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REVIEW

UNCOATED CAPILLARY COLUMN INLETS (RETENTION GAPS) IN GAS CHROMATOGRAPHY

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CONTENTS

1. INTRODUCTION

During the last few years, the use of uncoated capillary column inlets, usually consisting of pre-columns, has become important. Uncoated inlets or retention gaps are used for very different purposes, which are described in this review. Depending on the purpose of their use, the characteristics of pre-columns may be fairly critical and, as there are unsuitable pre-column materials on the market, the user should know what to ask for.

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The use of pre-columns is fairly new, starting around 1982[1]. However, Grob Sr. prepared capillary columns with uncoated end sections in 1960s, primarily in order to overcome problems related to overheating of the stationary phase in the column parts kept inside the injector and the detector block, and to avoid phase stripping due to flooding of the sample liquid. Many of the early commercial capillary columns were equipped with end sections free of stationary phase, but this changed with the introduction of commercial fused-silica capillary columns. The latter are mostly fully coated, in most instances even with an immobilized stationary phase, ruling out extraction of the stationary phase from the end sections.

2. FOUR PURPOSES FOR USING UNCOATED INLETS

2.1. Analysis of dirty samples

On-column injection introduces all the sample material, including non-volatile by-products, into the oven-thermostated column inlet. Non-volatile by-products, however, may cause deterioration of the column performance, as described below. Using vaporizing injection, only a small fraction of the non-volatile sample by-products enters the column. Hence, problems are likely to occur only with dirty samples or after injecting a larger number of samples. However, the phenomena that occur are basically the same.

The mechanisms that cause deterioration of the column performance are described below in some detail in order to show the extent to which pre-columns can solve the problems due to non-volatile sample by-products. However, before doing this, it is important to consider where the non-volatile materials are located within the column inlet. One must distinguish between injections causing flooding of the column inlet by the sample liquid (on-column injection and splitless injection under conditions causing solvent recondensation) and those which do not (split injection, splitless injection without solvent recondensation). In the latter instance non-volatile sample materials, entering the column as aerosols, are deposited in the first 3–10 cm of the column inlet. In the former instance, however, the flowing sample liquid carries the non-volatile material as far as the column inlet is flooded (Fig. 1A). Non-volatile materials remain at the location where they are deposited by the evaporating solvent (Fig. 1B). From the phenomenon of band broadening in space[2] we know that a film of limited homogeneity is formed. The material may be redissolved by the sample liquid introduced by a subsequent injection (Fig. 1D) and carried further towards the front of the flooded zone, with the effect that soluble materials are accumulated at the end of the flooded zone (as a droplet if injection volumes and injection conditions are reproduced), whereas poorly soluble materials remain spread throughout the flooded zone.

2.1.1. Increased retention power. Non-volatile sample by-products behaving as a liquid act as a stationary phase, retaining solute material in the column inlet. This extra retention causes broadening or distortion (tailing or splitting) of solute peaks by two mechanisms. First, the sample by-products are unevenly distributed (accumulated at the front end of the flooded zone, forming droplets due to the poor wettability of the surface of the stationary phase film). Molecules of the solutes of interest are chromatographed differently, depending on the location at which they are partitioning. Second, a film of retaining sample material accentuates band broad-

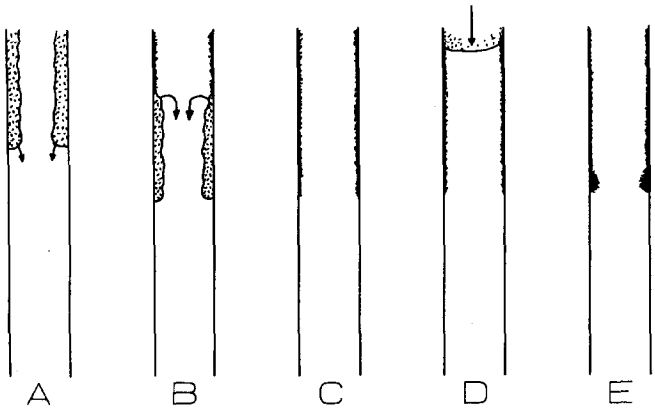

Fig. 1. Deposition of non-volatile sample by-products in the column inlet. The sample liquid flows first as a plug and subsequently along the capillary wall into the column (20–30 cm/μl of injected liquid) (A). Solvent evaporates from the rear to the front of the flooded zone (B), leaving non-volatile material as a more or less homogeneous layer on the capillary wall (C), provided that it wets the surface of the wall. The subsequent injection may redissolve the non-volatile material and carry it further into the column (D), with the result that such materials accumulate at the front of the flooded zone (E).

ening in space, reversing the reconcentration mechanism obtained when using a retention gap[3]. A few micrograms of retaining sample by-products (materials behaving as a liquid) are usually sufficient to affect noticeably the peak shapes.

The use of uncoated column inlets (with lengths at least corresponding to that of the flooded zone) reduces the effect of retaining sample by-products on the peak shapes. The uncoated inlet may be coated with an amount of sample by-products exhibiting a retention power similar to that of the stationary phase in the coated column without risking peak distortion — the retention gap must be "filled in" with dirt before the introduction of further amounts of dirt, which may cause peak distortion.

