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PEAK BROADENING OR SPLITTING CAUSED BY SOLVENT FLOODING AFTER SPLITLESS OR COLD ON-COLUMN INJECTION IN CAPILLARY GAS CHROMATOGRAPHY

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SUMMARY

Peak broadening or splitting in capillary gas chromatography may be due to condensed solvent flooding the inlet of the column. Solvent introduced by cold on-column injections or recondensed after a splitless injection (solvent effect) travels into the column as a liquid. This liquid carries the dissolved sample components into the capillary, and the sample materials are distributed over the whole length of the flooded zone. This causes the peaks to be broadened or, if the sample components are not evenly distributed, to be split. The broadening or splitting effect is stronger the longer is the flooded section of the capillary inlet. The peak distortion increases with the retention of the peak and does not disappear with temperature programming. The deficiency affects the peaks of all sample components with the exception of those which are sufficiently volatile to migrate at the column temperature during injection. It is shown that the separation efficiency of a 15-m column may decrease by a factor of five in terms of plates or by a factor of two if the resolution is calculated as Trennzahl.

INTRODUCTION

The injection of microlitre volumes of liquid samples onto capillary columns causes problems concerned with the band width of the sample at the beginning of the chromatographic process. Only the use of an appropriate splitting ratio results in sample introduction within a sufficiently short time to create a band width small enough to eliminate these problems. Other injection methods require special techniques to reduce potentially broad bands, *i.e.*, to reconcentrate the sample at the head of the column.

Estimations based on the final peak width show that under usual conditions the sample should be introduced within about 100 msec in order to prevent broadening by more than a few percent of an emergent peak of a few seconds width. The sample should be spread over less than 10 cm of the column inlet. Although, in practice, unknown effects make such theoretically derived values seem pessimistic, the problem has to be considered to be a real one for capillary gas chromatography (GC).

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A sample introduced within 100 msec amounts to about 5 μl of vapour (usually diluted with carrier gas), corresponding to less than 20 nl of liquid. However, for many applications there is a need to increase the sample size in order to attain detectable quantities of material. Techniques for the injection of liquid samples without splitting are vaporizing splitless and direct as well as cold on-column sampling. Peak broadening effects due to slow sample transfer have been investigated in detail for the splitless method[1,2]. A splitless injection of 1–2 μl of sample requires the transfer of 0.5–1 ml of vapour (diluted with carrier gas) from the vaporizing chamber to the column. Such a transfer is not linear but partly a dilution process. Therefore, the transfer time at a column flow-rate of around 3 ml/min is in the order of 30–50 sec, instead of the desired 100 msec. A reconcentration of the sample by a factor exceeding 100 is required at the head of the column.

In order to explain the origin of the solvent flooding to be discussed below, some aspects of splitless and on-column injections have to be reconsidered. Two techniques, "cold trapping" and the "solvent effect", have been used for a long time. Both create a temporary zone of high retention in the column inlet and thus concentrate the sample components of interest in a very short section of the column. The cold trapping effect will be the subject of a forthcoming paper and is of little importance here. The solvent effect requires conditions which recondense a large portion of the solvent in the first part of the column. The temporarily extremely thick layer of liquid in this zone blocks the sample components at its rear until the solvent is evaporated. At that moment the sample is released and chromatography is commenced, the delay corresponding to the time required to evaporate the solvent. A solvent effect is usually obtained by injecting 1–3 μl of sample at a column temperature which is at least 20°C below the boiling point (b.p.) of the solvent. Cold trapping is used for the analysis of high boiling materials injected at a column temperature well below that at which they migrate (but perhaps much higher than the b.p. of the solvent), whereas the solvent effect technique is required for analyses which must start below 100°C.

The cold on-column injection technique requires that the column be kept at a temperature below the boiling point of the solvent[3] to avoid a pressure increase in the column inlet due to an excessive volume of sample vapour. The sample components are blocked in the condensed solvent until the latter is evaporated. This means that cold on-column sampling is automatically carried out under conditions which lead to a solvent effect.

Both splitless injections with solvent effect and cold on-column sampling cause a large amount of solvent to be condensed at the head of the column. This solvent, useful for reconcentrating the sample, was found to be the cause of recently discovered peak broadening or splitting processes, which are described in this paper. These undesired effects may be avoided by a new type of band focussing which will be discussed in a future paper.

