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ON-COLUMN INJECTION OF LARGE SAMPLE VOLUMES USING THE RETENTION GAP TECHNIQUE IN CAPILLARY GAS CHROMATOGRAPHY

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1 INTRODUCTION

The use of long, uncoated column inlets as retention gaps[1] allows the on-column injection of much larger sample volumes than are routinely used today. The injection of volumes up to 100 μl is easy. The injection of several times larger sample volumes is also possible, but the technique becomes cumbersome and the gain in sensitivity is no longer dramatic. The injection of large sample volumes has important advantages, as discussed below.

1.1. Advantages of injecting large sample volumes

1.1.1. Increased sensitivity for analysis of volatile solutes

The injection of large sample volumes is a particularly valuable method for increasing sensitivity if the sample cannot be reconcentrated by the classical methods of solvent evaporation. If present in trace concentrations, solutes of surprisingly high boiling points are co-evaporated with the solvent. On evaporating, *e.g.*, *n*-pentane, the far less volatile naphthalene or alkanes such as *n*-hexadecane are lost to a considerable extent, even if the *n*-pentane is not evaporated to dryness.

Solvent evaporation in the column inlet prevents losses of solute material because all the injected material arrives at the detector. However, there are analogous phenomena of co-evaporation: solutes co-evaporating with the solvent form broad, distorted bands (partial solvent trapping[2]) that do not allow reasonable quantitation. The analogue of solute loss in solvent evaporation before injection is in fact peak distortion. However, co-evaporation in the column inlet occurs only for a far narrower range of solute volatilities; for instance, *n*-octane is completely retained in a layer of *n*-hexane until the latter is fully evaporated, whereas with conventional methods of solvent evaporation *n*-octane would be nearly completely lost. Further, if co-evaporation occurs (*e.g.*, for *n*-octane in *n*-heptane), the solute material can often be reconcentrated to a sharp band by the phase soaking effect[3]. Hence, mastering the solvent effects, *n*-octane can be determined quantitatively in *n*-heptane, although the two have boiling points that differ by only 25°C. In other words, solvent evaporation in the column inlet is far more selective than the classical methods of evaporation, as pointed out by Roeraade and Blomberg[4].

1.1.2 Handling of the complete sample

In trace analysis, it is often desirable to inject the whole sample in a quantitative manner. However, this is virtually impossible if conventional sample volumes are injected because the sample cannot be picked up by, *e.g.*, 2 μl of solvent. Therefore, it is common practice to work up an excessively large sample volume. After removal of the solvent used for the sample preparation (extraction, column liquid chromatography, etc.), the sample is rinsed from the walls of the round flask using 0.1–1 ml of solvent and transferred into a smaller vial. There the solvent is evaporated

again and the sample material washed from the walls, this time using perhaps only 20 μl of solvent. A 2-μl aliquot of this is injected; hence 90% of the sample is wasted. If the whole sample liquid can be handled, either ten times less sample material is needed to obtain the same sensitivity or a ten-fold increased sensitivity is achieved. The volume of solvent required for quantitatively picking up a sample from a flask is of the order of 30–100 μl, *i.e.*, in a range that can easily be handled by on-column injection into a retention gap of suitable length.

1.1.3. Convenience for sample preparation

The possibility of injecting large sample volumes often renders sample preparation more convenient. It may simply become unnecessary to reconcentrate the sample by solvent evaporation before the analysis (a step that frequently also introduces artefacts). In other instances extraction may be carried out with more solvent, improving the extraction efficiency, but primarily facilitating the separation of the extract from the extracted liquid or solid, *e g* , if the separation is hindered by the

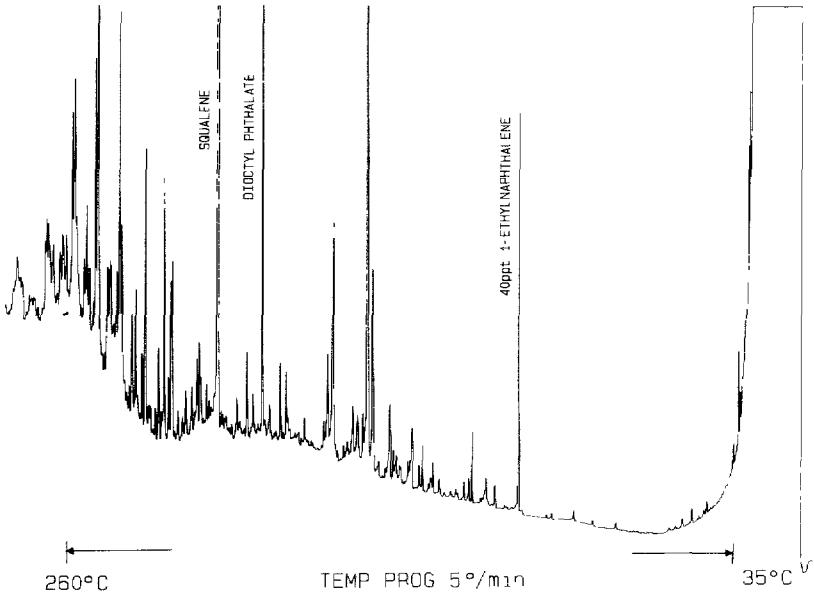

Fig 1 Analysis of higher boiling trace components in drinking water (Zürich tap water). A 1-l volume of water was extracted with 0 6 ml of pentane in a measuring flask[5] A third of the recovered extract, *i e* , 50 out of 150 μl, was injected on to a 15 m × 0 31 mm I D glass retention gap deactivated by trimethylsilylation (well wetted by pentane). This pre-column was attached to the separation column by fusing it to an intermediate piece of fused silica with an outer diameter (without polyimide coating) fitting into the butts of the retention gap and the separation column (see section 3 5). The sample was separated on a 10 m × 0.32 mm I.D glass capillary column coated with SE-54 of 0 3-μm film thickness. Carrier gas inlet pressure, 0.6 atm (H_2); injection at 25°C, followed by solvent evaporation at 35°C Owing to the high carrier gas flow-rate (7 ml/min), the solvent peak has a width of only 4 min A 40 ppt (40 ng) amount of 1-ethylnaphthalene was added to the water as an internal standard before the extraction. Injection of a 50-μl volume increased the sensitivity by a factor of 20 compared with earlier analyses carried out by the same method. From the point of view of solute volatility, reconcentration by solvent evaporation would have been possible However, it is technically difficult to evaporate the solvent down to about 6 μl, of which 2 μl were injected

formation of foam or emulsions. Another application in which the injection of large sample volumes is attractive is extraction of adsorbents or filters, particularly if used for trapping volatile components.

Fig. 1 shows an application involving a 50-μl injection of an aqueous extract.

1.2. Methods described in the literature

Vogt *et al.*[6] injected up to 250-μl volumes of liquid samples into packed inserts of a cold injector. The solvent was evaporated and vented through the split exit, then the injector was rapidly heated for elution of the solute material into the column. This technique, later termed "programmed temperature vaporizing" (PTV) injection[7] or "cold injection"[8], used in the "solvent split mode", provides a simple method of introducing large sample volumes, provided that the injector insert is sufficiently large to keep the sample. However, as in other solid injection techniques (*e.g.*, moving needle injection), volatile solutes are at least partly lost owing to co-evaporation with the vented solvent, whereby losses are known to affect solutes up to *n*-octadecane or the C_{12} methyl ester. Hence, the range of applications for such injection techniques is restricted to samples where reconcentration by conventional solvent evaporation before gas chromatographic (GC) analysis is possible also. Kirschmer and Oehme[9] described the use of a thermal desorption unit for injection of liquid samples of volume *ca.* 100 μl. The concept resembles that of PTV injection, whereby an intermediate cold trap was used for reconcentration of the solute bands.

Zlatkis and co-workers described the on-column injection of large volumes involving a trapping step and reversal of the column[10] or using a two-column system with a cold trap between them[11]. More elegantly, Fogelqvist and Larsson[12] injected up to 250 μl of a pentane extract of sea water for the analysis of C_1 and C_2 halocarbons, using conventional on-column injection into a fully coated (long) column. They were successful because they did not increase the column temperature for the elution of the solutes (no band broadening in space), and because they obtained a highly efficient phase soaking effect for reconcentration of solute bands distorted by partial solvent trapping.

Miller and Barringer[13] introduced extremely large sample volumes very slowly into a column kept at a temperature well above the boiling point of the solvent. Owing to the violent solvent evaporation in the hot column inlet, most of the sample liquid was pushed backwards out of the column inlet into the injector. The major part of the solvent evaporated there and the resulting vapours were blown through the injector head. However, the method does not seem highly reliable.

Etzweiler[14] described an injection device resembling a a very short retention gap. It contains a bulb volume of 80–200 μl into which the sample liquid flows from the bottom. The liquid cannot leave this bulb at the top, accumulates there and the solvent evaporates on bubbling carrier gas through it. Up to 100-μl volumes could be injected using this device.

