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MOVEMENTS OF THE SAMPLE PLUG IN THE COLUMN INLET AFTER ON-COLUMN INJECTION IN CAPILLARY GAS CHROMATOGRAPHY

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SUMMARY

The movements of the sample plug in the column inlet after on-column injection were observed visually, with special regard to transport of sample material backwards into the cool column section kept inside the injector or even out of the column. A rapid injection separates at least the first sample material 5–15 mm from the needle tip, but with low carrier gas flow-rates, column temperatures around the boiling point of the solvent and large sample volumes, the injected sample plug tends to return. If it touches the tip of the (still inserted) syringe needle, some liquid is sucked into the narrow space between the needle and the column wall, and is pulled back into the cool column section in the injector when the syringe is withdrawn. Rapid injections of large sample volumes may even cause some sample material to leave the column.

Ordinary sample volumes and carrier gas flow-rates are unlikely to cause back-flow, unless the column temperature is near the boiling point of the solvent. The tendency of the sample plug to flow backwards is accentuated with low carrier gas flow-rates, especially if combined with narrow-bore columns. The use of large bores and long retention gaps improves the situation considerably. Large sample volumes were almost inevitably pulled back up the column neck.

Errors due to back-flow of the sample, such as losses and discrimination, hardly reach 10%. Material lost in the cool column inlet in the injector may cause memory effects, transferring a few percent of the high-boiling components from one injection to the next. Quantitative deviations and memory effects become very small with large sample volumes.

INTRODUCTION

The on-column technique is a well established method of introducing samples into capillary columns. It is more precise and accurate and more reliable than other injection methods. Further, it has the important advantage of not exposing the sample to thermal stress.

The potential of the recently developed temperature-programmed vapourizing injector ("PTV")[1–3] is remarkable. The PTV might replace the classical vapourizing injection in the split and splitless modes. However, it hardly competes with on-column

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injection. The analysis of triglycerides by PTV injection, as frequently shown, demonstrates one of the limitations of the technique, namely that a considerable proportion of the sample is thermally degraded. The primarily resulting free fatty acids occur in the early part of the chromatograms.

The on-column injection technique is expected to be further developped in three directions: (a) injections of large volumes (10–100 μl), (b) injections on to narrow-bore columns and (c) automation, using a wide-bore retention gap and standard syringe needles[4]. All three techniques are known to be feasible under certain conditions, but their special requirements and limitations should be established before they can be recommended as applicable in every-day work.

It is risky to report on the limitations of a technique, because such information is often misused. On the other hand, a detailed knowledge about a technique is required in order to be able to understand all the phenomena that one may come across during practical applications. Many limitations, once they are known, may be eliminated by simple techniques (such as the band broadening in space by the retention gap[5]) and others are easily circumvented.

The limitations of the on-column technique discussed in this paper were of special interest in learning more about the background of two of the three techniques mentioned above, namely injection into narrow-bore columns and large sample volumes.

On-column injection is based on an exceptionally simple concept. The accuracy and precision produced by on-column injection depend only on the success of the sample transfer from the syringe needle to the column below the injection point. No sample material should be fractionated and lost on the needle tip and no material should return above the injection point.

The sample transfer in on-column injection is endangered by two well known and a third newly discussed mechanism. First, the sample must be mechanically and completely separated from the tip of the syringe needle. Materials that remain hanging on the needle tip are fractionated by the passing stream of carrier gas. Volatile parts are transferred to the column, high-boiling parts are pulled out of the column with the syringe needle. An at least moderately rapid injection solves this problem[6].

Second, if the evaporation of the sample in the column inlet is excessively rapid, more vapour is produced than can be carried further into the column by the carrier gas. This increases the pressure at the injection point above the carrier gas pressure in the injector and some sample material is thrown back out of the column, primarily in the liquid phase[6]. We determined the maximum column temperature needed in order to avoid excessive evaporation rates for a number of solvents and carrier gas flow-rates and came to the conclusion that a column temperature corresponding to the boiling point of the solvent can be recommended as a safe working rule. Galli and Trestianu[7] have shown that the temperature may be 10°C above the boiling point of the solvent if secondary cooling is used[8]. Later, a number of papers were published reporting on injections carried out at temperatures considerably above the boiling point of the solvent. However, they were lacking from experimental proof that there was no back-flow and, if it was to be recommended as a generally applicable method, that it would also be feasible under less favourable conditions.

The third process has been known to some extent almost since the on-column injection technique was first introduced, but it has never been studied. The sample

plug deposited in the column may return slightly and, if it touches the tip of the syringe needle, some liquid is pulled by capillary forces into the narrow space between the needle and the column wall. This mechanism has probably been neglected first because of its rare occurrence under ordinary conditions and second because the resulting errors are relatively small.