Peak broadening or splitting appear to be the result of a number of independent mechanisms, the best known of which is the slow sample transfer into the column. Peaks may be distorted by some solvent effects and by several kinds of inhomogeneities of the retention in the column, such as irregularities in the film thickness of the stationary phase or an unequal temperature distribution over the column. Unfortunately, little has been published on these subjects.

FLOODED COLUMN INLET

The injection of a large amount of solution into capillary columns creates a thick layer of liquid on the inner wall of the tubing. Such layers are not stable in the presence of a very rapid stream of carrier gas and most of the liquid is driven further into the column. The liquid carries all the dissolved sample components along and spreads them out over the whole length of the zone which is flooded by the solvent. This zone can be 30 cm long.

The flooded zone may be observed visually by the method described earlier[2]. Columns with a milky aspect, *i.e.*, those having a very rough inner surface, become transparent when wetted by a layer of liquid which fills the holes in this surface. Experimentally, it was found that this layer has to reach a minimum thickness of 0.5–1 μm in order to cause transparency. Immediately after a cold on-column or a splitless injection under solvent effect conditions, a relatively short wet zone is formed which rapidly expands into the column. After a few seconds the leading edge of the zone slows down and at the same time starts to move irregularly. Within the flooded zone, waves are built up and move forward and the front of the wet zone jumps forward whenever such waves arrive there. After some time the layer at the rear of the flooded zone becomes thin and finally evaporates completely. A fascinating development occurs when the wet zone moves at both edges; the rear moves quickly, thus reducing the length of the wet section of the capillary. As more and more of the solvent is evaporated the leading edge comes to a stop whereas the rear accelerates until it catches the front and the wet zone disappears.

The liquid flow as a transport mechanism to spread out the flooded zone is reinforced by a vapour phase transport, *i.e.*, an evaporation of solvent at the rear of the flooded zone and recondensation at the front. The importance of this transport is dependent on the solvent and on the column temperature. In most cases it is relatively small. Since it does not contribute to the spreading of the sample components, it is not considered further.

DISTRIBUTION OF THE INJECTED SAMPLE COMPONENTS

Ideally the sample should be deposited on the inlet of the capillary column as a sharp band of a few millimetres width in order to start the chromatographic process with a sharp "peak". However, the flood of solvent causes the sample to be spread out over several tens of centimetres. Since this spreading is a non-gas chromatographic process, even completely non-volatile materials move further into the column and undissolved components of turbid samples are carried in the flooded zone.

There are few techniques to investigate the distribution of the sample after the injection. Valuable information was obtained by direct visual observation. Visual observation has been used by Driessen and Lugtenberg[4] for the determination of adsorption. Concentrated solutions of perylene were injected into a milky column by the splitless and the cold on-column techniques, keeping the door of the GC oven open. The expansion of the flooded zone could be observed and the point of collapse of the wet zone marked with a fibre pen. The column was then taken out of the GC oven and the perylene distribution was observed in a dark box by its fluorescence under 366-nm UV illumination. Subsequently, a more dilute solution of perylene was

injected under the same conditions and chromatographed normally. The peak shape observed by recording the detector signal was compared with the perylene distribution in the column head.

Cold on-column injections into a column kept at a temperature between 25°C and the boiling point of the solvent always resulted in the perylene being spread out over a zone beginning a few millimetres below the injection point, the tip of the syringe needle, and reaching as far as the point of collapse of the flooded zone (see Fig. 1). Thus, the sample spread over the whole flooded zone, several tens of centimetres in length. The perylene distribution within this zone was seldom uniform. The amount of material deposited at a certain point is dependent on a number of parameters. It is finally determined by the quantity of liquid evaporated at this point, which is influenced by such factors as the thickness of the liquid layer, the viscosity of the solution, its surface tension and the replacement by liquid carried there by the flood. Accordingly, the maximum of perylene was sometimes located near the rear of the flooded zone. More often there were two maxima near the edges of the zone (Fig. 1) or a single maximum some 20–40 % of the length of the zone behind its front. This distribution was strongly dependent not only on the sample size and the solvent but also on chromatographic conditions such as column temperature and the speed of the injection. Most of the visual observations were in good agreement with the peak shapes obtained when the more dilute sample of perylene was analysed and recorded normally.