1.3. Advantages of the method involving retention gaps

The method described below, involving the use of uncoated column inlets of length 10–20 m as retention gaps, has a number of advantages:

(a) It is simple in terms of instrumentation. Once the pre-column is attached to a suitable separation column, injections are carried out as usual. No special equipment other than an ordinary on-column injector is needed.

(b) The method is applicable to all types of samples. Volatile solutes are not lost during solvent evaporation as in solvent split techniques, nor are solutes eluted at elevated temperatures broadened as a result of band broadening in space.

(c) The method provides results with the same accuracy and precision as on-column injection.

(d) The background of the technique has been thoroughly investigated. Hence, its limitations and sources of problems are relatively well known and can be explained in a straightforward way

This review discusses the important aspects of the technique and provides further guidelines on the preparation of a system suitable for the injection of large sample volumes and on the chromatographic conditions required. It places considerable emphasis on possible problems, not to discourage but to help the user to recognize symptoms if problems really occur.

2 CONCEPT OF THE METHOD

First, it is essential to realize that since the introduction of split injection, most newly developed injection techniques for capillary GC violate the basic principles of chromatography concerning the initial band widths. The injection of large sample volumes violates them considerably The initial band widths, measured in terms of column length (band broadening in space) and of time (duration of solute introduction into the column), must not contribute significantly to the width of the eluted band. Further, the sample should not influence its own chromatography (overloading effect). However, the situation on injection of large sample volumes is as follows:

(a) The flow of sample liquid in the column inlet spreads the solute material easily over half of the column length.

(b) The volume of vapour resulting from sample evaporation may exceed 50 ml, corresponding to several times the internal column volume. Hence, if co-evaporation of solute with the sample solvent occurs, extremely broad initial solute bands (easily exceeding 10 min) are formed.

(c) The amount of sample liquid introduced exceeds that of the stationary phase in the whole column by factors of up to 100. Hence, in the presence of condensed solvent, the sample liquid is by far the predominant stationary phase.

Nevertheless, there is no reason to be too concerned, as the resulting problems are known and can be mastered with mostly little effort. The subjects involved are the band broadening in space and the solvent effects, the essence of which is summarized below.

2.1. Solvent effects

The introduction of the sample into the column is relatively rapid. Hence, the injection time does not affect the initial band widths (with the exception of a few rather special cases). However, the problematic step just follows the injection, *viz.*, evaporation of the sample in the column inlet. The sample liquid forms a layer on

the column wall several metres long. The solvent evaporates from the rear to the front of this layer[15], which usually requires several minutes. Co-evaporation of solute material with the solvent is undesirable, as it produces initial band widths that are mostly equal to the solvent evaporation time. The bands have a "stool" or a "chair" shape, with the "back" of the "chair" eluted last. This phenomenon of "partial solvent trapping" was discussed in detail in previous papers[16].

The problem of peak distortion due to partial solvent trapping may be solved by the selection of a better solvent that retains the solute material more strongly in the sample layer and releases it only at the end of the solvent evaporation. This release of the "fully trapped" solute material is very rapid and ensures a sharp initial band (Fig. 2). Achievement of full trapping is surprisingly easy if the solutes have a clearly higher boiling point than the solvent, but creates problems if the boiling point of the solute is less than about 30°C above that of the solvent or if there is a polarity mismatch between the solvent and solutes[17]. Full trapping requires a volatile solvent with a polarity that provides a high retention power for the solute (which does not necessarily mean same polarity as that of the solvent).

If full solvent trapping cannot be achieved, there remains the possibility of reconcentrating solute bands distorted by partial solvent trapping by use of the phase soaking effect[18]. This second solvent effect is created by swelling the stationary phase film with solvent The swollen stationary phase has a several-fold increased film thickness and an accordingly increased retention power. During a dynamic process, solute

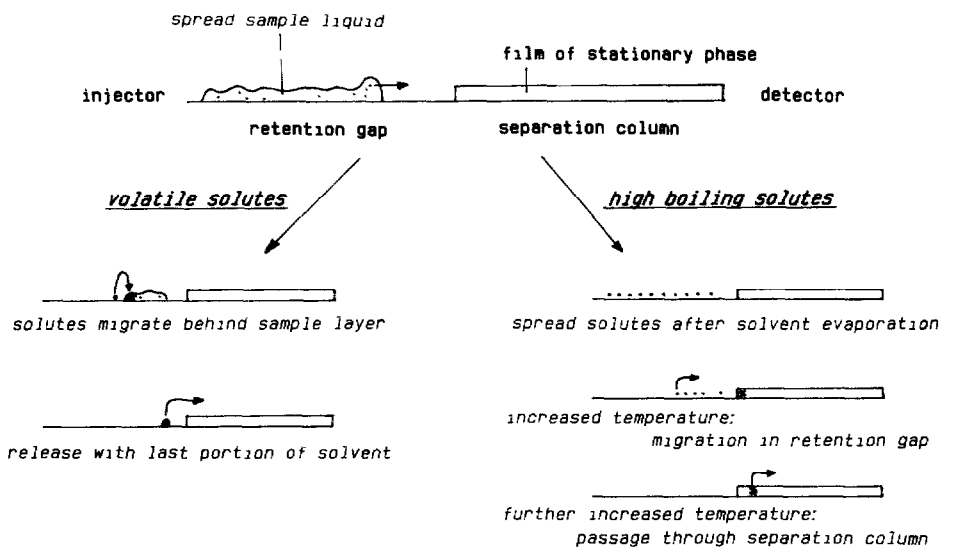

Fig 2 Effects involved in the reduction of the excessively long (broad) initial bands: injection forms a sample film in the uncoated column inlet (retention gap, one wall of which is shown schematically). Volatile and high-boiling sample constituents behave fundamentally differently: the volatile components are reconcentrated by the solvent effects, of which the solvent trapping is shown on the left. The solvent evaporates from the rear to the front of the sample layer The fully trapped solutes do not migrate noticeably within the solvent-coated inlet section, but they follow the rear of the layer and are reconcentrated at the point where the last portion of solvent evaporates. High-boiling solutes remain spread throughout the flooded zone However, as the temperature of the column is increased, they pass the retention gap much earlier than the separation column and are reconcentrated at the beginning of the separation column

band widths of several minutes were observed to be reconcentrated to a width less than 0.5 sec. The most effective reconcentration by phase soaking is achieved by using solvents that strongly retard the solute migration in the soaked stationary phase, either by strong swelling of the stationary phase film or by a shift of the polarity of the solvent–stationary phase mixture in the direction of producing a higher solute retention[18].

2.2. Band broadening in space

Sample liquid introduced by on-column injection forms a plug that closes the bore of the column. The plug is pushed into the column by the carrier gas, leaving a layer of liquid behind on the column wall[19]. It continues to move until all the sample liquid is spread as a film. This sample film is mostly too thick to be mechanically stable, and the liquid continues to flow along the wall further into the column, although with decreasing speed, until the sample solvent is evaporated (proceeding from the rear to the front of the sample layer). The volatile sample constituents follow the rear of the evaporating sample layer and are reconcentrated at the point where the last portion of solvent evaporates (Fig. 2). Solutes that do not migrate noticeably at the injection temperature, however, remain spread throughout the flooded zone. The phenomenon is called "band broadening in space" because all except volatile solutes have the same initial band width in terms of column length or column volume.

The length of the flooded zone in a coated column, and therefore of the initial solute bands, is of the order of 25 cm per microlitre sample volume injected. The maximum tolerable initial band length is between 5 and 30 cm, depending on the column length and the separation efficiency obtained. Hence the initial bands created by injection of large sample volumes must be shortened by factors up to 100.

If small sample volumes are injected, there are two possibilities of avoiding peak broadening by band broadening in space: the use of retention gaps or injection at column temperatures above the boiling point of the solvent[20]. However, for injection of sample volumes exceeding about 5 μl, there only remains the retention gap technique. This method involves the use of uncoated column inlets or pre-columns in which the sample is allowed to spread. The far lower retention power within this inlet accelerates solute migration, resulting in reconcentration of the initial bands at the beginning of the coated column part. The mechanism involved in the reconcentration effect is shown schematically in Fig. 2 as being chromatography in two steps. The solute material passes through the inlet of low retention power at a low temperature. It is stopped at the beginning of the stationary phase film, where the solute material is recombined to a sharp band. Chromatography of the solute continues only when the column temperature is further increased to give the solute the required volatility over the stationary phase. This simplified model requires some refinement, as indicated below.