In this paper, a number of experiments are first described that allowed visual observation of the movements of the sample plug and the determination of the critical conditions at which some liquid started to be pulled back up the column neck. In the second part, an attempt is made to establish the importance of this effect in a variety of respects.

EXPERIMENTAL AND RESULTS

Carrier gas behaving as a spring

To allow the ready observation of the sample in the column inlet, an on-column injector (Carlo Erba, Milan, Italy), which was not built into a chromatograph, was used. It was held with a clamp and fed via a pressure regulator and a pressure gauge with compressed air or hydrogen. Injections were carried out using an on-column syringe from SGE (Melbourne, Australia), equipped with a fused-silica needle of 0.17 mm O.D. and of 12 cm length. The syringe needle was long enough to pass the injector by 5 cm to allow easy observation of the events at the needle tip. The restriction of the injector above the rotating valve had a bore of 0.20 mm in order to avoid excessive leakage along the thin syringe needle. The column flow-rates for air were determined by a soap bubble meter and for hydrogen by displacement of water in a calibrated Pasteur pipette, which was sealed at the top.

The capillary columns were equipped with a retention gap[5] of glass with a well roughened internal surface, giving it a milky aspect. Such columns are transparent as soon as some liquid covers the roughened surface, which allowed a critical observation of the column parts that were wetted by the sample.

Unless very small sample volumes are injected, the liquid (pure solvents in our experiments) forms a plug in the column, built up by the many droplets that leave the syringe needle. The sample leaves the syringe needle with a high velocity, which gives the sample plug in the column a considerable initial speed. If the linear velocity of the carrier gas is at least equal to this, the plug carries on rushing into the column until it disappears owing to losses as it leaves material behind it on the column wall. However, if the carrier gas velocity is low, the sample plug behaves like a plunger. It compresses the gas in front of itself and is slowed down (Fig. 1). The impulse of the sample plug may be fully absorbed and the plug stopped for a moment. If the compressed gas ahead of the plug runs off rapidly enough, the sample plug starts to move forward again. However, the compressed gas may also push the plug backwards towards the syringe needle.

At a carrier gas flow-rate of 0.5 ml/min the first few tenths of a microlitre of injected solvent move rapidly several centimetres away from the needle tip into the column, but then the plug slows down and blocks the way for the following liquid. Whether the front of the plug remains still or moves backwards, the rear of the plug moves towards the tip of the syringe needle owing to the further liquid elongating the sample plug. During a fraction of or even a full second the plug hardly moves,

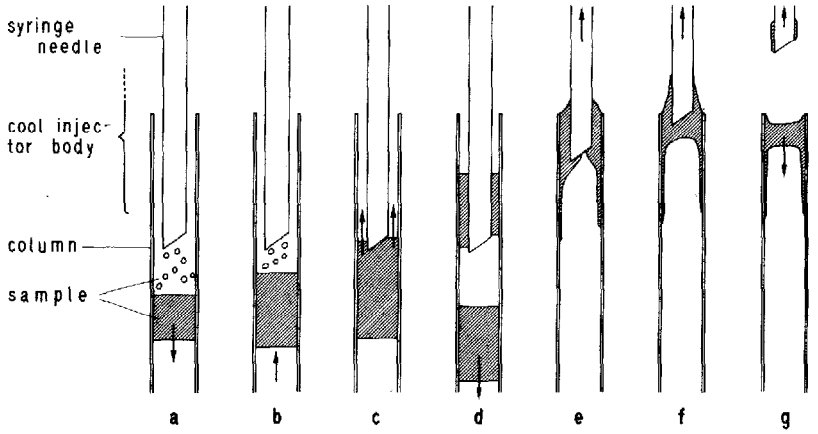

Fig. 1. Steps observed when the sample was pulled backwards into the cool section of the column inlet kept in the on-column injector by the withdrawn syringe needle. (a) The first sample material rushes into the column with the linear velocity of the liquid at the exit of the syringe needle. (b) The sample plug, acting as a plunger, compresses some carrier gas at the front of itself and is slowed down or stopped by the counter pressure built up this way. (c) The further liquid leaving the syringe needle prolongs the sample plug backwards. The liquid comes into contact with the needle tip and is sucked backwards between the needle and the column wall by capillary forces. (d) After the injection is completed, the compressed carrier gas in front of the sample plug runs off. The sample plug moves into the column, but the liquid between the needle and the column wall remains. (e) The liquid between the needle and the column wall is pulled backwards by the withdrawn syringe needle. (f) The needle pulls nearly all the liquid to the entrance of the column. The liquid even protrudes out of the column but, provided there is no surface in the injector near enough to serve as a bridge, the liquid is pulled off the needle tip and falls back into the column. (g) A plug of liquid returns from the entrance back into the column, leaving a film of sample behind itself on the column wall. Of all the liquid pulled backwards by the syringe needle, only the material contained in this film remains in the cool inlet section in the injector, which is in the range of a few tens of nanolitres.

until the carrier gas on its front side has flowed away and the plug starts to move further into the column, coating the column inlet with a sample layer until the plug is exhausted.