Baktir *et al.*[4] demonstrated the usefulness of uncoated inlets for the determination of pharmaceuticals in plasma. Whereas the column already eluted strongly broadened peaks on the first injection of the sample if the column was fully coated, the column did not show a deteriorated performance after the sixth injection when it was equipped with an uncoated pre-column. On-column injection only became feasible for these extremely dirty samples when using uncoated inlets.

2.1.2. Increased bleeding. A drifting baseline resulting from an increasing oven temperature does not necessarily indicate column bleeding, *i.e.*, elution of stationary phase fragments generated by degradation of the stationary phase throughout the column. More often than is generally recognized, baseline drifts are due to a local deficiency, namely degradation of the stationary phase (or of sample by-products) within the flooded column inlet. Aggressive dirt (*e.g.*, alkali) degrades the stationary phase in the inlet, whereby degradation is accelerated with increasing oven temperature, creating a baseline drift as observed for general column bleeding. However, in contrast to the latter, the problem can easily be solved by removing the contaminated inlet section. Of course, baseline drift due to degraded stationary phase from the flooded column inlet is ruled out when using uncoated inlets. There remains, however,

the possibility of baseline drift due to degraded dirt material from the same inlet section, *e.g.*, due to free fatty acids released from triglycerides.

2.1.3. Phase stripping. Flowing sample liquid tends to dissolve at least part of the stationary phase in the column inlet, to carry the latter further into the column and to pile it up at the point where the last portion of the sample liquid evaporates. Phase stripping was a considerable problem when stationary phases were not immobilized (bonded). However, it would be wrong to believe that immobilization of the stationary phase film completely solved the problem. First, many of the commercial columns are not rinsed before supply, and as immobilization is usually not complete, some extractable material remains. Second, depolymerization of stationary phase macromolecules due to oxygen, moisture or aggressive sample by-products constantly produces additional extractable material (which becomes visible as bleeding if the fragments are small enough to be volatile).

2.1.4. Increased adsorptivity. Sample by-products may increase the adsorptivity of the separation system, whereby dirty spots act as adsorptive sites. This problem cannot be solved by using uncoated column inlets. For instance, if the samples introduce hydroxides (Fig. 2), acids are adsorbed whether the alkali is located on a bare surface or within a coated inlet.

2.1.5. Degradation of labile solutes. Decay of thermally labile solutes often occurs within a dirty column inlet. If, for instance, triglycerides and the unsaponifiable matter of fats (usually containing traces of alkali) are analysed on the same column, enhanced degradation of triglycerides can be expected. Triglycerides are "grilled" on the alkali-coated inlet up to an oven temperature of about 250–280°C, *i.e.*, that required for noticeable movement of triglycerides in a coated column.

The use of uncoated inlets strongly reduces the degradation of thermolabile solutes within the flooded column inlet. For instance, triglycerides move away from the uncoated inlet — and a possibly aggressive dirt layer on the latter — at around 120–170°C, instead of the 250–280°C if the inlet is coated. This is due to the far lower retention power in the uncoated inlet (retention gap). Needless to say, temperature differences of 100–130°C have a drastic impact on chemical reactions.

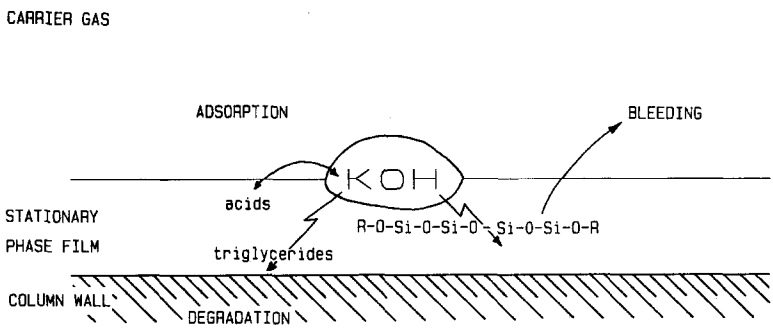

Fig. 2. Some effects of dirt (potassium hydroxide, KOH, serving as an example) on chromatographic performance. KOH adsorbs acids, degrades (saponifies) triglycerides and cleaves macromolecules of the stationary phase into volatile fragments which, on evaporation, cause baseline drift (bleeding). An important detrimental effect of other kinds of dirt is hardly observed with KOH, *viz.*, broadening and distortion of solute peaks due to extra retention power. Effects of non-volatile sample by-products cannot be eliminated but often substantially reduced using uncoated pre-columns.

2.1.6. Easy replacement of column inlet. All of the problems listed above can be solved by removing the contaminated column inlet. As the tolerance for non-volatile sample by-products in the column inlet is relatively small, frequent replacement of the inlet may be necessary. Removal of a contaminated inlet is easy if interchangeable pre-columns are used. The pre-columns initially installed may be longer than required, allowing the contaminated inlet section to be removed several times before a new pre-column must be installed. The length of the column inlet flooded by a 1 μl volume of sample liquid is at most about 30 cm if a capillary of 0.32 mm I.D. is used, and up to 20 cm if the pre-column is of 0.53 mm I.D.[5]. For instance, injecting 2 μl volumes, flooding an inlet section of at most 60 cm length, a 1.8 m $\times$ 0.32 mm I.D. pre-column can be used in three steps.