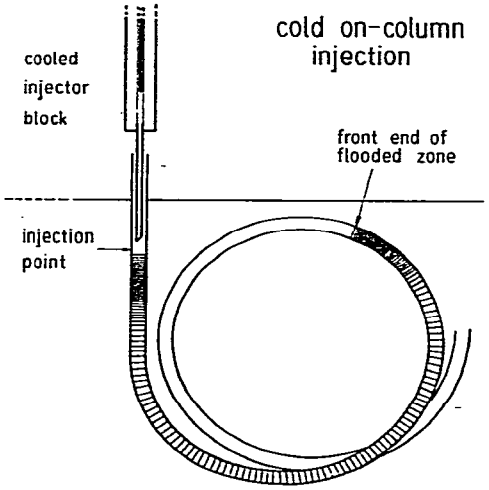

Fig. 1. Possible distribution of a sample component (*e.g.*, perylene) in the capillary column inlet after cold on-column injection. The liquid introduced by the syringe flows into the column and floods some 10–60 cm of it. The dissolved sample components are spread out over the whole length of the flooded zone. The distribution of the material within this zone depends on the amount of solvent which evaporates at a certain point. It is rarely homogeneous. If the amount of material deposited has two maxima, one near each edge of the flooded zone, as is often observed experimentally, the peak will be split as shown in Fig. 4.

Unfortunately, the variety of compounds allowing direct visual observation is very restricted. The results for perylene are typical for substances boiling far above the column temperature at the injection. For more volatile components (or for high boiling substances such as perylene in higher boiling solvents injected at elevated

column temperature) the situation is different because these substances are able to migrate through the gas phase at the column temperature during the injection. These substances tend to follow the rear of the wet zone, which moves due to evaporation, either by their own volatility or by coevaporation with the solvent. The rear of the wet zone dries with a typical speed of around 1 cm/sec. Since the linear carrier gas velocity is usually around 50 cm/sec, a chromatographed substance having a capacity ratio of about fifty will still follow the wet zone on the basis of its own volatility. Thus a relatively wide range of volatile sample components can be reconcentrated at the point of collapse of the wet zone, the leading edge of the flooded zone. During isothermal runs, no peak broadening is noticeable. In temperature programmed chromatograms the first peaks after the solvent are sharp and the following ones become broad or split only gradually (Fig. 4).

The factors determining the distribution of the sample components during splitless injections with solvent effect are somewhat different. The experiment with perylene showed that a significant portion of the material was deposited in the capillary section which is within the ferrule and nuts of the attachment to the injector. The remaining perylene passed that area and, once in the cold part of the capillary in the GC oven, it spread out in the flooded zone (Fig. 2).

When injected into a vaporizing sampler, perylene passes through the hot part of the capillary located in the injector. The following section of the column is located in the bottom of the injector where the temperature drops from, *e.g.*, 300°C to the

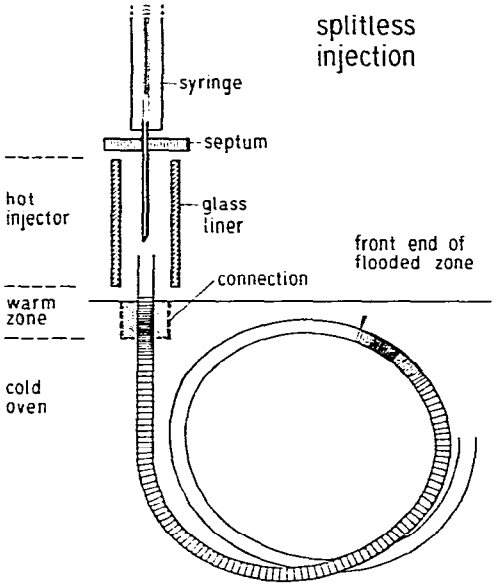

Fig. 2. Distribution of a medium volatile sample component after splitless injection into a cold column to recondense the solvent (solvent effect). One portion of the component is trapped in the relatively cool bottom of the injector (containing the fitting and the nut). Another portion passes into the cold section of the column in the GC oven. There the solvent is condensed, redissolves the sample components and carries them further into the column. The distribution of the sample component within the flooded zone is not predictable.

25°C of the oven over a few centimetres. In principle, the perylene migrates to the point where the column temperature is sufficiently low to condense it, but, since this section is very short, not all of the perylene is trapped. Some of the material passes on into the cold part of the capillary in the GC oven. There the solvent condenses and floods the following section of the capillary. This solvent is responsible for the fact that the perylene reaching the oven was spread out over a length of several tens of centimetres, similar to what was observed for the cold on-column sampling.