The flooded zone must be restricted to the uncoated column inlet. If the sample liquid flows into the separation column, the extractable parts of the stationary phase coating (present event if the film is immobilized) are carried towards the front of the flooded zone, causing phase stripping. However, more important, the solute material spread in the separation column is not reconcentrated. Fig. 3 shows a chromatogram

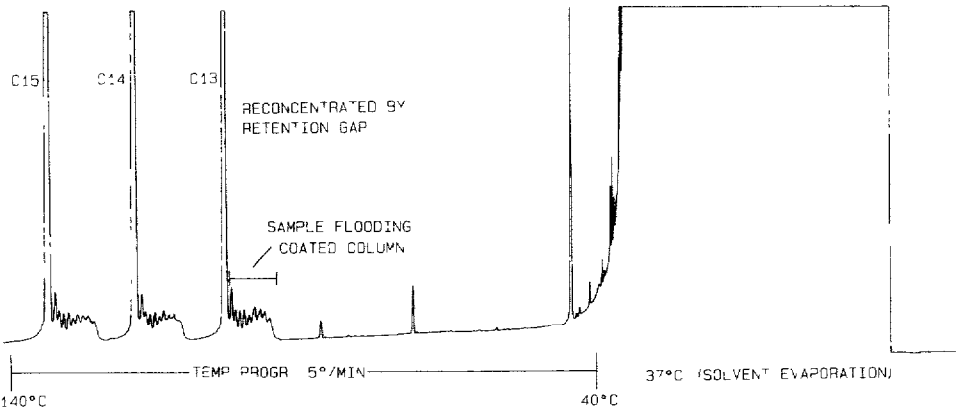

Fig 3 Effect of using retention gaps of insufficient length sample liquid penetrates into the separation column and spreads the dissolved solute material there. This material elutes earlier than the bulk of the material deposited in the retention gap because it starts chromatography from an advanced position. The reason why the early eluted material forms a band with small maxima with a constant distance between each other is discussed in the text. The finally eluted large peaks represent the solute material originally spread in the retention gap, then reconcentrated at the beginning of the separation column. A 15 m × 0 30 mm I.D glass capillary column coated with SE-54 of 0.3-μm film thickness was coupled by means of a butt connector (Carlo Erba) to a wide-bore, 10 m × 0 50 mm I D. fused-silica retention gap with a trimethylsilylated internal surface, inlet pressure (hydrogen), 0.3 atm. The coupling is not perfect as the baseline after the solvent peak does not return to the level before injection A rapid increase in the oven temperature shortly after the solvent peak would have resulted in a "hump" of the type shown in Fig 5

obtained by injection of 120 μl of a pentane solution on to a 10 m × 0.50 mm I.D. retention gap. This wide-bore retention gap was too short to keep the large volume of sample liquid from the separation column. The solute material remaining in the retention gap was reconcentrated at the entrance of the separation column and eluted as sharp peaks. The earlier eluted pre-peaks are due to solute material deposited ahead of the material forming the large peaks, *i.e.*, deposited in the separation column by the flowing sample liquid. The maxima of the pre-peaks represent material deposited in those parts of the glass capillary coils where the sample liquid tended to flow backwards against the stream of carrier gas (at those points more solvent is evaporated per unit column length, which also means deposition of more solute material). From the fact that there are nine maxima, it may be concluded that the sample material penetrated 3.5 m into the separation column or that the sample volume injected was too large by about 20 μl. In Fig. 3 the pre-peaks are obvious. However, in a complex mixture and for smaller peaks they would probably be overlooked. Hence care is required in order to detect such deficiencies.

3 TECHNICAL ASPECTS TO BE CONSIDERED

3.1. Wettability of the retention gap surface

The formation of a sample film in the retention gap is a prerequisite, which requires that the retention gap surface be wettable by the sample liquid. Samples that do not wet the internal wall of the retention gap unregularly leave droplets behind

the moving sample plug and easily flow 10–20 times further into the column than liquids that form a film (4–5 m per microlitre of liquid).

The most commonly applied methods of deactivating the support surface for non-polar capillary columns result in trimethylsilylation of the silanol groups. Such surfaces are wetted only by alkanes or ethers as solvent, but not, *e.g.*, by dichloromethane, benzene or acetone. Phenyldimethylsilylated surfaces, however, are wetted by virtually all commonly used solvents, excluding water. Methanol is spread over an elongated flooded zone, indicating critical wettability. The even more polar diphenylmethylsilylated surfaces are wetted by the same range of solvents. As their inertness is usually inferior to that of phenyldimethylsilylated surfaces, this type of deactivating retention gap is not attractive for our purpose[21].

Methanol and water are the most difficult solvents regarding wettability of retention gap surfaces. Silylated surfaces are not wetted by water or aqueous alcohols. Carbowax-deactivated capillary tubes would be expected to exhibit better wettability. However, methanol and water wash out the deactivating layer of Carbowax. Finally, it must be noted that the problem is of a fundamental nature: surfaces with a sufficiently high activity to be wetted by polar solvents tend to be active also towards the solutes in the sense of adsorptivity.

3.2. Required length of the retention gap

The uncoated column inlet must be long enough to prevent flow of sample liquid into the separation column. On the other hand, excessively long retention gaps slow the analysis and possibly contribute to peak broadening. Therefore, some guidelines are required to help to estimate the length of the flooded zone.

The length of the flooded zone is strictly proportional to the volume of injected sample liquid[22]. This allows the length of the flooded zone to be expressed as length per unit sample volume injected (cm/μl).

3.2.1. Factors affecting the length of the flooded zone

The length of the flooded zone per unit sample volume depends primarily on three factors. First, it may depend on the speed of the sample plug in the column inlet. As is well known from the dynamic coating of capillary columns with stationary phases, a slowly moving plug leaves a thin layer behind it. Accordingly it moves further than a rapidly moving plug until all its liquid is spread on the column wall, *i.e.*, the flooded zone is elongated[21]. However, above a low, critical plug velocity the thickness of the final sample layer (and hence also of its length) becomes independent of the plug velocity because the layer approaches the maximum of what is mechanically stable. In order to avoid elongated flooded zones, plug velocities below 10–15 cm/sec should be avoided.

Unfortunately, there is no straightforward relationship between the carrier gas velocity and the velocity of the sample plug. The two are not equal because of the resistance to the movement of the sample liquid and the resulting pressure drop over the plug. This pressure drop over the sample plug depends on the length of the plug, the number of included gas bubles, the nature of the sample liquid and the plug velocity,but easily reaches 0.2–0.3 atm. In order to avoid a considerable reduction in the flow-rate during sample introduction or even complete stoppage of the sample plug, elevated column head pressures should be used (see section 3.9.2).

TABLE 1

EXPECTED LENGTHS OF THE FLOODED ZONE AT VARIOUS INJECTION TEMPERATURES RELATED TO THE BOILING POINT OF THE SOLVENT

0.3 mm I.D. retention gaps, wetted by the sample liquid; conditions resulting in a plug velocity of at least 10 cm/sec are used

Injection temperature	*Length of flooded zone (cm/μl)*
>20°C below b.p.	30
5–20°C below b.p.	25
Up to 10°C above b.p.	20

Second, the length of the flooded zone depends on the inner diameter of the retention gap or rather the internal surface area per unit length of the uncoated inlet. In a wide-bore (*e.g.*, 0.5 mm I.D.) retention gap the length of the flooded zone is markedly reduced.

Third, and most important, the length of the flooded zone is influenced by the column temperature in relation to the boiling point of the solvent. At relatively high column temperatures the flooded zone is shortened owing to the accelerated solvent evaporation and the accordingly reduced time available for the flow of liquid.

3.2.2. Guidelines

Table 1 gives some values recommended as guidelines for estimating the length of the flooded zone as a function of the column temperature relative to the boiling point of the solvent. The values actually measured are usually lower, and hence the guidelines are on the safe side. Accordingly, a 20 m × 0.30 mm I.D. retention gap allows the injection of 100-μl sample volumes if solvent evaporation occurs at or slightly above the boiling point of the solvent, of 80 μl at up to 20°C below the boiling point of the solvent and of 70 μl at even lower column temperatures. However, volumes of more than 150 μl were repeatedly injected into such retention gaps using an inlet pressure of about 1.5 atm, flow-rates of around 7 ml/min and solvent evaporation temperatures 20°C above the boiling point of the solvent.

3.2.3 Visual control?

It is helpful to have direct visual control over the length of the flooded zone rather than being blindly dependent on data from the literature. Visual control, however, is possible only if glass capillary retention gaps are used. In order to permit observations at elevated oven temperatures, the oven door is left open and the oven is closed by a pane of glass. Window glass panes can be used up to around 80–100°C. At higher oven temperatures, panes of borosilicate glass are required.

3.3. Retention power in the retention gap

The reduction of the initial band length by use of retention gaps is the result of the different retention powers in the retention gap and the separation column, although the chromatographic conditions applied are also of importance (see below).

The lower the retention power in the retention gap, the more efficiently are the bands reconcentrated during the transition from the retention gap into the separation column.