Optimal accuracy of on-column sampling requires that the rear of the sample plug does not touch the tip of the syringe needle. If it touches, some of the liquid is drawn by capillary forces into the space between the needle and the column wall (see below).

Using the same visual method, conditions were tested, that would just avoid the return of the sample to the needle tip. For a given column and carrier gas flow-rate, the volume of sample was varied to determine the maximum volume that was not sucked between the needle and the column wall. The carrier gas was hydrogen, but for equal flow-rates of air similar results were obtained. The results varied considerably for different solvents. Diethyl ether gave small maximum sample volumes, owing at least partly to its high volatility. It was used in our determinations together with *n*-hexane, a typical solvent with a boiling point far above the column temperature (25°C).

The geometry of the column greatly influences the return of the sample plug. Table I shows results obtained with a column of length 30 m and I.D. 0.32 mm. At

TABLE I

MAXIMUM SAMPLE VOLUMES WITHOUT BACK-FLOW FOR A 30 m × 0.32 mm I.D. COLUMN

Pre-column	*Carrier gas flow-rate (ml/min)*	*n-Hexane (μl)*	*Diethyl ether (μl)*
None	1.8	1.5	1
	3.5	3	2
1.8 m × 0.52 mm I.D.	1.8	4	2.5
	3.5	5	3

an inlet pressure (hydrogen) of 0.3 atm, giving a flow-rate of 1.8 ml/min, the maximum volumes were 1.5 μl for *n*-hexane and 1 μl for diethyl ether. At double this flow-rate these volumes were also doubled. The use of a wide-bore pre-column (1.8 m × 0.52 mm I.D.) strongly increased the maximum volumes, especially at low carrier gas flow-rates.

Table II shows the maximum sample volumes determined with a narrow-bore main column (11 m × 0.17 mm I.D.). Pre-columns of 0.32 and 0.52 mm I.D. and of various lengths were compared at different carrier gas flow-rates (0.45 and 1 ml/min, requiring inlet pressures of 0.3 and 0.6 atm, respectively). In agreement with the results in Table I, the following conclusions can be drawn:

(a) An increasing I.D. of the pre-column allows larger sample volumes to be injected (at a carrier gas flow-rate of 1 ml/min and for pre-columns of 0.32 and 0.52 mm I.D. the difference corresponded to a factor of about five).

(b) A long pre-column gives larger maximum sample volumes than a short one (a factor of about 2 for an increase from 0.25 to 6 m).

(c) The maximum volume depends on the I.D. of the main column (a factor of 3 if main columns of 0.32 and 0.17 mm I.D. are compared, both with pre-columns of 1.4 m × 0.32 mm I.D.).

TABLE II.

MAXIMUM SAMPLE VOLUMES WITHOUT BACK-FLOW FOR A NARROW-BORE CAPILLARY COLUMN (11 m × 0.17 mm I.D.)

Pre-column	*Carrier gas flow-rate (ml/min)*	*n-Hexane (μl)*	*Diethyl ether (μl)*
1.4 m × 0.32 mm I.D.	0.45	<0.5	<0.5
	1	0.5	<0.5
	4	1.5	1
0.25 m × 0.52 mm I.D.	0.45	1	<0.5
	1	1.5	1
1 m × 0.52 mm I.D.	0.45	2	<0.5
	1	3	1
6 m × 0.52 mm I.D.	0.45	3	0.5
	1	4	1

(d) Volatile solvents (such as *n*-pentane or ether) give lower maximum volumes than solvents with boiling points considerably above the column temperature.

These results suggest that wide and long pre-columns should be used. A large volume of easily accessible carrier gas ahead of the sample plug builds up less pressure if some of its volume is displaced by the sample plug, so it shows less tendency to push the sample plug backwards. The carrier gas represents a softer "spring".

The result that the volatile solvents have a greater tendency to return suggests that the evaporation of the solvent contributes to the pressure build-up at its front. If the solvent is able to saturate the carrier gas in front of the sample plug in a fraction of a second for an appreciable distance, a volatile solvent creates a significant volume of vapour that adds to the volume of already compressed carrier gas. Although there are some similarities, this situation differs from that of the returning sample material owing to the excessive evaporation rate. First, the mechanism working below the boiling point of the solvent does not reject sample material as far as out of the column (as is common for excessive evaporation). Second, losses due to excessive evaporation rates are hardly dependent on the geometry of the column and may occur even at very high carrier gas flow-rates. The rejection pressure is not built up in front of, but in the centre of the sample plug.