The proportion of perylene in the capillary section warmed by the fittings and in the flooded zone in the column, respectively, depends on the injector temperature and the shape of the temperature gradient from the injector to the oven (*i.e.*, on the geometry of the connecting parts, their heating characteristics and on the time the oven is cooled before injection. Usually, more material is retained in the warm capillary section in the bottom part of the injector the higher its boiling point is (see Fig. 4).

In practice the broad pre-peaks of high boiling sample components, *i.e.*, the portion of the material reaching the flooded zone, may be overlooked in complex chromatograms or neglected by regarding them as disturbed baseline. For quantitative analyses, however, this causes serious errors.

To summarize, for splitless injection there are not only the flooding phenomena to be considered as observed during cold on-column injections, but also a splitting due to the trapping effect of the (short) warm zone of the capillary connection.

LENGTH OF THE FLOODED ZONE

The length of the capillary section flooded by the solvent determines the initial band width of a substance, hence the broadening of a peak due to the flooding phenomenon. The determination of the length of the flooded zone was of interest for its correlation with the peak broadening observed after a chromatographic run.

Table I summarizes results obtained by cold on-column injections at 25°C into a milky column of 0.32 mm I.D. with a carrier gas flow-rate of about 50 cm/sec. Surprisingly, the length of the wet zone was found to depend little on the volatility of the solvent, as may be seen for the alkanes pentane, hexane and heptane. This does not mean that the time required to evaporate these solvents would be similar; in fact the time required for heptane is approximately ten times that for pentane. This contradicts intuition. It means, for example, that it is not true that the choice of a more volatile solvent reduces the length of the column which may suffer phase stripping. This notwithstanding, the higher boiling solvent probably exerts a stronger phase stripping due to the increased duration of the flooding. The polarity of the solvent influences the speed of the evaporation more than the length of the flooded zone (due to the evaporation energy required).

As expected from the similarity of the lengths of the flooded zones for solvents of different volatilities, the column temperature cannot be used to shorten the flooded zone in cold on-column injections for column temperatures below the b.p. of the solvent. Table II shows some results for chloroform. At column temperatures below the boiling point of chloroform the length of the flooded zone is approximately constant. At 20°C above the boiling point, the flooded zone was still 40 % as long as it

TABLE I

LENGTH OF THE FLOODED ZONE AND TIME REQUIRED TO DRY THE WET ZONE

Cold on-column injection, oven at 25°C.

Solvent	*0.5 µl*		*1 µl*		*2 µl*	
	Length (cm)	*Time (sec)*	*Length (cm)*	*Time (sec)*	*Length (cm)*	*Time (sec)*
Pentane	10	2	16	4	30	7
Hexane	11	6	21	11	41	20
Heptane	12	17	22	31	55	63
Benzene	10	13	20	22	50	55
Toluene	17	37	30	70		
Dichloromethane	7	4	22	7	50	16
Chloroform	10	7	22	16		
Diethyl ether	8	2	17	4	30	7
Acetone	10	6	20	12	47	26
Methanol	14	26	23	48	55	100
Ethanol	10	35	22	70	35	150

TABLE II

EFFECT OF TEMPERATURE ON THE FLOODED ZONE

Cold on-column injection of 1.5 µl chloroform.

Column temperature (°C)	*Length of flooded zone (cm)*
27	30
60 (b.p.)	25
80	10

was at the b.p. However, these temperature conditions are unsuitable for cold on-column injections[3].

The carrier gas flow-rate did not significantly influence the length of the flooded zone. An increase of the carrier gas inlet pressure by a factor of four shortened the flooded section of the capillary by 10%.

The speed of the injection does not greatly affect the length of the flooded zone. A 1.5-µl volume of chloroform injected at 55°C during 10 sec gave a flooded zone 20 cm long. This is not markedly different to the 25 cm found for rapid injection. It is nevertheless interesting that for slow injections perylene was mainly deposited in the first part of the flooded zone, indicating that the expansion of the wet zone during slow injections occurred primarily through the vapour phase. However, slow injections in cold on-column sampling may be discriminative and should be avoided[3].

The length of the flooded zone obtained by splitless injections was generally similar to that for cold on-column sampling (Table III). Differences were observed for the most volatile solvents. Their slow transfer from the vaporizing chamber to the

TABLE III

LENGTH (cm) OF THE FLOODED ZONE FOR SPLITLESS INJECTIONS

Column at 28°C. n.d. = Not detected.