The retention power in an uncoated capillary tube is obviously not zero. It depends on the pretreatment (deactivation) of the internal wall and the polarity of the solutes of interest. Such retention powers have been measured for various types of retention gaps[23], expressing retention power in terms of "apparent film thickness of an apolar stationary phase", *i.e.*, assuming that the retention power is due to a film of a non-polar stationary phase. This was preferred to the more commonly used phase ratio, β, because the latter also depends on the bore of the capillary tube, whereas it was required to describe a surface property only. Further, "apparent film thickness" allows an easy comparison with the retention power in the separation column, as the latter is usually also expressed in terms of film thickness. If polar stationary phases are involved, the increase or decrease in the retention power compared with non-polar stationary phases must be estimated, which is not too difficult if it is considered that only rough estimations are required for combining retention gaps with suitable separation columns.

Table 2 lists some approximate retention powers typically found for retention gaps deactivated by silylation with hexamethyldisilazane (HMDS) or diphenyltetramethyldisilazane (DPTMDS) or by treatment with Carbowax. Deactivation by Carbowax is simple and rapid, but the resulting retention powers are very high and the reconcentrations obtained are sufficient only if the flooded zones are relatively short. Silylated surfaces exhibit lower retention powers than the untreated surfaces whereby the trimethylsilylated (HMDS-treated) surfaces have 2–4 times lower retention powers than the phenyldimethylsilylated (DPTMDS-treated) surfaces. On the other hand, the phenyl groups may be needed in order to obtain a satisfactory wettability of the surface (see above). The values shown in Table 2 represent averages also between glass and fused-silica capillaries; the retention powers in fused-silica retention gaps tend to be clearly (up to a factor of 2) below those of glass retention gaps.

3.4. Film thickness in the separation column

The retention power in the retention gap is more or less a given quantity. If very long flooded zones (resulting from the injection of very large sample volumes) require high reconcentration factors, it may become necessary to use separation col-

TABLE 2

TYPICAL RETENTION POWERS (APPARENT FILM THICKNESSES OF A NON-POLAR STATIONARY PHASE) IN DIFFERENTLY PRE-TREATED RETENTION GAPS, MEASURED FOR DIFFERENT CLASSES OF SOLUTES

Pre-treatment	*Apparent film thickness (nm)*			
	Alkanes	*Aromatics*	*Esters*	*Alcohols*
Silylated with HMDS	0.5	0.5	0.5	1.5
Silylated with DPTMDS	2	1	2	3
Treated with Carbowax	4	8	7	27

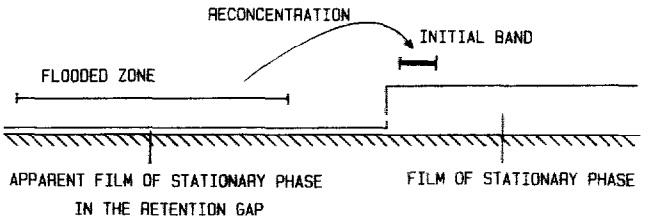

Fig. 4. Reconcentration of solute bands spread within the flooded zone occurring during the transition of the material from the uncoated inlet of low retention power (the apparent film thickness of stationary phase is a calculated unit for expressing the retention power in the inlet) to the more strongly retaining separation column. The reconcentration efficiency is related to the difference in retention powers in the two column parts.

umns of increased retention power (*i.e.*, of elevated film thickness) in order to achieve a sufficient difference in the retention powers in the two column parts. This compels us to consider more closely the required reconcentration factor and the consequences for the selection of the separation column.

3.4.1. Maximum tolerable residual initial band length

The reconcentration obtained by the transition from an inlet of low retention power into a separation column of high retention power (Fig. 4) must be sufficient to result in a residual initial band length in the separation column that does not broaden the solute peaks excessively. The maximum tolerable residual initial band length is estimated on the basis of the terminal band length[24], the length of the solute band when passing from the separation column into the detector, under conditions ensuring ideally sharp initial bands. For most applications, the initial band length may correspond to 30–60% of the terminal band length[25].

Usually, initial band lengths of around 20 cm can be accepted in the separation column. Band lengths of only 10 cm can be accepted if the ultimate of the separation power must be exploited or if the separation column is very short. On the other hand, residual initial band lengths between 50 cm and 1 m can be tolerated if the separation efficiency is not critical, if long separation columns are used or if the separation efficiency of the column is poor (*e.g.*, if wide-bore separation columns are involved).

3.4.2. Required reconcentration factor

Division of the length of the flooded zone by the tolerated residual initial band length yields the required reconcentration factor. For instance, if injections of 200-μl volumes create flooded zones of 40 m length and if the acceptable band length in the inlet of the separation column is 20 cm, the required reconcentration factor is 200.

3.4.3. Achievement of reconcentration

The mechanism of reconcentration during the passage of the solute material from the retention gap into the separation column is discussed in detail in ref. 25. As an approximation, the reconcentration may be considered as a phase-ratio focusing (a term suggested by Takeoka and Jennings[26]), assuming a reconcentration that is equal to the difference in retention powers in the two column parts[1]. This model

neglects the dead time (gas hold-up time) of the flooded zone, *i.e.*, the fact that the rear molecules need a considerable amount of time to cover the distance to the front, even if the retention gap exerts no retention power at all. Owing to this neglect, the model of phase-ratio focusing is optimistic; the real reconcentration is less effective, although the difference becomes small if the chromatographic conditions allow the solute material to pass the retention gap far below the elution temperature (see section 3.12).

Applied to the example above, the required reconcentration by a factor of 200 calls for a separation column with a retention power exceeding that of the retention gap by a factor of 200. If we further assume that the sample is dissolved in a solvent of intermediate polarity, compelling us to use a phenyldimethylsilylated retention gap with a retention power for polar solutes corresponding to an apparent film thickness of 3 nm, a non-polar separation column must be coated with a 0.6-μm thick film (possibly only 0.2 μm if a polar separation column is used). On the other hand, a sample of hydrocarbons dissolved in an aliphatic solvent could be introduced into a trimethylsilylated retention gap with a six-fold lower retention power. In this instance, the minimal film thickness in the separation column required for a sufficient reconcentration would be only 0.1 μm.

The example discussed above indicates that the retention power (film thickness) in the separation column requires special attention only if long flooded zones are involved. Solutes spread in flooded zones of length 10 m (resulting from injections ranging between 30 and 50 μl) are sufficiently reconcentrated under virtually all conditions.

The use of pre-columns with inner diameters exceeding that of the separation column presupposes enhanced reconcentration factors due to the elongation of the vapour plug when entering the narrower bore separation column. The required increase in reconcentration is equal to the ratio of the inner diameters of the two column parts[27]. However, the flooded zone per unit volume of sample is shortened when using wide-bore inlets, with the effect that there is no increase in the required reconcentration efficiency per unit sample volume injected.

A mathematical treatment[27] allows calculation of the minimal film thickness in the separation column for a given tolerance in peak broadening as a function of the length of the flooded zone, the retention power in the retention gap and the dimensions of the two column parts. However, in practice, rough estimations according to the above guidelines are sufficient.

3.5. Coupling the pre-column to the separation column

The best column equipped with a retention gap is that with the uncoated inlet section built into the same piece of capillary tube as the separation column, circumventing the need for a coupling. A relatively long piece of capillary tube (*e.g.*, 40 m) is deactivated by the appropriate silylation method (considering the wettability of the surface for the samples of interest), but only part (*e.g.*, 15 m) of this capillary is actually coated with stationary phase. This approach presupposes that the analyst prepares his own columns, which is easy, rapid and economical with today's column preparation methods.

It is impossible to discuss all the techniques for connecting pre-columns to

separation columns as proposed in recent years. We therefore only summarize our own experiences.

Shrinkable PTFE tubing still allows the easiest preparation of joints between pre-columns and separation columns; the resulting connections have the additional advantage that the pre-column may be disconnected and reconnected to the separation column without demounting the column from the instrument, just by slipping the butt out of the sleeve. This is particularly useful if the pre-column requires frequent rinsing with solvent (see below). The drawbacks of PTFE connections were discussed in ref. 28, and are related to restricted thermal stability (220°C to be safe), tailing solvent peaks, owing to solvent penetrating into and slowly returning from the PTFE, and also significant losses of solute material above about 150°C. Oxygen diffusing through the PTFE into the column is a problem only for oxygen-sensitive stationary phases such as the polyglycols. The poor mechanical stability (particularly at elevated temperatures) can easily be overcome by strengthening the joint by means of a tube, such as a glass tube taken from the tip of a Pasteur pipette.

Butt connectors, consisting of unions containing one or several ferrules, have been used successfully in many applications by many laboratories. The resulting joints are as thermostable as the columns; losses of solute material by adsorption on the ferrule material are surprisingly small. Solvent peaks are mostly acceptable although not perfect. However, the cost of such devices is still very high.