Large sample volumes

Sample volumes above about 4 μl accentuate the effect described above to such an extent that some sample material may be rejected back out of the column. The problem arises if a volume of liquid is introduced that is greater than the volume run off the column by the carrier gas in the same time. If 100 μl of liquid are injected within 1 sec, this is equivalent to a flow-rate of 6 ml/min, thus exceeding high carrier gas flow-rates.

If the flow-rate of the introduced liquid exceeds that of the carrier gas, the first liquid moves into the column for a certain distance until it is stopped by the compressed gas at its front. In the following moments it cannot return because of the pressure of the further liquid pushed into the column by the syringe. At the injection point a pressure is built up that may become sufficient to push the following liquid backwards through the space between the column and the needle wall out of the column.

If sample volumes of only few microlitres are injected, the liquid is hardly ever rejected far behind the tip of the syringe needle. Even if the carrier gas flow-rate is zero (a column plugged at its exit and at ambient pressure at its entrance), the front of the sample plug is not pushed backwards behind a point about 5 mm ahead of the needle tip. Under these extreme conditions the sample volume must exceed about 2 μl in order to be pushed out of the column.

Capillary forces between column and needle

If the liquid sample is rejected far enough to come into close contact with the syringe needle, some of the liquid is sucked into the narrow space between the outer needle and the column wall (Fig. 1c). The same occurs at any carrier gas flow-rates if the plunger of the syringe is pushed slowly, because the liquid is not separated from the needle tip and forms a drop. This drop touches the column wall, which

causes some liquid to be sucked back up against the flow of the carrier gas into the space between the column and the needle walls.

It is difficult to observe what happens to the liquid sucked between the column and the needle wall if the column inlet is attached to an injector. We observed it in a freely hanging column inlet. A flow of carrier gas (air) was created by applying a vacuum to the exit of the column.

The movement of the liquid into the narrow space between the needle and the column wall is rapid. In most instances the liquid did not reach the entrance of the column. It may plug the column for a short time, but the carrier gas rapidly opens a channel. The remainder of the liquid remains there for a long time, often longer than is required for the bulk of the liquid in the column to evaporate. Even *n*-pentane evaporated only extremely slowly out of the narrow space in question. When the syringe needle is withdrawn from the column, the liquid trapped in the narrow space is pulled backwards also (Fig. 1e). First the (interrupted) ring of liquid moves until its rear end reaches the column entrance. As the needle moves further up, the liquid cannot follow except for the small amount coating the outer wall of the needle, and the rear of the liquid stops moving. At the front end of the liquid, the needle tip appears to try to slip out of the liquid ring, but in vain, because the liquid does not remain as a thick film on the column wall. Most of it sticks to the needle tip and is pulled up towards the column entrance. At the column entrance all the liquid is accumulated to form a plug, even protruding, but there it breakes its contact with the needle tip. The plug is immediately pushed back into the column and most of this liquid runs back into the oven-thermostated part of the column. In the cool column section kept in the injector there just remains a sample film coating the column wall.

This detailed description explains an important fact: if some liquid is pulled backwards into the narrow space between the needle and the column wall, it does not matter how far it is sucked back up and it is not important how much liquid is affected. The liquid is pulled back to the column entrance in any case and the amount of liquid left in the cool column section (about 1.5 cm in the injector used) is dependent only on the thickness of the deposited film. Thus losses back up the column neck either occur or do not occur, and are not a matter of degree.

In the experiment with the free column inlet there were no losses of sample material out of the column if the small amount of sample hanging as a film on the outer needle wall is neglected. All the returning sample plug did was to deposit a relatively small proportion of the sample as a film in the cool column section kept in the injector. However, the situation inside the injector may differ from our experiment if there are surfaces very close to the column entrance that become available to the protruding liquid. If the liquid touches such surfaces, the syringe needle is likely to pull it fully out of the column inlet up into the valve or septum area. It is expected that only a relatively small proportion of such liquid would return into the column and that consequences on quantitative analyses would be strongly accentuated. However, evidence discussed below allowed to conclude that such losses did not occur for the injector used and glass capillary columns (with relatively thick walls).

Some chromatographic experiments

Some chromatographic experiments were carried out to establish the effect of the visually observed phenomena on real analyses. Fig. 2 concentrates on the effects that must be expected if some sample is sucked back up into the space between the column and the needle wall.

A mixture containing equal amounts of C_{10}, C_{13}, C_{18}, C_{22} and C_{26} *n*-alkanes in *n*-pentane (1:20,000) was injected on to a 6 m × 0.17 mm I.D. glass capillary column coated with 0.08 μm of SE-52. The column inlet was equipped with a 1.5 m × 0.32 mm I.D. pre-column without a coating (retention gap). Injections were carried out with the door of the oven open (28°C); after 2 min the column temperature was programmed at 15°C/min to 225°C.