The presence of large amounts of non-volatile material in the samples influences the choice of the injection technique. As a rule of thumb, dirt concentrations above 0.1% render on-column injection laborious as the inlet may need frequent replacement. Vaporizing injection (splitless injection) tolerates far higher dirt concentrations provided matrix effects are considered for quantitative analyses[6]. On the other hand, replacement of the column inlet or of the pre-column is much easier than the interchange, washing and reconditioning of glass inserts of vaporizing injectors. Furthermore, column inlets are also contaminated by splitless injection, although not as rapidly as when using on-column injection.

2.2. Reconcentration of bands broadened in space

In both on-column and splitless injection under conditions causing recondensation of the sample solvent, sample liquid flows through a considerable length of the column inlet, forming a more or less mechanically stable film on the capillary wall. The dissolved solute material ends up being spread throughout this flooded column inlet. Volatile solutes are reconcentrated by solvent trapping[7], but solutes eluted more than about 50°C above the injection temperature form broad initial bands (approaching a band length corresponding to the length of the flooded zone). Excessive initial band lengths cause broadening and often severe distortion (*e.g.*, splitting) of peaks, as shown in Fig. 3. This "band broadening in space"[2,8–11], observed at elevated elution temperatures, has nothing to do with the broad initial bands resulting from slow sample introduction in splitless injection, and reconcentration techniques used to overcome the latter band broadening (solvent effects and cold trapping) are inefficient.

In splitless injection peak distortion due to band broadening in space is seldom substantial. However, losses in separation efficiency are often considerable, especially when using short columns[6,12]. In on-column injection the extent of peak broadening primarily depends on the sample volume injected. Peak broadening is significant for sample volumes exceeding 0.8–1 μl if the column is short (around 15 m) and 1.5–2 μl if the column has a length of about 50 m[13]. Band broadening in space causes severe problems, even if small sample volumes are injected, if the sample liquid does not wet the stationary phase surface, *e.g.*, if methanolic solutions are injected on to apolar columns.

In on-column injection band broadening in space can be strongly reduced or even eliminated by injecting at a column temperature near the solvent boiling point at the current carrier gas inlet pressure[14]. This is impossible in splitless injection, and

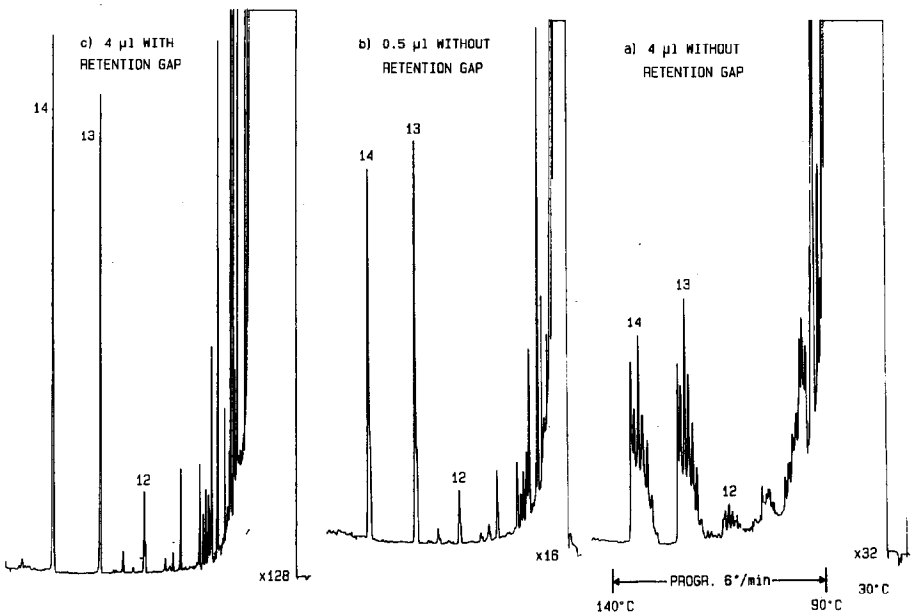

Fig. 3. Peak distortion due to band broadening in space and reconcentration of initial bands using uncoated column inlets (retention gaps). (a) 4 µl on-column injection of C_{13} and C_{14} *n*-alkanes, 10 ppm in acetone, on to a fully coated 7 m × 0.32 mm I.D. capillary column coated with OV-1 of 0.15 µm film thickness. Injection at 30°C, followed after 2 min by temperature programming as indicated. As acetone poorly wets OV-1 surfaces, the sample liquid entered the column by about 2.5 m (60 cm/µl, instead of the usual 15–20 cm/µl with good wettability). Peaks are split into as many maxima as there are flooded coils of the column. (b) As (a), but only 0.5 µl injected. Band broadening is strongly reduced as the initial band length is eight times shorter than in (a). (c) 4 µl injection as in (a), but using a 1.2 m × 0.32 mm I.D. pre-column deactivated with DPTMDS (wettability!) as a retention gap that fully reconcentrates the initial bands.