A very promising method was recently proposed by Etzweiler[29]. He melted glass capillary butts on to bare fused-silica butts, mechanically stabilizing it with a polyimide coating. The resulting connections are thermostable and have nearly perfect chromatographic properties; there is virtually no dead volume, and contact between solvent or solute material and plastic seals is excluded.

The commonly observed phenomena created by poor connections are shown schematically in Fig. 5. The solvent peak may be broadened, but often this broad-

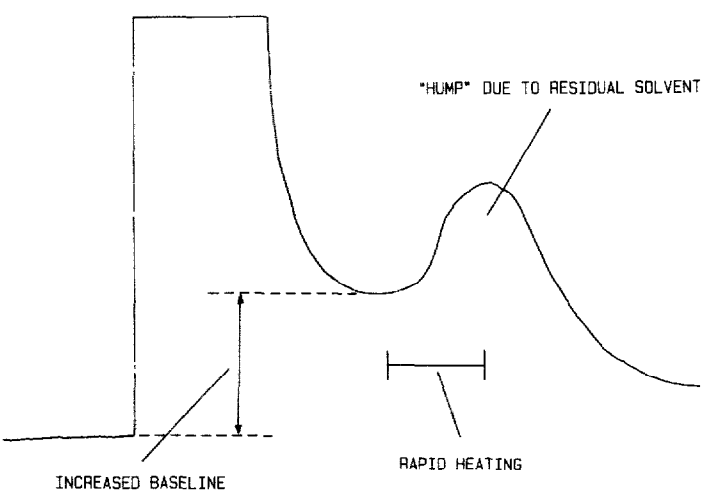

Fig. 5. Symptoms of poor connections between the pre-column and the separation column: baseline not returning to the level before injection and a "hump" created by the rapid increase in the oven temperature. Such deficiencies become more pronounced if large sample volumes are injected, presumably owing to the prolonged contact time between the solvent vapour and the adsorbing plastic material in the connection.

ening is not important. Tailing of the solvent peak also is mostly modest. However, the baseline does not return to the level before elution of the solvent peak. This is due to solvent slowly leaving a dead volume or being desorbed from plastic material (ferrule, adhesive or PTFE tubing). When the coupling is heated specifically or by heating the GC oven, a "hump" is produced owing to accelerated elution of solvent vapour from the connection. Such phenomena are virtually absent if fused couplings are used such as those proposed by Etzweiler[29]. On the other hand, it must be added that the increased baseline after the solvent elution and the "hump" created by a rapid increase in oven temperature are to a lesser extent also visible when using columns without any connections.

3.6. Cleaning of retention gaps

Involatile sample by-products remain spread within the flooded zone in the retention gap and mostly increase the retention power (and/or adsorptivity) of this section. An increased retention power, however, results in reduced reconcentration of the solute bands broadened in space at the beginning of the separation column. Solute peaks start to become broad and to tail, mostly combined with a reduction in the peak areas.

Cleaning of retention gaps by rinsing with solvent is easier than cleaning coated columns because there is no film of stationary phase preventing contact between the extraction solvent and the dirt to be removed, being adsorbed on the support surface underneath the stationary phase. It is recommended to rinse pre-columns with methanol, dichloromethane and pentane, pushing plugs of the corresponding solvents (0.5–1 ml each) one behind the other relatively slowly through the capillary tube. If washing with solvent is not successful, silylation analogous to resilylation of coated columns[30] may facilitate the dissolution and removal of the hindering material by the subsequent rinsing with solvent.

3.7. Choice of sample solvent

The choice of the sample solvent should take into account the following implications for the analysis:

(a) If solutes are analysed that elute near to the injection temperature, the solvent must be suitable for the required solvent effects, *i.e.*, providing full solvent trapping of the solutes of interest or strong phase soaking if full solvent trapping cannot be achieved (see section 2.1.).

(b) The solvent must wet the internal surface of the uncoated column inlet.

(c) Depending on the length of the uncoated column inlet, injection should be carried out near to the boiling point of the solvent in order to keep the flooded zone relatively short.

(d) Solvent evaporation in a column kept near the boiling point of the solvent accelerates volatilization of the solvent and reduces the width of the solvent peak. Injection and solvent evaporation near the boiling point of the solvent does not improve the separation of early eluted solute peaks from the solvent peak, but shortens the isothermal period required for solvent evaporation. The latter argument is not unimportant if large sample volumes are injected: at column temperatures far

below the boiling point of the solvent the solvent peak has a width that rapidly exceeds 30 min!

(e) The solvent must be acceptable for the detector. Use of benzene or toluene cannot be recommended for flame-ionization detection because of the formation of thick, black smoke. Other solvents (*e.g.*, chlorinated solvents) may create a health hazard, calling for pumping off the gases leaving the detector. Switching off the detector may be a solution to some problems However, especially for specific detectors, one should consider means of bypassing the solvent vapour.

3.8. On-column syringes for injection of large volumes

On-column syringes suitable for on-column injection of up to 100-μl volumes are available from Hamilton, whereby the fused-silica needle and the ferrule system of a 10-μl on-column syringe (701 RN FS) must be transferred to a 100-μl syringe, replacing the standard removable steel needle. Use of 0.23 mm O.D. fused-silica needles is preferable to the 0.17 mm O.D. needles because the wider bore facilitates the picking up of large volumes of liquids.

On-column syringes for injection of larger volumes are not available commercially. However, they can easily be prepared using a standard 500-μl syringe and fitting a fused-silica needle into the exit of the steel needle with adhesive.

A major problem with all syringes with removable fused-silica needles is the memory effect: sample material from previous injections returns from dead volumes or is desorbed from the plastic ferrule in the needle attachment part into the following sample. Rinsing of syringes is inefficient in cleaning dead volumes or extracting ferrules. Memory effects can be avoided by placing pure solvent in the needle attachment zone immediately after an injection. The pure solvent extracts the problematic parts, but first of all prevents sample material from diffusing into the ferrule and the dead volumes during the possibly long periods between two injections.

3.9. Injection speed

The injection speed must be adjusted to the capability of the carrier gas to carry sample liquid away from the injection point. If sample introduction is forced at excessively high speeds, sample liquid is pushed backwards out of the capillary inlet into the injector.

3.9.1. Effects due to excessively rapid injection

If sample liquid returns into the injector, three effects must be expected. The first is concerned with the solvent peak: a substantial amount of sample liquid remains in the dead volumes or as a film on the surfaces in the injector. The solvent evaporates from there and is rinsed back into the column during an extended period of time, creating solvent peaks of the type shown in Fig. 6. The solvent peak is broadened to a width easily exceeding 30 min, and the pen returns only slowly, resulting in broad tailing. If the carrier gas supply enters the injector below the column inlet, the zone creating problems is located above the column entrance including the valve area. In this event, return of the pen to the baseline is achieved by opening the valve, creating a modest leak that causes the solvent residues to be purged from

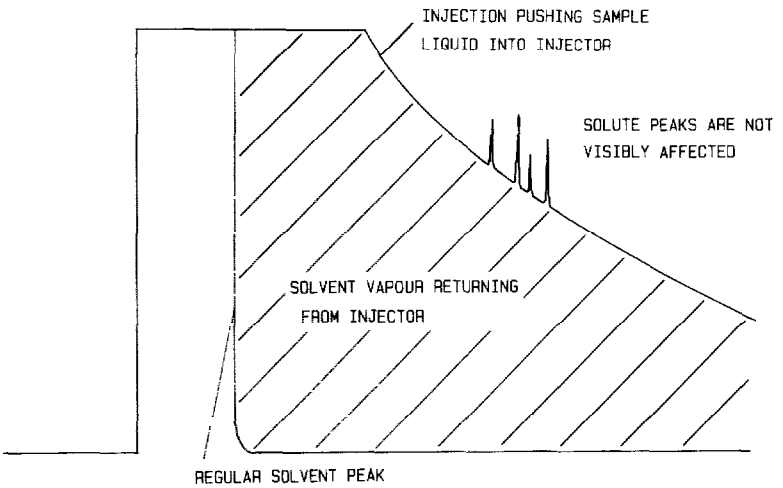

Fig 6 Solvent peak after an excessively rapid injection if the sample liquid is introduced more rapidly than the carrier gas is able to carry away liquid further into the column, some of the liquid is pushed through the narrow space between the needle and the column wall backwards out of the column inlet into the injector If the injector is not equipped with a built-in purge, such a back-flow is immediately detected by the shape of the solvent peak, which becomes broad and tailing.

the injector rather than allowing them to diffuse into the carrier gas stream entering the column. Often a small purge flow-rate must be maintained over a considerable period of time until a low and stable baseline is restored. The effects of returning sample liquid on the solvent peak are drastic because only the (tailing) base of the solvent peak is shown on the chart paper. Volatile solutes behave in the same manner, but owing to their much smaller size, peak distortion is not visible.