Fig. 2A shows a chromatogram obtained by on-column injection of 0.5 μl of the above test solution at a carrier gas inlet pressure of 0.2 atm (hydrogen). After 30 sec the inlet pressure was increased to 0.8 atm to elute the sample more rapidly. Visual observation confirmed that at the low carrier gas flow-rate used (about 0.25 ml/min) some sample was pulled between the needle and the column wall. Some sample must therefore have been deposited in the cool column section in the injector.

The high-boiling components were expected to be lost in the cool top section of the column in the injector, whereas the more volatile material was assumed to return into the main column by evaporation. This was checked by a comparison of the peak areas of this and six identical injections with areas obtained by injections at an inlet pressure of 1 atm, for which no returning sample could be observed. Between the injections the column inlet was washed with *n*-pentane to avoid carry-over from one injection to the next. The peak areas of *n*-C_{10} and *n*-C_{13} were indeed identical for the two sets of injections. On the other hand, the injections at the low column inlet pressure produced peak areas for the *n*-C_{22} and *n*-C_{26} peaks that were 4–8% smaller than the reference peak areas where back-flow of the sample was avoided. The missing 4–8% of this material must have been lost in the cool top section of the column and on the outer wall of the syringe.

Fig. 2B shows the memory effect of the injection in Fig. 2A. After an injection of 0.5 μl of sample at 0.2 atm, the same amount of pure *n*-pentane was injected under identical conditions. Some *n*-pentane was sucked between the needle and the column wall, as the sample was previously; it was pulled back to the entrance of the column, released by the needle tip and pushed back into the column, washing down most of the material left in the cool column section by the previous injection. There are no peaks for *n*-C_{10} and *n*-C_{13}, indicating that these components left the cool inlet section during the analysis of its own sample —according to the peak areas even before the bulk of their material started the chromatographic migration. The peak areas of *n*-C_{22} and *n*-C_{26} corresponded to 4% of the areas in Fig. 2A. A second injection of pure *n*-pentane did not show any peaks for *n*-C_{18} to *n*-C_{26}.

A second experiment produced the same proportion of memory material as shown in Fig. 2B. After the first injection of the sample, the column inlet was removed from the injector and the column pressure was allowed to stabilize for 5 min, then pure *n*-pentane was pushed into the column by a syringe attached to the column with plastic tubing. The column was reinstalled, and the material washed down from the first few centimetres of the column analysed normally. This experiment confirmed that the memory effect was not caused by material that had left the column.