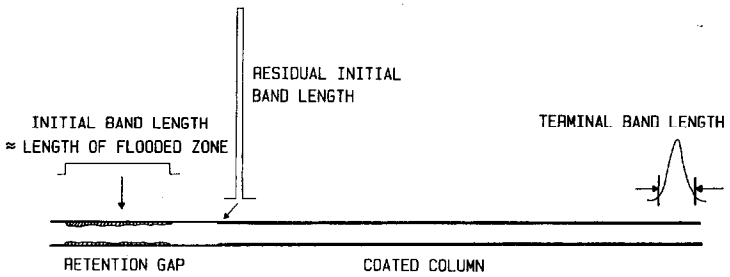

Fig. 4. Reconcentration of bands broadened in space using retention gaps. The initial band length of solutes (except that of volatiles at the injection temperature) corresponds to the length of the flooded zone. Owing to rapid movement of the solutes within the column inlet of low retention power and to the passage of the inlet at oven temperatures far below the elution temperature, the initial band length is strongly reduced at the entrance of the coated column (residual initial band length). The residual initial band length should not exceed 30–50% of the terminal band length in order to avoid peak broadening.

in on-column injection the method often proved to be unreliable.

A very powerful technique for avoiding peak distortion involves the use of uncoated column inlets (retention gaps). The sample liquid is allowed to spread within the uncoated inlet, *i.e.*, within a column section of very low retention power (Fig. 4). The resulting broad initial bands are reconcentrated by the transition into the coated column of far higher retention power. The residual initial band length in the inlet of the coated column should be clearly below the terminal band length[15], the length of the solute band leaving the column, if peak broadening is to be avoided. Terminal band lengths range between 20 and 80 cm, depending on the length and performance of the column. As a rule of thumb, residual initial band lengths may correspond to up to 30–50% of the terminal band lengths without producing peak broadening of practical relevance[13].

Reduction of the initial band lengths during transition from the uncoated into the coated column easily corresponds to a factor of 100 and in some instances even of 1000[13], which allows us to work with flooded zones several tens of metres long. This was the basis for developing the on-column injection of large sample volumes. It was found that reconcentration of band broadening in space by retention gaps allowed an increase in the injection volumes from around 1 μl to far more than just a few microlitres[16]. For instance, the use of a 15 m × 0.32 mm I.D. or a 9 m × 0.53 mm I.D. pre-column allows on-column injection of up to 50–80 μl of liquid samples, depending on the column temperature during solvent evaporation. Using longer retention gaps, this maximum injection volume can be increased to several hundred microlitres, which was found to be useful for on-line coupled high-performance liquid chromatography–gas chromatography (HPLC–GC). HPLC fractions of up to 400 μl were transferred into pre-columns 50–60 m long[17,18].

Fig. 3 shows a far more modest application: a 1.5 m × 0.32 mm I.D. pre-column served for reconcentration of the initial bands formed by an on-column injection of a 4-μl volume.

2.3. Wide-bore pre-columns

Uncoated pre-columns may be of wider bore than the separation column. Of course, there is considerable band broadening during passage through such wide-bore pre-columns, particularly if the latter are long, exhibiting internal volumes exceeding several-fold that of the separation column. However, such band broadening is more than compensated for by the reconcentration obtained at the entrance of the coated column. As a rule of thumb, no special precautions are necessary as long as the inner diameter of the pre-column does not exceed that of the coated column by more than a factor of 2–2.5[13]. Larger differences in diameter compel us to adjust the conditions (high gas flow-rate, slow temperature increase).

Wide-bore pre-columns facilitate injection. The use of 0.53 mm I.D. pre-columns (connected to separation columns of at least 0.25 mm I.D.) allowed the construction of on-column autosamplers using rugged and reliable gauge 26 syringe needles (the same as used for manual injection into vaporizing injectors). Further, if it is of advantage to use pre-columns, one can consider using relatively wide-bore pre-columns, facilitating manual injection. This certainly facilitates the introduction of the needles of on-column syringes if a 0.32 mm I.D. pre-column is attached to a 0.25 mm I.D. coated column. Alternatively, for those who hesitate to use standard

on-column syringes with their fine needles for routine work, a 0.53 mm I.D. pre-column allows the use of gauge 26 syringe needles, *i.e.*, of the type of needles used for vaporizing injection (except that the needle might need to be longer).

Wider bore inlets allow the use of narrow-bore columns with on-column injection where direct introduction of the needle into the column is impossible. Pre-columns of 0.32 mm I.D. are suitable for columns down to 0.15 mm I.D., whereas 0.25 mm I.D. pre-columns even allow work with 0.10 mm I.D. columns. This is of particular interest as (conventional) splitless injection into narrow-bore columns is impossible owing to insufficient carrier gas flow-rates[19].