Solvent	*Volume injected*		
	1 µl	*2 µl*	*3 µl*
Pentane	n.d.	20	30
Hexane	15	40	60
Heptane	20	60	110
Benzene	20	50	75
Toluene	30	90	
Dichloromethane	7	25	50
Chloroform	12	40	65
Methanol	25	60	
Diethyl ether	n.d.	10	25

column shortens the flooded zone. The partial vapour pressure may not even be sufficient to create a flooded zone at all, as seen for 1-µl injections of pentane and diethyl ether.

PEAK DISTORTION

The solvent flooding the capillary inlet causes the sample components (except the most volatile compounds) to spread out over 10–60 cm of the column. The shape of this band determines the "peak" at the beginning of the chromatographic process and will usually be reflected in the shape of the final peak also. As described for the distribution of the sample material within the flooded zone, there may be a single, distorted peak with a tail or a shoulder on either side. More often the peak is split into two or even more maxima.

To describe the peak distortion caused by the solvent flooding, it is necessary to clearly differentiate between the well known peak broadening due to slow sample transfer (band broadening in time) and the broadening caused by local dispersion by the spreading liquid (band broadening in space). The two phenomena have completely different characteristics.

The importance of peak broadening caused by slow sample transfer is dependent on the time. If a sample enters the column within 5 sec, this is disastrous for an early, narrow peak since its width may be multiplied. For a late peak, which may have a width of 20 sec anyway, the distortion is hardly noticeable. It is of no importance whether the slow elution of a broad peak was achieved by using a long column, a low column temperature or a low carrier gas flow-rate. It is also characteristic that the broadening effects disappear during temperature programmes due to the cold trapping effect, *i.e.*, due to the fact that the substances eluting at elevated column temperature do not migrate noticeably into the column during the time of sample transfer. Peak broadening in time is therefore most accentuated at the beginning of chromatograms.

Band broadening in space as a result of the solvent flooding is dependent on the

length of the flooded zone in relation to the total length of the column. The distortion is related to the retention time, *i.e.*, peak broadening measured as millimetres on the chart paper increases linearly with the retention time. Thus, instead of disappearing for chromatographically broad peaks, the distortion is even more drastic. The peak distortion also does not disappear during temperature programming, since the substances of different volatilities are all spread out in the column inlet in the same manner. Instead of being a deficiency of the early part of the chromatograms, the band broadening in space is most accentuated in the later section of the runs. Components which are volatile at the column temperature during the injection are not affected at all since they are reconcentrated at the point of the collapsing wet zone. Therefore chromatograms run isothermally at the injection temperature do not show any peak distortion. In temperature programmed runs or in isothermal runs carried out at temperatures above the injection temperature, peak distortion will be most visible for the late peaks. The only parameter able to mask the peak distortion caused by solvent flooding is the length of the column. Peak broadening due to diffusion during chromatography in long columns reduces the defects in the peaks.

The importance of the influence of the column length on the band broadening in space becomes obvious when considering a hypothetical column of length equal to that of the flooded zone. Since all sample components would be spread out as far as the exit of this column, their elution would commence at the very first moment of the chromatographic process. Hence there would be no resolution at all. If the column were twice as long, the peaks would be very broad. They would begin to be eluted at half of their normal retention time since the material eluted first would reach the middle of the column at time zero, *i.e.*, during the injection. Since the last material would be eluted at the correct retention time, the peaks would be spread over half of the (isothermal) chromatogram (not considering the broadening by diffusion in the column during chromatography). The expected peak broadening may be calculated from the length of the flooded zone and the length of the column. As mentioned above, the broadening effect has to be expressed in relation to the retention. The peak broadening as a percentage of the retention (seconds or millimetres on the chart paper) is equal to the flooded zone as a percentage of the total column length.

A 30-cm flooded zone (obtained by an on-column injection of somewhat less than 1.5 μl of sample) in a 15-m capillary column causes the front of the materials to pass 2% of the column length at the start of the chromatographic process. This causes the final peak to be broadened by 2% of the retention (provided it is not due to a substance which is volatile at the injection temperature). If the width of a given peak is equal to 2% of its retention, the system has an efficiency of less than 13,000 plates. For a 15-m column with 50,000 plates this means a reduction in the separation efficiency by a factor close to five. The resolution of the column (measured as "Trennzahl", TZ) decreases by more than a factor of two. For a 50-m column, however, the losses in separation efficiency due to the same peak broadening are more than three times smaller.