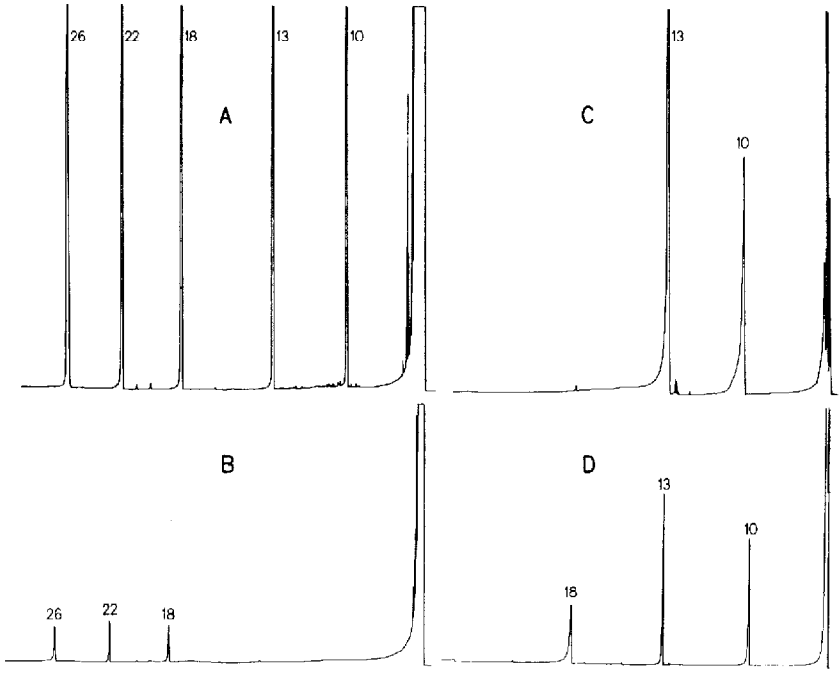

Fig. 2. Chromatographic effects due to the deposition of sample material in the inlet section of the column kept in the cool on-column injector. Peak numbers refer to *n*-alkane chain lengths. (A) Test sample of C_{10}–C_{26} *n*-alkanes in *n*-pentane. Injection of 0.5 μl at a very low carrier gas flow-rate (0.25 ml/min of hydrogen). Some of the injected liquid is drawn backwards between the needle and the column wall. Although about 5% of the sample is deposited in the cool column inlet section (1.5 cm), the peaks do not show significant tailing. However, the *n*-C_{22} and *n*-C_{26} peak areas are 4–8% too small compared with the peak areas of *n*-C_{10} and *n*-C_{13} (discrimination). (B) Memory effect seen after injection A by an injection under the same conditions as in A but using pure *n*-pentane as a sample. The *n*-C_{18}–*n*-C_{26} material of the proportion of sample A that was deposited in the cool part of the column inlet is washed back down into the oven-thermostated column by the *n*-pentane pulled backwards to the same point. A second identical injection of pure *n*-pentane no longer produced any memory signals. (C) The outer wall of the first 10 mm of the column is coated with a drop of sample (mixture as in A). In a temperature-programmed run the most volatile solutes are swept into the column, but their peaks are broad and distorted. Components with a higher boiling point follow much more slowly, during hours or days, or not at all. (D) The first 15 mm of the column, the section kept in the cool injector, is coated with sample on the internal wall, to demonstrate what happens to sample material deposited there if the syringe needle pulls some liquid backwards to the column entrance. The volatile components were carried back into the chromatographic process, but part of *n*-C_{18} and all of the higher boiling components were lost there. These losses caused the discrimination in A and the memory effect in B.

The amount of the sample material carried over from one injection to the next may be established by another consideration also. As the moving sample plug leaves behind itself a sample film of constant thickness, the proportion of the sample material left in the cool column inlet must correspond to the proportion of the cool column section on the total length of the sample-coated zone. At the low carrier gas

flow-rate used, the 0.5 μl of *n*-pentane coated 35 cm of the retention gap. The cool section in the injector was about 15 mm long, which corresponds just to the expected 4%.

The absolute size of the memory peaks were (as expected) independent of whether 0.5, 1 or 2 μl of sample were injected. This means that the losses during the analysis and the memory effects during the next run are inversely proportional to the sample size, as long as sample is pulled back up. Analyses with small sample volumes give less accurate results than with large sample volumes. On the other hand the small sample volumes seldom cause the sample plug to return to the needle tip.

Fig. 2C and D show the sample parts that return by evaporation from the injector into the oven-thermostated column. Chromatogram D refers to material deposited inside the first 15 mm of the column. The column inlet was taken out of the injector. Some sample was pushed 15 mm into the column by a syringe attached to the column by plastic tubing. This was done before the carrier gas pressure in the column was fully equilibrated to ambient pressure. Thus the sample plug left the column as soon as the pressure from the syringe was taken off. As a result, the cool top section of the column was coated as after an injection with returning sample. The components up to *n*-C_{13} left this inlet completely, and *n*-C_{18} to a considerable extent. This result was certainly influenced by the fact that the column inlet was uncoated and by the short transfer time due to the rapid temperature programming.

Fig. 2C refers to material deposited outside the first 10 mm of the column. If some sample were to be pulled out of the column by the syringe needle, it would probably be sucked by capillary forces into the narrow space of the column guide. At least part of the sample would be deposited on the outside of the column inlet. The injector used introduces the carrier gas from below the column entrance, such that volatile material on the outside of the column is carried back into the column. The column inlet was taken off the injector together with the screw and the fitting. A drop of the sample was deposited ahead of the fitting on the outer column wall, the inlet mounted back on the injector and a chromatogram (C) eluted normally. Broadened and tailing peaks of *n*-C_{10} and *n*-C_{13} are observed; the higher boiling components were not transferred into the column. Chromatogram C confirms that volatile components of sample material that left the column are at least partly carried back into the analysis, forming distorted peaks. The peaks are even broader than in Fig. 2C if the material diffuses back from dead volumes, *e.g.*, around the rotating valve or the septum. Further, corresponding peaks are often clearly separated from a sharp peak representing the material that remained inside the column.

On the injector tested, we did not find any signs that the syringe pulled sample material fully out of the column. This was tested under extreme conditions: carrier gas flow-rates of 0.1 ml/min with a column (described above) with a geometry provoking return of the sample plug, sample sizes up to 2 μl and a column temperature 5°C below the boiling point of the solvent. The most stringent tests were carried out isothermally at 145°C without secondary cooling with a test solution containing *n*-heptadecane in *n*-nonane. Both components would have returned into the column with strong delay or not at all, once having left the column.

DISCUSSION

The visual observation of the sample plug in the column inlet after on-column injection provided information on the mechanism that may cause memory effects and general or selective losses of sample. Below are listed a number of aspects where these effects may be of importance. It should be stressed that in most instances the problem is not dramatic. It was shown that with the injector tested only about 30 nl of sample were deposited in the cool column inlet kept inside the injector body, with two consequences: (a) the high-boiling components in this part of the sample are lost for the analysis and cause an according distortion of the sample composition (discrimination) if the sample contains more volatile components also, and (b) the lost material returns into the oven-thermostated column on the next injection, bringing liquid into the cool head of the column, and causes a memory effect.