2.4. High oven temperature on-column injection

In high oven temperature on-column injection, uncoated inlets are advantageous if the elution temperatures are below about 160°C[20]. As the retention power is low, solutes rapidly leave the temporarily cooled inlet as soon as solvent evaporation is completed (solvent trapping), even if heating of the inlet is delayed. This is of interest as it is difficult to find the correct moment to heat the inlet. For analyses involving high elution temperatures (above about 240°C), uncoated inlets are again useful as they cause rapid elution of the solutes from the temporarily cooled inlet even if the latter reaches the oven temperature fairly slowly.

3. RECONCENTRATION EFFECT OBTAINED USING RETENTION GAPS

As mentioned above, transition of solutes from an inlet section of very low retention power to a coated column of higher retention power causes a reconcentration of initial bands broadened in space. A comprehensive description of the reconcentration mechanism is complex[13]. The following three aspects are important.

3.1. Two-step chromatography

Reconcentration of bands broadened in space is necessary and possible only if solutes elute at least about 50°C above the injection temperature. In chromatographic runs involving a temperature increase, solutes pass the retention gap zone at lower temperatures than the coated column. As the retention power in the uncoated inlet is about 100–1000 times lower than in the separation column, solutes pass this inlet 100–150°C below the elution temperature. They arrive at the entrance of the coated column at a temperature that is too low for noticeable migration. They remain in the inlet of the coated column, and are reconcentrated there, until the oven temperature is increased to near the elution temperature.

3.2. Phase ratio focusing

"Phase ratio focusing", an expression proposed by Takeoka and Jennings[21], gives another simplified picture of the phenomenon[22]. As a realistic example we assume that the retention power in the uncoated inlet is 100 times lower than that in the separation column, *i.e.*, that solutes migrate 100 times more rapidly in the pre-column than in the coated column. The solute band moves with a constant length until its front starts to enter the coated column. There, the advanced material moves 100 times more slowly than the rear material, giving the latter the possibility of catching up. The phase ratio focusing model predicts that the reconcentration ob-

tained corresponds to the ratio of the retention powers (commonly expressed in terms of the phase ratio, β) in the uncoated inlet and the coated column, *i.e.*, to a factor of 100 in this instance. The model indicates correctly that the reconcentration is the more efficient the lower is the retention power in the pre-column and the higher is that in the separation column (*i.e.*, the thicker the film of the stationary phase).

3.3. Column-internal cold trapping

The phase ratio focusing model is deficient in that it neglects the gas hold-up time (dead time) of the inlet section which is flooded by the sample liquid. This becomes obvious if we assume hypothetically that there is no retention power at all within the uncoated inlet. The above model would predict reconcentration to infinitely narrow bands. However, this is incorrect as it takes the rear material some time to reach the entrance of the separation column after the front of the band has arrived there. The rear material is not retained by any partitioning, but is behind the advanced material by the time required by the carrier gas to flow through the inlet section of the length corresponding to that of the flooded zone. If the flooded zones are long and the carrier gas velocities low (especially if the pre-column is of a wider bore than the coated column), such gas hold-up times are far from negligible.

The contribution of the gas hold-up time of the flooded zone to the residual initial band width cannot be reduced by the phase ratio focusing mechanism, *i.e.*, by the selection of the properties of the two column parts, because it is of another nature (band broadening in time)[23]. However, it is reduced by column-internal cold trapping, the fact that the solutes arrive at the entrance of the coated column at temperatures below the elution temperature (see Section 3.1). As in splitless injection[6], cold trapping shortens solute bands broadened in time by a factor of approximately two for each 15°C the solutes arrive at the entrance of the coated column below the elution temperature. For instance, if the solutes are reconcentrated in the inlet of the coated column 100°C below the elution temperature, the contribution of the gas hold-up time to the residual initial band width is reduced by a factor of 100, *i.e.*, to less than 1 s even if the gas hold-up time of the flooded zone is around 1 min.

The reconcentration power given by the phase ratio focusing model is optimistic. The reconcentration obtained in reality approaches the latter only if solutes arrive at the entrance of the coated column far below the elution temperature. This is automatically the case if the uncoated inlet is rapidly swept by the carrier gas, *i.e.*, if the inlet section is relatively short (less than about 10–15 m) and the carrier gas velocity is high. Special attention is required if the gas hold-up times of the flooded inlet section and of the whole uncoated inlet are long.

There is little point in listing detailed specifications of cases where reconcentration by column-internal cold trapping requires special attention. In the relatively rare cases where peak broadening is observed (examples are given in ref. 24), column-internal cold trapping is reinforced, *e.g.*, by avoiding very rapid (*e.g.*, ballistic) temperature increases within the critical range of temperatures around 130–80°C below the elution temperature or by increasing the carrier gas flow-rate.

4. CRITICAL CHARACTERISTICS OF PRE-COLUMNS

4.1. Inertness

Uncoated inlets must be sufficiently inert to rule out adsorption or decompo-

sition of solutes. Deactivation of the internal wall of the pre-column is the more important the longer is the pre-column. However, activity problems are easily overestimated. For many applications even untreated fused silica provides completely satisfactory results.