The two other effects caused by sample liquid pushed backwards into the injector are related to losses of higher boiling solute material in the cooled injector. This material is lost from the analysis, resulting in smaller solute peaks but also in discrimination of the later eluted components if mixtures are analysed that contain solutes with a wide range of volatilities. However, such material is not lost for ever: it is collected by the subsequent injections bringing sample liquid into the injector. As some of the liquid returns into the column, high-boiling material is rinsed back into the separation column. As this washing effect is inefficient, the memory effects are observed over many injections. Solutes of intermediate volatility (typically eluting between 150 and 230°C from standard non-polar columns) also return via the gas phase.

Memory effects due to transport through the gas phase can be reduced by heating the oven (and the injector) to an elevated temperature and keeping the injector opened. More efficient, however, is rinsing with solvent: the capillary column and the ferrule of the column attachement are dismounted. A small carrier gas flow-rate from the regulator into the injector precludes solvent penetrating into the gas supply line and the manometer. Then several millilitres of solvent, *e.g.*, dichloromethane, are passed through the injector.

3.9 2. Resistance to the movement of the sample plugs

For a better understanding of the background determining the suitable injection speed, the events during the injection process must be considered more carefully (Fig. 7). A 50-μl volume of liquid forming a single plug fills a 0.3 mm I.D. capillary to a length of about 70 cm. However, in practice this sample liquid is split into many smaller plugs: in order to rule out back-flow into the injector, the flow of sample liquid leaving the syringe needle must be below the flow rate leaving the injection point. Therefore, the sample plug is interrupted by numerous gas bubbles of various sizes. The first plugs of the sample liquid still move at a speed near to the linear velocity of the carrier gas, but on continuing the sample introduction, this speed is reduced as it becomes more difficult for the carrier gas to push the longer train of liquid plugs and air bubbles further into the column.

At a carrier gas flow-rate (before injection) of 3 ml/min, a gas volume of 50 μl passes the exit of the syringe needle per second. If this flow-rate could be maintained, a 50-μl volume of liquid could be injected within 1 sec. However, this speed is possible at best during the first moments of the injection.

No specific data can be given on the reduction of the flow-rate at the injection point as a function of the sample volume injected, the column inner diameter and the previously adjusted carrier gas flow-rate. As a simplification, it may be assumed that the speed of a long sequence of sample plugs depends on the carrier gas inlet pressure rather than its flow-rate. The higher the inlet pressure, the greater may be the pressure drop over the plugs of liquid, still leaving a pressure drop over the

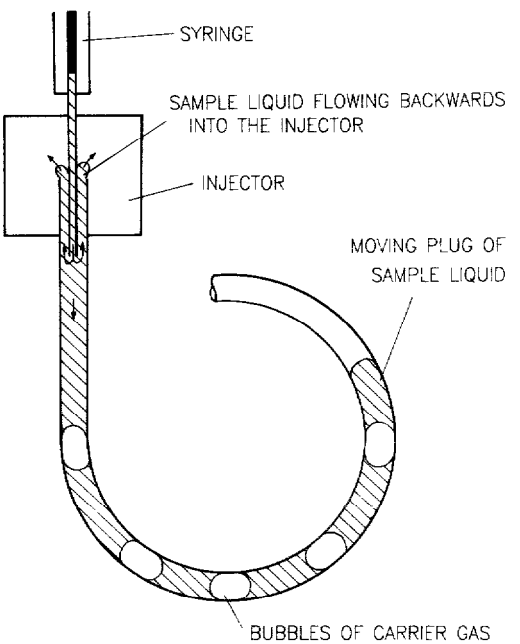

Fig. 7. Return of sample liquid into the injector upon excessively rapid injection. The flow of sample liquid leaving the syringe needle must be restricted to that which the carrier gas can move further into the column.

remainder of the column, which ensures that the carrier gas ahead of the sample plugs continues to flow through the column. In fact, if the carrier gas inlet pressure is low, the plugs of liquid may come to a complete stop, regardless of the carrier gas flow-rate before injection. On the other hand, the flow-rate is only slightly reduced if the inlet pressure is high.

Injection must be extremely slow to create some negative effects. There is no band broadening due to slow injection because of the solvent effects. Furthermore, sample evaporation on the needle tip, resulting in deposition of high-boiling solute material on the needle and their removal from the column inlet[31], becomes important only if extremely slow injection is combined with a high gas flow-rate and a column temperature near the boiling point of the solvent.

Injection of large sample volumes always results in return of the sample liquid to the needle tip and liquid being sucked into the narrow space between the needle and the column wall above the injection point[32]. However, losses of solute material are negligible, and memory effects are also small.

3.9.3. Practical recommendations

As a rule of thumb stemming from practical experience, the carrier gas inlet pressure for a 50-μl injection should be at least 0 5 atm and for a 100-μl injection at least 0.8 atm. At the beginning of the injection the flow of sample liquid released from the syringe needle may approach the carrier gas flow-rate before injection, corresponding to several tens of microlitres per second. Later, the speed must be reduced to around 5–10 μl/sec. It is important to depress the plunger in a smooth fashion. Steps introducing more than about 5 μl at a time easily cause back-flow of liquid into the injector

On-column injectors equipped with a well designed, permanent injector purge allow perfectly shaped solvent peaks to be produced under nearly all circumstances. However, their effect is "cosmetic". At least for some injections it is advisable to close the purge exit (and to leave it closed until the solvent peak is fully eluted) in order to ensure that the injections are carried out correctly.

3.10. Injection temperature

The flooded zone is the shorter the higher is the column temperature during the flooding process (see section 3.2.1), which allows a further increase in the sample volume injected or the use of a shorter retention gap. Further, high temperatures accelerate evaporation of the solvent, reducing the width of the solvent peak. However, what is the maximum tolerable injection temperature?

We distinguish between the injection temperature, *i.e.*, the temperature during the sample introduction, and the solvent evaporation temperature because their selection is guided by somewhat different arguments.

At injection temperatures considerably below the boiling point of the solvent the injected sample liquid must replace an equivalent volume of carrier gas (which forces one to inject at a reduced speed). At injection temperatures near the boiling point of the solvent, the volume of carrier gas to be replaced increases by the volume of solvent vapour diffusing into the carrier gas at the front and rear of each plug of sample liquid. This solvent vapour diffusing into the bubbles of gas between the plugs

of liquid elongate the latter and compel one to inject more slowly to allow the extra volume to run off the front of the flooded zone. On approaching the boiling point of the solvent at the column inlet pressure (which is above the boiling point of the solvent at ambient pressure), the vapour pressure of the solvent becomes large and renders injection very difficult. Above the column temperature corresponding to the actual boiling point of the solvent under the carrier gas inlet pressure, injection becomes impossible because the gas bubbles continuously expand and push the injected sample liquid backwards out of the column inlet. If large sample volumes are injected, secondary cooling or other systems for independently cooling a section of the column inlet are no longer of any help: the injection temperature must be below the boiling point of the sample at the gas inlet pressure[33].

The column temperature during the introduction of the sample liquid should be at least 20°C below the actual boiling point of the sample (solvent). This means keeping the column about 15°C below the boiling point of the solvent at ambient pressure if the carrier gas inlet pressure is relatively low and to keep it near the boiling point of the solvent if the inlet pressure is at least 2 atm.

3.11. Solvent evaporation

An 80-μl volume of solvent produces about 20 ml of vapour (about 50 ml for a low-molecular-weight solvent such as methanol). If the gas flow-rate through the column is assumed to be 5 ml/min and if the solvent vapour were to replace the carrier gas in the column completely, the solvent evaporation would take 4–10 min. On the other hand, if the solvent vapour constitutes only 20% of the gas passing through the column and if the carrier gas flow-rate is only 1 ml/min, solvent evaporation would take 100–250 min. This demonstrates that the conditions used during solvent evaporation deserve special attention.

The rate of evaporation of a given solvent in the column inlet depends on the flow-rate through the column (which may differ considerably from the carrier gas flow-rate adjusted previously owing to the changed viscosity of the gas–vapour mixture). Further, it depends on the proportion of solvent vapour in the gas passing through the column, this proportion corresponding to the partial vapour pressure of the solvent at the actual column temperature. Theoretically, the solvent vapour could

TABLE 3

RECOMMENDED MAXIMUM COLUMN TEMPERATURE DURING SOLVENT EVAPORATION IN THE COLUMN INLET AS A FUNCTION OF THE CARRIER GAS INLET PRESSURE: DIFFERENCES BETWEEN THE SOLVENT EVAPORATION TEMPERATURE AND THE BOILING POINT OF THE SOLVENT AT AMBIENT PRESSURE

Inlet pressure (atm)	*Temperature difference (°C)*
0 5	0
1 0	+ 8
1.5	+15
2 0	+20
3.0	+30

completely replace the carrier gas. Undiluted solvent vapour would pass through the column if the vapour pressure of the solvent is equal to the inlet pressure of the carrier gas, *i.e.*, if the column temperature corresponds to the actual boiling point of the sample (solvent). However, this is not realistic: the vapour pressure of the solvent must remain below the inlet pressure of the gas in order to prevent back-flow of sample material into the injector.