Since it is a constant amount of sample that causes these problems, the effects are smaller the larger is the sample volume. For a minimum volume that may be injected accurately by on-column sampling[6] (about 0.3 μl), the troublesome sample part would constitute 10% of the total with a corresponding discrimination and memory effect. For a sample volume of 3 μl this proportion is reduced to 1%. On the other hand, a sample volume of 0.3 μl is rarely drawn backwards by the syringe needle (see Table I), whereas for 3 μl this is fairly common.

Conventional conditions

By "conventional conditions" we understand columns of I.D. around 0.3 mm and carrier gas flow-rates of several ml/min. Table I shows that under conventional conditions there may be a return of sample liquid, but it can easily be avoided. A sample volume of 1 μl may be injected without liquid pulled backwards, down to a carrier gas flow-rate of about 1.5 ml/min, provided that the temperature of the column inlet below the injection point is at least 15°C below the boiling point of the solvent. At 4 ml/min, a common carrier gas flow-rate if the "rapid" hydrogen is used, sample volumes of 2–3 μl are still "safe".

For narrower bore columns, *e.g.*, with an I.D. of 0.25 mm (requiring a syringe with a fused-silica needle of 0.17 mm O.D.), the return of some sample material is probable. This can be avoided by injections of small sample volumes and by using relatively high carrier gas flow-rates. However, the situation improves drastically if wide-bore retention gaps are fitted to the column inlet.

If the column temperature approaches the boiling point of the solvent, the sample plug tends to return to the needle tip even at relatively high carrier gas flow-rates and for small sample volumes. On-column injection at a column temperature 5–10°C above the boiling point of the solvent to avoid band broadening in space[9] requires the use of a secondary cooling or an equivalent device because this allows the sample plug to be kept inside the column inlet at least 10°C below the boiling point of the solvent while the remainder of the column is at a higher temperature.

Our previous tests on the accuracy and precision of on-column sampling[6,10] were carried out under conventional conditions, including a column temperature of around 30°C, using *n*-hexane as the solvent. Thus a return of the sample plug was excluded. As far as specified, the excellent characteristics of on-column injection reported by others in the literature were obtained under similar conditions.

Galli and Trestianu[7] reported detailed data for injections carried out at column temperatures around the boiling point of the solvent (*n*-hexane). They compared absolute and relative peak areas when injecting with and without secondary cooling. At a column temperature of 55°C the absolute peak area of the most volatile component (n-C_9) was about 1% smaller without than with secondary cooling, a discrimination (selective loss) of the high-boiling test components up to 5% was observed and the relative standard deviations of the absolute peak areas increased from 1.5 to 2.8%. At column temperatures of 65 and 70°C the results with and without secondary cooling were somewhat accentuated.

Galli and Trestianu[7] interpreted their results as being due to excessively rapid evaporation of the sample, which could be slowed by the secondary cooling. However, there was no satisfactory explanation of the fact that the observed deviations did not increase strongly when the column temperature increased from 55 to 70°C. With the background of the observations described in this paper, it is tempting to assume that there was no back-ejection by excessive evaporation but that the secondary cooling decided whether the sample plug returned to the tip of the syringe needle or not.

Injector design

The observations described in this paper indicate some points to be considered in the design of on-column injectors. The length of the cool column section inside the injector should be minimal to minimize the negative effects if sample is pulled back up to the column entrance. There is a direct relationship between the length of the cool top section of the column inlet and discrimination as well as memory effects. It would be desirable to have the column inlet fully thermostated by the oven.

The design of the injector around the column entrance requires special attention to prevent contact between liquid sample pulled back up the column inlet and the internal surfaces of the injector next to the column entrance. Such a contact would act as a bridge and would allow the syringe needle to pull the liquid from the column neck fully out of the column into the channel serving as a needle guide and towards the closing device (septum or rotating valve). To avoid this contact the column entrance should be located in a cavity with a minimal distance of perhaps 1 mm between the column and the nearest injector walls. We are aware of the fact that this contradicts the requirement for a column guide to align the column accurately with the syringe needle. The column guide usually consists of a conical cavity, becoming narrow just above the column entrance.

The amount of liquid pulled out of the column, if there is a bridge between the column and the injector, depends on the space between the needle and the column wall (or on the difference between the I.D. of the column and the O.D. of the syringe needle). The larger this space, the more liquid is pulled out of the column, which may be many times the amount deposited in the head of the column if there is no such a bridge (in the latter instance the excess of liquid returns as a plug into the column; see Fig. 1f and g). In addition to the important losses, the solvent peak is broadened, and the material pulled out of the column contributes to the "ghost" peaks often observed in on-column injection, typically eluting at column temperatures between 100 and 200°C. These "ghost" peaks are due to contaminants in the injector with a

volatility allowing them to return into the column during many days (for alkanes a range between about C_{15} and C_{20}).