Fig. 3 shows some separation efficiencies measured on a capillary (4.5 m × 0.31 mm) coated with 0.15 μm of OV-73, using the cold on-column sampling technique. The test mixture contained the even numbered *n*-alkanes C_{12} to C_{24}, diluted 1:300,000 in hexane. During the injection the column was kept at 30°C. TZ values were calculated for each pair of alkanes, then divided by two to compensate for the

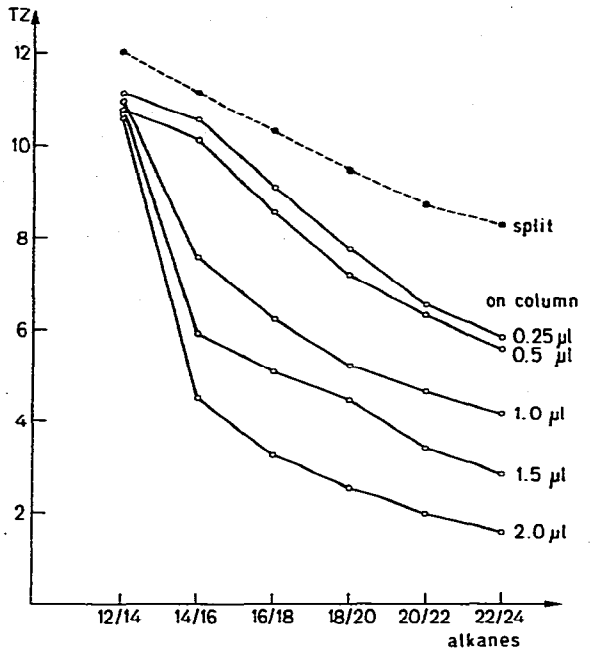

Fig. 3. Separation efficiencies in terms of Trennzahl (TZ) from a capillary column (4.5 m × 0.31 mm) coated with 0.15 µm of OV-73. TZ values are determined from two consecutively even numbered *n*-alkanes ranging from C_{12} to C_{24} (diluted 1:300,000 in *n*-hexane) and divided by two to account for the two methylene units by which the alkanes differ. The values of the vaporizing split injection (ratio 1:60) reflect the column's efficiency under the (non-optimized) conditions used (0.1 atm H_2 as carrier gas, temperature programmed from 30 to 240°C at 12°C/min). Cold on-column injections reduced the separation efficiency due to the band broadening caused by solvent flooding. The larger the sample volume, the longer is the flooded zone and the broader the band at the beginning of the chromatographic process. The *n*-dodecane peak was not measurably broadened even for the large sample volumes injected by the cold on-column technique, because dodecane was sufficiently volatile to follow the rear of the wet zone and to reconcentrate at the point where the last liquid disappears (at the front of the flooded zone).

fact that the neighbouring alkanes used differed by two methylene units. In order to characterize the separation efficiency under ideal sampling conditions, TZ values were also calculated for a vaporizing split injection. All TZ values obtained by cold on-column injections were clearly lower and indicated peak broadening by the flooding process. With the exception of the early peaks, the loss of efficiency rapidly increased with the sample volume injected, because an increased amount of liquid prolongs the flooded zone. For a sample volume of 2 µl, the separation efficiency in terms of TZ was lowered by a factor of 5 (in terms of plates, by a factor 25). Obviously, this disastrous result must be viewed in terms of the shortness of the column. Nevertheless, the broadening effect cannot be neglected, except for extremely long columns. The losses in separation efficiency observed were in good agreement with peak broadening effects estimated from the length of the flooded zone.