The opinion that column activity is due to holes in the stationary phase film, and that uncoated pre-columns are therefore highly active, has no experimental support.

4.2. Wettability

Activity problems are as easily exaggerated as wettability problems are underestimated. The surface of the uncoated inlet must be wetted by the sample in order to obtain a film of sample liquid on the capillary wall. A lack of wettability results in only some unregularly scattered droplets of sample liquid remaining behind the plug of sample liquid pushed into the capillary by the carrier gas, with the consequence that the plug moves much further into the column until it disappears (see Fig. 5). Non-wetting sample liquids easily flood the capillary for a length exceeding 5 m for each microlitre volume of sample injected, instead of the 25–30 cm/μl typically observed for wetting liquids.

Table 1 gives the wettabilities of various fused-silica and glass surfaces. It should be remembered that wettability is related to surface energies which, in turn, are not directly related to the polarity of solvents. Further, there is a range of critical wettabilities where the wettability depends on uncontrolled side-effects. The indications in Table 1 are pessimistic, *i.e.*, only reliable wettabilities are given.

Raw silica surfaces are wetted by all solvents except aromatics, methanol and water. Wettability by chlorinated solvents is critical, but mostly satisfactory.

Carbowax-deactivated pre-columns are of interest because their preparation is extremely simple: the capillary tube is rinsed with a solution containing about 1% of a Carbowax in dichloromethane, baked under inert gas (*e.g.*, carrier gas) at 280°C for 1 h and rinsed with dichloromethane in order to rinse extractable Carbowax material out of the capillary. The wettability of the surface is excellent: Carbowax-

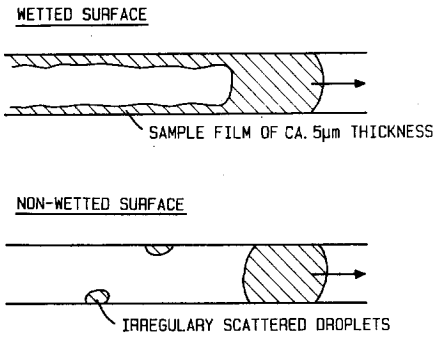

Fig. 5. Uncoated column inlets must be wetted by the sample liquid in order to allow the latter to form a film on the capillary wall. If the surface is not wetted, the plug of sample liquid irregularly leaves some droplets behind. Far less liquid is deposited per unit capillary length and the liquid flows much further into the column. The length of the flooded zone is easily increased by a factor of 10 and depends on factors that are hardly under control.

TABLE 1

WETTABILITIES OF DIFFERENTLY PRE-TREATED CAPILLARY TUBES (PRE-COLUMNS)

Solvent	*Raw silica*	*Carbowax deactivation*	*Trimethyl-silylation*	*Phenyldi-methyl-silylation*
Alkanes	+	+	+	+
Ethers	+	+	+	+
Aromatics	–	+	–	+
Acetone, ethyl acetate	+	+	–	+
Chlorinated solvents	?	+	–	+
Methanol	–	+	–	?
Isopropanol	+	+	–	+
Water	–	?	–	–

deactivated pre-columns can be used for solutions in all solvents except water (water extracts the deactivating Carbowax).

The most thorough deactivation is achieved by leaching of the silica surface followed by silylation, identical with or similar to the procedures used for preparing apolar columns. However, the silylating reagent must be selected with care. Trimethylsilylation creates a surface of low energy[25], wetted only by alkanes and ethers. Solutions in most solvents, even those of only moderate polarity, easily flow through the whole pre-column into the separation column.

Substitution of one methyl group by a phenyl group (phenyldimethylsilylation) strongly increases the surface energy. Surfaces are wetted by all common solvents except water. The wettability by methanol is critical: dry methanol floods a capillary length corresponding to about twice the normal values (40–60 cm/μl).

Diphenylmethylsilylated surfaces exhibit no improvement in wettability. However, deactivation is less complete. Therefore, phenyldimethylsilylated surfaces appear to be optimal for general purposes. There remains the problem that at present there is no satisfactory deactivation method that produces a surface wetted by water.

4.3. Retention power

As indicated by the model of phase ratio focusing (Section 3.2.), reconcentration of bands broadened in space is the more efficient the lower is the retention power within the uncoated inlet. Commercially available deactivated capillaries exhibit greatly varying retention powers. If pre-columns do not exceed a length of a few metres, reconcentration is satisfactory even if pre-columns of relatively high retention power are used. However, larger pre-columns must be of low retention power unless the difference in retention powers between the pre-column and the coated column is created by using thick film coated columns[27].

Retention powers in uncoated pre-columns are conveniently expressed in terms of "apparent film thickness of an apolar stationary phase", *i.e.*, the retention power caused by the interaction between solutes and the deactivated capillary wall is quantitated as if it were due to a film of an apolar stationary phase. This facilitates the comparison of retention powers as the film thickness of the stationary phase in the coated column is known.