The problem of the returning sample plug adds a new argument to use a secondary cooling or equivalent devices. Such a cooling should reduce the vapour pressure of the solvent at the front of the sample plug, decreasing the risk that the plug may move backwards to the needle tip. The effect is considerable (see Tables I and II) and efficient just in a range of conditions that are important for common analyses. A secondary cooling is an indispensable tool if samples are injected at column temperatures near the boiling point of the solvent (and if highly accurate results are required), but it is not a general solution to the problem of the returning sample plug.

For a number of other aspects the present subject is not important. The length of the syringe needle does not influence the losses in the cool head of the column because even a very long needle, depositing the sample far into the oven-thermostated column, cannot prevent material sucked between the needle and the column wall being pulled backwards as far as to the entrance of the column. There is no new argument on whether the injector should be equipped with a septum or a rotating valve. In particular, there is still no objection to a leakage during the injection as long as the leakage remains constant during the manipulations.

Narrow-bore columns

It has been known for a long time that a reduced column diameter increases the separation efficiency of the system. This was recently demonstrated for microbore columns in an impressive way by the Eindhoven group[11]. However, the applicability of such columns is severely hindered by injection problems. Split injections with a high splitting ratio are feasible, but preclude applications that require high sensitivity. Splitless injections are impossible because the very low flow-rates are unable to transfer a reasonable proportion of the sample from the injector to the column[12] (resulting in low sensitivity, high discrimination and poor reproducibility). It is expected that there will be little difference whether the splitless injection is carried out with a classical vapourizing injector or by the PTV.

The on-column injection technique is a promising solution for sampling at low carrier gas flow-rates. It requires a retention gap at the column inlet with an I.D. of at least 0.25 mm to take the syringe needle. Although this subject has not been studied in the necessary detail, the limitations appear to come from two sides. A long and wide-bore retention gap allows injection in a perfect manner, *i.e.*, without pulling some sample material backwards, for columns with I.D.s down to 0.20 mm, but at carrier gas flow-rates below 0.5 ml/min and column diameters below 0.15 mm it becomes difficult to prevent some sample from returning. This back-flow probably must be accepted, which is undesirable but not dramatic, because the negative effects are small compared with errors caused by other injection techniques. Second, the dead volume of the large retention gap tends to broaden the initial bands. Whether the material is sufficiently reconcentrated at the beginning of the stationary phase in the main column depends to a large extent on the depth of the retention gap[4] and on the temperature difference of the column between the injection and the elution of the components of interest.

Large sample volumes

Sample volumes above about 5 μl cannot be injected by pushing the plunger of the syringe at the maximum possible speed. First, some sample material may be pushed backwards out of the column unless the carrier gas flow-rate is very high (see above) and, second, the resistance of the liquid in the narrow-bore syringe needle increases the pressure inside the barrel to such an extent that most syringes start to leak.

If large sample volumes are injected, the flow-rate of the sample into the column must not exceed the flow-rate of the carrier gas. At a carrier gas flow-rate of 3 ml/min (measured at the column exit at ambient pressure) it is expected that about 25 μl of liquid may be introduced per second, which technically is not a serious problem. However, at a carrier gas flow-rate of 0.3 ml/min (narrow-bore columns) the injection rate is reduced to 2.5 μl/sec, which is difficult to achieve manually if the sample volume exceeds about 20 μl.

At the reduced injection speeds the sample is no longer thrown off the syringe needle. A droplet is formed that almost immediately causes some liquid to be pulled backwards between the needle and the column wall. This deficiency, however, is not important because the proportion of the sample deposited in the cool head of the column is small, *e.g.*, 0.1% for a sample volume of 30 μl. In certain instances it might be important to consider the memory effects (with a carry-over also in the range of fractions of a percent). For checking and elimination it is recommended to inject pure solvents under conditions that cause some solvent to be pulled back also.

Hydrogen as carrier gas

The phenomena discussed above show once again the importance of a high carrier gas flow-rate. The tendency of the sample to be pulled backwards is strongly reduced and large sample volumes may be injected at a higher rate. The carrier gas flow-rate is determined by the column. Hydrogen produces a similar separation efficiency at double the flow-rate compared with helium, and at triple the flow-rate compared with nitrogen. As hydrogen sensors have been available for a several years (*e.g.*, from Brechbühler, Schlieren, Switzerland, or Carlo Erba), the safety aspect should no longer preclude the best carrier gases to be used for nearly all applications (except with detection by with alkali flame-ionization detectors or in some cases with mass spectrometry).

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