In practice, peaks are seldom broadened without being deformed at the same time. Fig. 4 illustrates one possible type of deformation, peak splitting, for the two types of injections discussed in this paper. The sample contained the even numbered

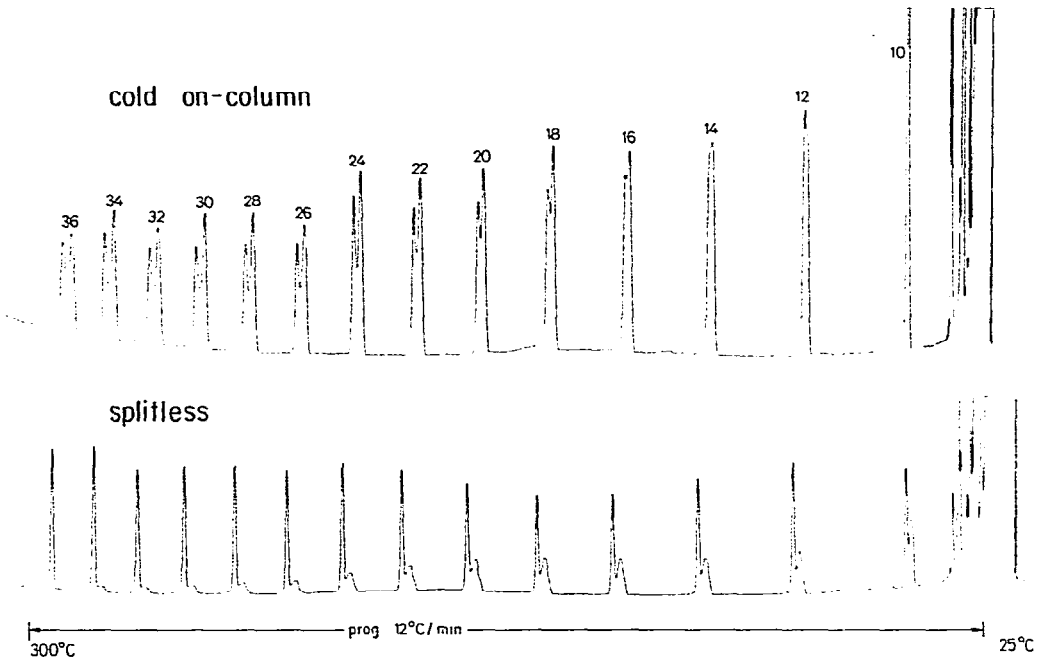

Fig. 4. Typical chromatograms with split peaks for a sample containing the even numbered *n*-alkanes C_{12} to C_{36}, diluted 1:200,000 in acetone. Capillary column (5 m × 0.30 mm) coated with 0.6 μm SE-52; 0.1 atm H_2 as carrier gas; temperature programmed from 30 to 300°C at 12°C/min. The split peaks in the cold on-column injection (1.5 μl) are due to an uneven distribution of the sample components within the flooded zone (similar to Fig. 1). The material in the splitless injection (2.5 μl) is divided into a portion trapped in the relatively cool part of the injector and a broad pre-peak spread out over the flooded zone.

alkanes from C_{10} to C_{36} dissolved in acetone and was introduced into a column at 30°C. Both chromatograms were obtained using the same column (5 m × 0.30 mm) coated with 0.6 μm of SE-52. Note that the apparently similar splittings obtained by the two injection techniques arise from different causes. The splitless injection divided each sample component into a broad (early) peak of the material in the flooded zone and an almost unbroadened peak of material trapped in the relatively cool, non-flooded inlet of the column. The proportion of the material passing the barrier of the warm capillary section decreased with increasing boiling point of the component. The material within the flooded zone was distributed to give a broad maximum at the centre of the zone. The split peaks produced by the cold on-column injection are due to the uneven deposition of the sample material within the flooded zone. Hence in this case there were two maxima at the end of the flooded zone.

CONCLUSIONS

Cold on-column injection and splitless sampling at low column temperature take advantage of the large amount of solvent condensed on the wall of the column inlet, which serves as a barrier to the volatile sample components migrating into the

column during the sampling process. This "solvent effect" indeed solves the problem of "band broadening in time" due to the slow sample introduction during the splitless injection and the slow evaporation of the sample in the area of the injection point of cold on-column sampling. However, it was not realized that the large amount of condensed solvent creates a new problem, *i.e.*, it spreads out the less volatile sample components. This new problem of "band broadening in space" may be at least as serious as the known band broadening in time, especially if short columns are used.

For splitless injection, band broadening in space is eliminated as soon as recondensation of solvent is excluded, *i.e.*, by using cold trapping rather than the solvent effect to correct for the band broadening in time. Obviously, this solution can only be applied to a restricted range of analyses and has no value for the cold on-column injection technique.

A general solution to the problem of band broadening in space will be described in a future paper. A "retention gap" at the inlet of the column as long as the flooded zone is kept free of stationary phase. The sample components are then reconcentrated where the stationary phase begins.

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