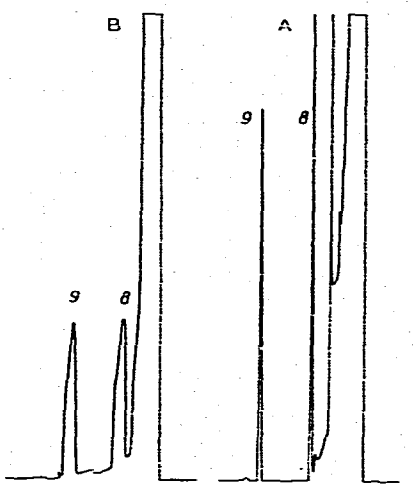

Fig. 9. Example of the particularly large difference in the efficiencies of two solvents. Injection without splitting at room temperature; 2 μl of 1:10,000 solution. Column 40 m × 0.34 mm, Carbowax 600. Solvent: A, carbon disulphide; B, *n*-pentane.

solvents) is opposite to that in Fig. 3C (acetone solvent). We have observed this characteristic difference in many similar instances. It shows that the detailed mechanism of plug concentration is different for polar and non-polar solvents.

INFLUENCE OF TECHNIQUE AND EQUIPMENT

Of the many technical and instrumental details, we wish to discuss two basic aspects: the absolute amount of solvent and the injection time.

Absolute amount of solvent

All analyses reported in this paper were started by injecting 2 μl of a 1:10,000 solution. It is interesting to consider what will be obtained after injecting 0.2 μl of 1:1000 solution, containing the same amount of sample components in ten times less solvent. The immediate answer will probably be that a similar or better result will be produced, as the original length of the vapour plugs will be much smaller. Thus a much lower concentration effect should be needed. The experimental results (Fig. 10), however, indicate the contrary: the result is even worse than that obtained with 2 μl of 1:10,000 solution in isopentane (Fig. 2D). The reason is excessive dilution of the small amount of solvent vapour in the vapourizer chamber. Under these conditions, the partial pressure of *n*-hexane in the carrier gas becomes so low that, even at 25°, condensation of solvent in the stationary phase is not sufficient to yield an observable solvent effect. We emphasize this example because it probably explains some failures that have been experienced with our method in other laboratories. No positive result can, in fact, be expected if the vapourizer volume is too great, or if additional dilution occurs in the mixing chambers or in long transfer lines between the vapourizer and the column.

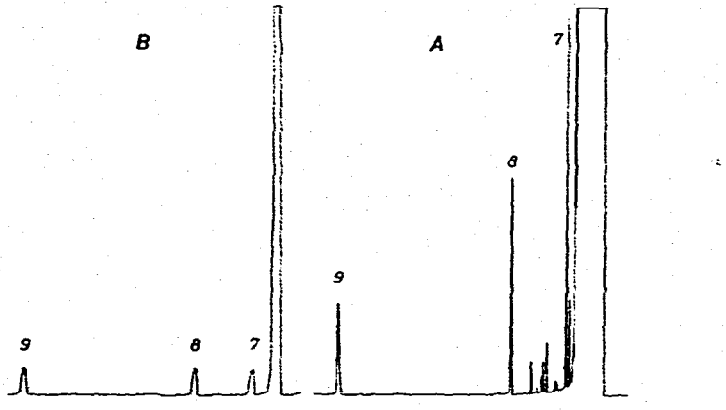

Fig. 10. Effect of concentration of solvent vapour in the carrier gas. Injection without splitting at room temperature of identical amounts of sample components in different amounts of solvent (carbon disulphide). Volume of glass liner in the vapourizer: 0.8 ml. Column and technique as in Fig. 2. A, 2 μl of 1:10,000 solution; B, 0.2 μl of 1:1000 solution.

Injection time

In our method, the splitting system is not used for stream splitting but for venting all parts around the vapourizer that may be reached by back-diffused traces of solvent vapour during the period in which the splitting valve is closed. As it would take an infinite time for 100% transfer of sample on to the column, a practical injection time is normally determined, during which, for example, 90 or 95% of the sample enters the column. As can be seen from runs with an unsuitable solvent, or without solvent (*e.g.*, Figs. 1B, 1C and 2D), in this work *ca.* 90% transfer was used. The corresponding injection time is four times the time that would be necessary to transfer the undiluted sample vapour. The injection time influences the peak shape only in the case of an insufficient or missing solvent effect, while under properly selected conditions it affects the peak height.