TABLE 2

RETENTION POWERS (nm APPARENT FILM THICKNESS) OF DIFFERENTLY DEACTIVATED CAPILLARY TUBES (RANGE CONSIDERING DIFFERENT RETENTION POWERS MEASURED FOR SOLUTES OF DIFFERENT POLARITIES)

Deactivation	*Retention power*
Hexamethyldisilazane (HMDS)	0.3–1
Diphenyltetramethyldisilazane (DPTMDS)	1–2.5
Octamethylcyclotetrasiloxane (D_4)	2–6
Triphenyltrimethylcyclotrisiloxane	2.5–7
Carbowax	4–30

Table 2 lists the retention powers of differently deactivated capillary tubes in nanometres of apparent film thickness. The lowest retention powers were measured on surfaces trimethylsilylated with hexamethyldisilazane (HMDS)[27]. Phenyldimethylsilylation with diphenyltetramethyldisilazane (DPTMDS) resulted in surfaces exhibiting roughly three times higher retention powers. Silylation with analogous cyclic siloxanes, often used for preparing apolar fused-silica columns, is less suitable for our purpose than silylation with monofunctional reagents, as the retention powers are substantially increased, by a factor of at least 6 for methylsilylation and 2.5–3 for phenylmethylsilylation. Apparently, the cyclic silylation reagents tend to polymerize, forming a thin layer on the capillary wall. Carbowax deactivation produces the highest retention power, particularly for polar solutes, which is easily understandable considering that a thin layer of Carbowax is deposited on the surface.

4.4. Evaluation of pre-column deactivation methods

The size of the pre-column has a strong influence on how critical are the surface properties — except that wettability is an important prerequisite independent of the size of the pre-column. If pre-columns are small (up to a few metres long and with inner diameters not far exceeding that of the coated column), availability and price often play a predominant role. If solutes are not very adsorptive or labile, usually untreated fused silica serves the purpose. Carbowax deactivation is attractive because it can be effected with little effort. The adsorptivity of the resulting pre-columns for polar solutes is low. However, general inertness (*e.g.*, chemical inertness) is clearly inferior to that of silylated pre-columns and the thermostability of the deactivation is limited to about 280°C. The use of silylated pre-columns usually means a dependence on commercial suppliers. For small pre-columns wettability is the only characteristic of importance, *i.e.*, methylsilylation is not suitable if sample solvents other than alkanes or ethers are used.

The surface properties of pre-columns become more critical if they are large (exceeding about) 10 m in length if the inside diameter corresponds to that of the coated column, shorter if it exceeds the latter). Inertness is more critical and a more efficient reconcentration of bands broadened in space is required, calling for a low retention power within the pre-column. This usually precludes the use of raw fused silica or of Carbowax-deactivated pre-columns, and disilazane-deactivated pre-columns are preferable to pre-columns treated with cyclosiloxanes. The latter can be illustrated by the following example. If the flooded zone has a length of 15 m and

the retention power within the cyclosiloxane-deactivated pre-column corresponds to a 6 nm film thickness (for polar solutes), reconcentration to a residual initial band length of 15 cm requires the use of a column coated with a film of 0.6 μm thickness. The same residual initial band length is obtained from a column coated with only a standard film thickness (about 0.2 μm) if a DPTMDS-silylated pre-column is used. Use of an HMDS-deactivated pre-column combined with a standard thin-film column would result in a residual initial band length of only 5 cm. However, this is of no practical advantage as residual initial band lengths of 15–20 cm can be tolerated without significant peak broadening, while the phenyldimethylsilylated capillary offers the advantage of the far more general wettability.

If very long pre-columns are used for handling very large volumes of liquid, the retention power within the pre-column becomes very important[26]. HMDS-treated, *i.e.*, trimethylsilylated, pre-columns are preferable whenever wettability allows their use, as such pre-columns of 50 m length satisfactorily reconcentrate bands broadened in space even when combined with columns coated with films of standard thickness only. DPTMDS-silylated pre-columns, which must be used if wettability is a problem, presuppose columns coated with films of at least 0.7 μm thickness.

If commercial pre-columns are used, it is recommended that DPTMDS-silylated capillary tubes that combine good wettability with reasonably low retention power should be sought. HMDS-treated capillary tubes are of interest for applications involving very long pre-columns and samples dissolved in alkanes or ethers.

5. CONNECTION BETWEEN PRE-COLUMN AND COATED COLUMN

The connection between the pre-column and the separation column is critical and can easily ruin the performance of the separation system. Therefore, several groups have invested considerable effort in the preparation of such connections. Of the resulting many propositions[13], only a few have found general acceptance.

5.1. Butt connectors

Most commonly, connections between capillaries are prepared using butt connectors, the first ones being introduced by Supelco around 1981. Such connections are satisfactory for some applications, but not for others. Their tightness is not very reliable (leakage possibly after several weeks of use due to the continuous expansion and contraction of the ferrule caused by heating and cooling); their performance varies, depending on how well the connection was prepared (poor control as visible inspection is precluded); connections between capillaries of different diameters are particularly critical; and for many laboratories their price prevents their use in large numbers.