Recommended differences between the evaporation temperature of the solvent and the boiling point of the solvent at ambient pressure are listed in Table 3 as a function of the carrier gas inlet pressure. This solvent evaporation temperature must be maintained until the solvent is completely evaporated. As the end of the solvent evaporation cannot be easily detected, it is recommended that the temperature is maintained until the solvent peak is completely eluted. This period of time is in excess of what is required by the dead time of the non-flooded part of the column and the width of the solvent peak related to the phase soaking process of the last solvent passing through the column. However, this extra time is comparatively small.

3.12. Rate of temperature increase

This section is of interest only if long retention gaps (exceeding 10–20 m) are used and the carrier gas velocities applied in the retention gap are below about 30 cm/sec.

In section 3.4 it was mentioned that the reconcentration obtained by using retention gaps also depends on the chromatographic conditions applied. This dependence on the operational parameters is related to the dead time of the flooded column inlet section, *i.e.*, the time required by the rear solute material to cover the distance to the front of the flooded zone, even if the solute is not retained by the surface of the uncoated pre-column. This dead time, which easily exceeds 1 min if long flooded zones and low carrier gas velocities are involved, creates a corresponding peak broadening unless the band is reconcentrated by a type of column-internal cold trapping effect. It was shown[34] that the peak broadening due to the dead time of the flooded column inlet section becomes negligible if the solute material is transferred to the entrance of the separation column at least about 80–120°C below the elution temperature. This, however, may compel us to adjust the temperature increase in analytical runs to the speed of the solute migration through the retention gap.

As an example, a solute is assumed to be eluted from the separation column at 250°C and solvent evaporation is carried out at 50°C. The most rapid analysis of this solute would involve ballistic heating from the solvent evaporation temperature to the elution temperature immediately after completion of solvent evaporation in the column inlet. However, when at what temperature is the solute passed through the retention gap?

At low temperatures the solute does not move within the retention gap because, despite of the absence of stationary phase, the retention by the internal walls is excessive. It is realistic to assume that the solute is chromatographed through the uncoated inlet with a capacity ratio, k, between 2 and 5 at a temperature of about 150°C. If the retention gap is assumed to be 40 m long and if the average linear gas velocity within this inlet is 40 cm/sec, several minutes are required to pass the solute through the inlet. However, during ballistic heating (40°C/min for a powerful oven),

the column is at about 150°C only for a few tens of seconds. As a result, the last solute material arrives at the entrance of the separation column near to (in more extreme cases even at) the elution temperature (250°C). At these high temperatures the most advanced solute material (from the front of the flooded zone) migrates into the separation column instead of "waiting" for the rear solute material at the entrance of the separation column, and the resulting peak is broadened.

If the retention gap is only 20 m long and the carrier gas velocity is 80 cm/sec, the solute passes through the uncoated inlet within about 1 min (again assuming a temperature at which there is some retention in the inlet). This allows transfer of all the solute material to the entrance of the separation column at sufficiently low temperatures even if ballistic heating is applied

The transfer of solute through the uncoated inlet requires special consideration if the inlet is long, if its inner diameter exceeds that of the separation column (resulting in a low carrier gas velocity in the inlet) and if the carrier gas flow-rate is low. The problem can be solved in two ways. First, a temperature programme is applied, starting at the solvent evaporation temperature or at least 120°C below the elution temperature; the programme rate must be adjusted to the longer of the two column parts. In this way the solute selects a suitable temperature for passing through the retention gap by itself. Second, the solute may be transferred to the entrance of the separation column by an intermediate isothermal step at a temperature 80–120°C below the elution temperature. The latter method results in shorter analysis times because heating may be carried out ballistically, but the determination of a suitable intermediate temperature and of the duration of this step is more demanding, usually requiring some experimentation. Further, this method is only suitable for samples containing solutes with a narrow range of elution temperatures.

3.13. Carrier gas flow-rate

The rate of evaporation of the solvent in the column inlet is proportional to the carrier gas flow-rate. In fact, at low carrier gas flow-rates solvent evaporation may become unreasonably time consuming. Further, the dead time of the flooded zone becomes a hindering factor if the solutes are slowly transferred through the uncoated inlet. Hence, there is considerable interest in a high carrier gas flow-rate.

The most important factor determining the applicable carrier gas flow-rate is the type of carrier gas used. Hydrogen is much preferred to helium or nitrogen, first because the flow-rate providing the maximum separation efficiency is twice or three times that of the alternative gases, and second an increase in the flow-rate beyond the optimum results in the smallest reduction in the separation efficiency if hydrogen is used. For 0.3 mm I.D. capillaries, carrier gas velocities of up to several metres per second can be used without sacrificing much in terms of column efficiency.

The applicable carrier gas flow-rate also increases if the column inner diameter is increased, but not linearly. The optimal flow-rate through a 0.5 mm I.D. column is not much above that of a 0.3 mm I.D. column, and excessive flow-rates cause a particularly rapid reduction in column efficiency if wide-bore columns are used. If flow-rates, of, *e.g.*, 10 ml/min are required, 0.3 mm I.D. capillaries are still preferable to the 0.5 mm I.D. tubes.

The 0.3 mm I.D. capillaries are optimal for our purpose also because of the

pressure drop along the column they create the desirable high pressure drop for the fairly high flow-rate required. For instance, 20-m separation columns equipped with 20–30-m retention gaps can be used with a 2 atm inlet pressure (hydrogen).

3.14. Flow-regulated carrier gas supply?

The main advantage of a flow-regulated carrier gas supply is facilitated solvent evaporation. First, flow regulation precludes a back-flow of sample material upon excessive temperature increase after completion of the sample introduction causing (which may occur, *e.g.*, if the boiling point of the sample is considerably below that of the pure solvent). The flow regulator reacts to high solvent vapour pressures by an increase in the inlet pressure.

Second, flow regulation allows accelerated solvent evaporation owing to the possiblity of using high solvent evaporation temperatures. The regulated flow of carrier gas may force a large volume of solvent vapour through the capillary, provided that the gas pressure delivered to the instrument allows the flow regulator to supply the necessary inlet pressure. Experiments with strongly forced conditions, however, indicated that exaggerated conditions may affect the solvent effects. Third, flow regulation allows reasonably short solvent evaporation times (narrow solvent peaks) to be achieved even with relatively low carrier gas flow-rates, as a large volume of solvent vapour may be moved with a small carrier gas flow-rate.

However, flow regulation is not capable of preventing a back-flow of sample liquid into the injector upon injection at excessively high column temperature or at excessive speed because it reacts too slowly. Injection still requires column temperatures clearly below the boiling point of the solvent and the injection speed cannot be substantially increased. After completion of the sample introduction, the column temperature is increased to the solvent evaporation temperature by programming at 10–30°C/min, adjusted to the capability of the flow regulator to fill the gas volume behind the column inlet with the necessary gas pressure (thus depending on the regulated flow-rate and the gas volume between the regulator and the column entrance).

4 SUMMARY OF RECOMMENDATIONS

The above sections explain the background of the technique and of the guidelines concerning the conditions to be adopted. Below we summarize these guidelines by suggesting a set-up to start with.

A 15–20 m × 0.30–0.35 mm I.D. pre-column of glass or fused silica, deactivated by a phenyldimethylsilylating reagent, is coupled by a butt connector to a separation column of about 0.3 mm I.D. and with a length that can vary between 10 and 60 m, coated with any stationary phase of at least 0.4-μm film thickness. Hydrogen is used as the carrier gas at an inlet pressure of 0.8 atm if the separation column has a length of 10 m and up to 2.5 atm if the separation column is very long.

The 15-m retention gap allows the injection of up to 50-μl volumes of any samples except solutions in methanol and water at any temperature below the boiling point of the solvent For solutions in methanol the maximum sample volume is only 30 μl; aqueous solutions cannot be handled in this way. If the sample is introduced at a temperature 5°C below the boiling point of the solvent at ambient pressure using

a 0.8-atm inlet pressure, or 10°C above the boiling point of the solvent at a 1.5–2.5-atm pressure, the maximum sample volume can be increased to 80 μl. For 20-m retention gaps the corresponding sample volumes are 70 and 100 μl. This does not mean that this composite column can only be used for injections of large sample volumes — even split injection is possible.

For the introduction of 50–100-μl volumes of liquid, one starts with an injection speed of at most 20 μl/sec and reduces it to 5 μl/sec towards the end of the injection. After completion of the sample introduction the oven temperature may be increased by 10°C above the the maximum injection temperature given above. This temperature must be maintained until the pen of the recordeer returns from the solvent peak.