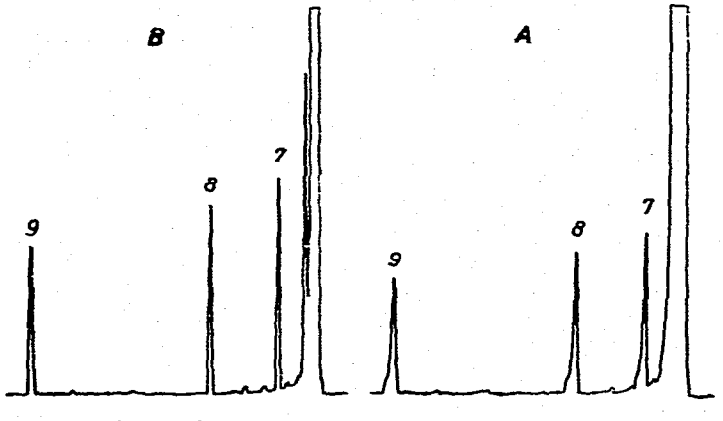

Fig. 11. Effect of injection time (time between injection and reopening of splitting system) on peak shape, using conditions with poor solvent effect (solvent, isopentane at room temperature). Column and procedure as in Fig. 2. Injection time: A, 30 sec; B, 10 sec.

The only difference between the two chromatograms shown in Fig. 11 is the injection time. This example shows that a reduced injection time (10 sec), under conditions with a poor solvent effect may simulate to some extent the successful concentration of sample plugs. Comparison of the peak heights shows, however, that almost no concentration effect did occur.

CONCLUSIONS

(1) It is a common error (*e.g.*, see ref. 11) to interpret the mechanism of injection without splitting as a type of cold trapping. Provided that the main variables, namely stationary phase, sample solvent and column temperature, are suitably selected, no temperature difference between injection and separation is necessary, *i.e.*, isothermal analysis is possible.

(2) The solvent should be considered as an essential tool, instead of a disturbing by-product. Ingeniously designed devices (*e.g.*, see refs. 12 and 13) with the aim of eliminating the solvent are not necessary.

(3) Under proper conditions, the solvent peak will obscure only the peaks of those substances which would be eliminated together with the solvent. Thus the previous elimination of the solvent does not extend the information given by the first section of the chromatogram.

(4) On capillary columns with a stable coating, large amounts of solvent can be injected daily for several years without adverse effects on the column*. In this respect, again there is no need to eliminate the solvent. Non-volatile, aerosol-like particles transfered on to the column are easily removed by periodically burning out the column inlet, as described previously[3].

(5) The most universal solvents for injection without splitting at room temperature, yielding an ideal solvent effect on most stationary phases and for most sample types, are carbon disulphide and methylene chloride.

(6) Instrumental pre-conditions for successful injection without splitting are direct transfer of the vapourized sample on to the column, with minimum dilution with carrier gas; furthermore, an effective venting (splitting) system is needed, which separately vents the septum area[3].

(7) We consider that the solvent effect, although with less importance, will also be valuable on packed columns. Particularly in this case, the same venting system should be used to avoid broad solvent peaks. We also consider that venting back-diffused solvent vapour after injection would be of great value in regular work with packed columns.

(8) Actual cold trapping is needed only for injections without solvent (gaseous samples), or in instances where a perfect solvent effect cannot be obtained.

ACKNOWLEDGEMENT

This work was made possible through the generosity of F. J. Burrus & Cie, Boncourt, Switzerland.

* Column damage is possible only by heavily exaggerated solvent effects, as *e.g.* by injecting 2 μl of toluene on to a colnmn at 25°.

REFERENCES

1 K. Grob and G. Grob, *J. Chromatogr. Sci.*, 7 (1969) 584.
2 K. Grob and G. Grob, *J. Chromatogr. Sci.*, 7 (1969) 587.
3 K. Grob and G. Grob, *Chromatographia*, 5 (1972) 3.
4 A. B. Littlewood, *Gas Chromatography*, Academic Press, New York, London, 1962, p. 164.
5 J. A. Völlmin, *Clin. Chim. Acta*, 34 (1971) 207.
6 F. Etzweiler and N. Neuner-Jehle, *Chromatographia*, 6 (1973) 503.
7 A. J. Artho and R. Koch, *Ann. Tabac*, (1974) in press.
8 K. Grob, *J. Chromatogr.*, 84 (1973) 255.
9 D. R. Rushneck, *J. Gas Chromatogr.*, 3 (1965) 318.
10 W. E. Harris, *J. Chromatogr. Sci.*, 11 (1973) 184.
11 J. A. Luyten, *Gas–Liquid Chromatography of Steroids with Glass Capillary Columns*, Thesis, Technical University Eindhoven, Eindhoven, 1973, pp. 63–73.
12 P. M. J. van den Berg and Th. P. H. Cox, *Chromatographia*, 5 (1972) 301.
13 A. L. German and E. C. Horning, *J. Chromatogr. Sci.*, 11 (1973) 76.