5.2. Fused connections

Etzweiler[28] found that small pieces (such as capillaries) of hard glass and fused silica can be fused together despite their different coefficients of thermal expansion. This was the basis for preparing connections between two glass capillaries, a glass and a fused-silica capillary and two fused-silica capillaries[29]. The main advantage of fused connections is their perfect performance even under extreme conditions such as when used for coupled HPLC–GC where such connections were in contact with liquid for up to more than 10 min and no distortion of the solvent peaks was observed.

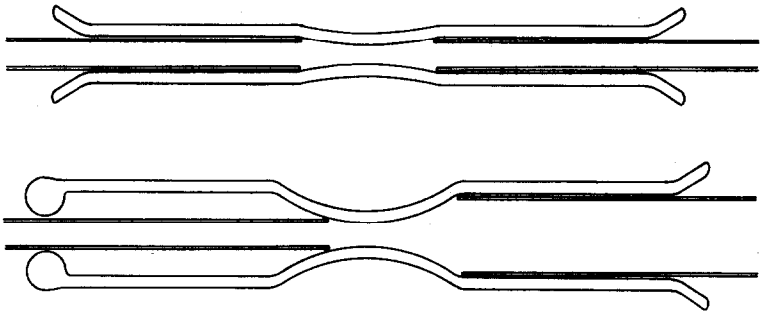

Fig. 6. Press-fit connections between fused-silica capillaries, forming a seal between the polyimide coating of the capillary and a flat conical seat within the glass connection tube of about 2 cm length. Top connection between two fused-silica capillaries of equal outer diameter; bottom connection between a wide-bore pre-column and a standard-bore column.

5.3. Press-fit connections

Recently, Rohwer *et al.*[30] published an idea that some others had successfully used but without recognizing its potential. In fact, we believe that press-fit connections can replace all others, including the butt connectors, for connecting two fused-silica capillaries. Fused-silica capillaries bring the ferrule for a seal along in the form of their outer polyimide coating. If fused-silica capillaries are gently pressed into a flat conical seat of a glass tube (Fig. 6), reliable tightness is achieved without any further reinforcement. In fact, the preparation of such connections takes only a few seconds. We have tested such connections thoroughly and can confirm the findings reported by Rohwer *et al.*[30]. Tightness is no problem provided that the capillary is cut squarely and the conical seat is sufficiently flat. In contrast to Rohwer *et al.*, we prepare connection glass tubes by pulling them out in a flame of suitable size. By this means, two zones of flat cones are formed, the edges of the zone of reduced diameter and the centre of the latter. Optimal seals and mechanical stability of the connection are achieved if the connection tubes are adjusted to the outer diameters of the capillaries to be joined. If two capillaries of equal outer diameter are combined, they form the seals at the edges of the constriction. If two fused-silica capillaries of different diameters are joined, the smaller capillary diameter should fit the diameter in the centre of the constriction (see Fig. 6). It is another advantage of press-fit connections that tightness can be controlled visually: a brown ring is formed within the zone of the seal which must be complete.

When freshly prepared, some connections can be pulled apart again, although only with forces corresponding to enormous gas pressures within the connection. After some heating above 200°C, strong forces trying to pull the connection apart break the fused silica rather than pull the column butts out of the seat.

The GC performance is nearly as good as that of fused connections. Even injections of very large sample volumes or use in coupled HPLC–GC did not cause distortion of the solvent peak. The activity of the accessible glass surface is negligible. For testing purposes we introduced ten connections into the column inlet and another ten into the outlet of an excellent fused-silica column. Without any conditioning, the column test indicated slightly increased adsorptivity. However, after conditioning at 250°C for 30 min the column performance was completely restored.

Press-fit connection tubes must not have sharp edges at their entrances, as such edges damage the polyimide coating of fused-silica capillaries and cause them to break. This problem was solved by opening up the entrances of the connection tubes. In the case of the connection tubes for joining capillaries of different outer diameters, the entrance for the smaller capillary was heated in a small flame, rounding the edges and reducing the inner diameter of the tube. By this means the capillary is more tightly retained by the glass tube, preventing movement of the capillary butt in the connection seat.

Connection glass tubes have become available from ICT (Frankfurt, F.R.G.) and Carlo Erba (Milan, Italy).

6. SUMMARY

Uncoated but deactivated pre-columns have become a widely used tool in capillary gas chromatography (GC), serving strongly differring purposes. Pre-columns are often used as guard columns, reducing the effects of involatile sample by-products on chromatographic performance and rendering exchange of contaminated column inlets simple. Wide-bore pre-columns facilitate introduction of the syringe needle and open the way for a relatively robust on-column autosampler. Other pre-columns are used for re-concentrating solute bands that are broadened due to the flow of sample liquid in the column inlet (retention gap). Long pre-columns allow on-column injection of large sample volumes (*e.g.*, 50–80 μl when a 15 m $\times$ 0.32 mm I.D. pre-column is used). The background of the various uses of pre-columns is discussed, concluding with an evaluation of different deactivation methods for the internal wall of the pre-columns. Critical parameters are inertness, wettability and retention power. Press-fit connections are recommended for coupling pre-columns to the coated columns.

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