If dirty samples are analysed, the pre-column requires frequent rinsing.

The above guidelines contain considerable safety margins, which should ensure the success of the experiments.

5. CONCLUSION

There is no natural law which states that the ideal sample volumes of on-column injections are between 0.5 and 3 μl. On the one hand, even these amounts of sample liquid severely overload capillary columns, compelling us to rely on reconcentration by solvent effects or measures against peak broadening due to band broadening in space. On the other hand, as efficient techniques are available for solving

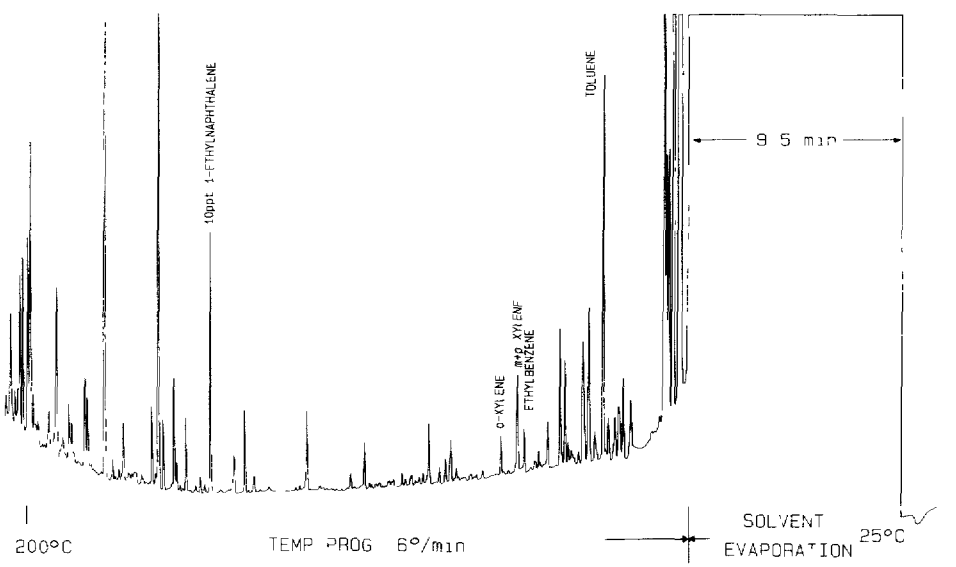

Fig 8 Pentane extract of Zurich drinking water prepared as the extract in Fig 1 The water, containing less than 20 ppt of gasoline, was spiked with 100 ppt (100 ng/l) of gasoline and 10 ppt of internal standard (1-ethylnaphthalene), 80 μl were injected on to a 20 m × 0 32 mm I D. glass capillary coated with 1 5 μm of immobilized PS-255 (a methylsilicone), equipped with a 20 m × 0 32 mm I D. glass retention gap, leached and silylated with HMDS as described previously[35] The two column parts were coupled by fusing their butts on to a 10 cm × 0 20 mm I.D fused-silica capillary Inlet pressure (hydrogen), 0.8 atm.

these problems, why should one not exploit them much better than is currently done? If we could lose the habit of injecting sample volumes of around 1 μl, how many analyses could be facilitated by injecting 10–50 times larger sample volumes?

Fig. 8. shows the analysis of an extract of drinking water spiked with 100 ppt (100 ng/l) of gasoline and 10 ppt of 1-ethylnaphthalene (internal standard). The injection of large volumes increased the sensitivity obtained by a factor of 40 and provided a very rapid method for determinations of trace concentrations of gasoline in ground water.

5.1. Broad solvent peaks obscuring solute peaks?

Solvent peaks become considerably broadened on injection of large sample volumes, owing to the necessity to transfer all the solvent through the column to obtain the required solvent effects Such broad solvent peaks may create the impression that many solute peaks may be obscured. However, at least for fully trapped solutes (the majority of solutes) this is not true, as shown in Fig. 9, where the early peaks of a 100-μl on-column injection are at least as well separated from the solvent peak as by split injection of a 3000-fold more concentrated solution using the separation column without the uncoated pre-column. Peaks of solutes that are only partially trapped and reconcentrated in the separation column by the phase soaking effect are not as efficiently shifted to higher retention times. However, they are still not affected by the broad solvent peak.

Another problem that must be faced is the effect of the sample on the absolute

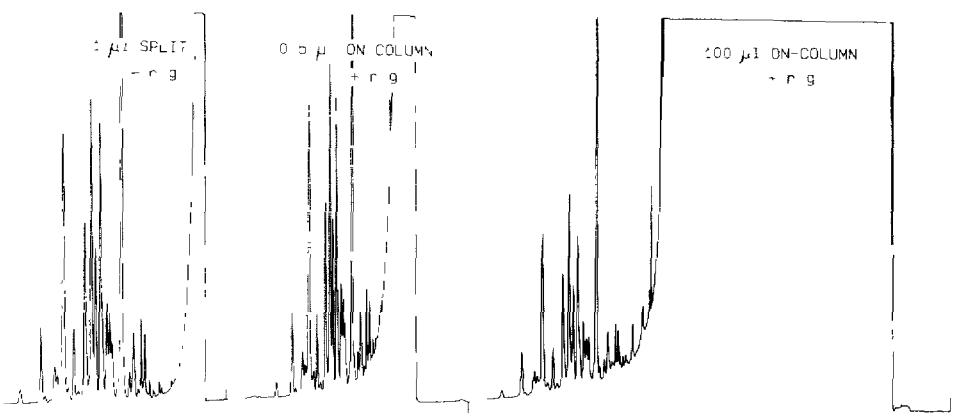

Fig 9 Do broad solvent peaks obscure early solute peaks? "Nonane fraction", a mixture of isomeric C_9 and C_{10} alkanes, in pentane Left chromatogram split injection on to a 12 m × 0 32 mm I D glass capillary column coated with OV-73 of 0.3-μm film thickness Centre chromatogram 0 5 μl on-column injection on to the same separation column equipped with a wide-bore, 10 m × 0 50 mm I.D. pre-column (butt connector), it is a very similar chromatogram except that the solvent peak is broadened owing to longitudinal diffusion in the retention gap (r.g.) (having an internal volume exceeding that of the separation column by a factor more than two) The 100-μl injection resulted in improved separation of the early peaks compared with the 0.5-μl injection owing to the solvent trapping process the solutes were released near the exit of the pre-column at the end of the solvent evaporation period (see Fig 2). Isothermal runs at 40°C with the exception that the 100-μl volume was introduced at ambient temperature (returning to 40°C after withdrawal of the syringe).

retention times, primarily of the early eluted peaks. The retention time of the major peak in Fig. 9 is at least doubled if the sample volume is increased from 0.5 to 100 μl. As in many other applications in capillary GC, it is strongly recommended to use relative retention times.

5.2. Preparation of retention gap capillaries

The preparation of capillary columns was a mysterious task for many years and this outdated impression sometimes still leads to the opinion that the preparation of columns should be left to commercial producers. The availability of commercial columns is indispensable (and the columns available today are indeed excellent compared with the often very poorly made glass capillaries on the market a few years ago). However, laboratories with a high consumption of columns should reconsider preparing columns themselves because of the arguments put forward by Grob[36] and also because it is highly economical and allows one to have many more columns than could be purchased.

The use of long retention gaps is another strong argument favouring the treatment of capillaries in the laboratory. The price of commercial fused-silica retention gap tubing inevitably is high. Fused-silica retention gaps are convenient if frequent removal of a contaminated inlet is necessary, as glass retention gaps necessitate straightening of the inlet whenever a piece of the pre-column is removed. However, if longer retention gaps are used for injection of large sample volumes, this practice is no longer possible as fused silica becomes an unreasonably expensive form of capillary tubing. Glass capillary retention gaps are of almost the same quality and, if prepared by the user, are available in almost unlimited numbers. Deactivation can be carried out with long pieces and the actual work involved in the preparation of a pre-column 100-m length is about 2 h.

Deactivation methods for uncoated pre-columns are identical with those used for the preparation of coated columns[35]. It is therefore an obvious step to coat part of the deactivated tube to produce a tailor-made column for injection of large sample volumes. It is unlikely that columns with built-in retention gaps for large injections will be available from stock for a wide range of interesting applications because there are far too many parameters to be adjusted: the type of stationary phase, the film thickness of the stationary phase, deactivation by trimethylsilylation or phenyldimethylsilylation, the length of the retention gap and the separation column and possibly their inner diameters.

6. SUMMARY

The technique of injecting liquid samples of volumes up to more than 100 μl is described. It relies on the reconcentration of the solute bands by well known mechanisms: the solvent effects for volatile sample components and the retention gap technique for higher boiling solutes. The involved technical parameters are discussed and recommendations given concerning selection of the retention gap, separation column, injection speed and chromatographic conditions.

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