Journal of Chromatography, 287 (1984) 1–14
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,448

EFFECT OF "DIRT" INJECTED ON-COLUMN IN CAPILLARY GAS CHROMATOGRAPHY; ANALYSIS OF THE STEROL FRACTION OF OILS AS AN EXAMPLE

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(Received November 22nd, 1983)

SUMMARY

On-column injection deposits high-boiling or non-volatile byproducts of the samples ("dirt") in the flooded column inlet. The major problems with such deposits are due to the retention power. The retention power of the dirt layer accentuates the peak broadening due to band broadening in space because it retards the migration of the solute material in the rear part of the flooded zone and increases its delay on the advanced material. A second mechanism, causing peak distortion due to inhomogeneous retention power in an inlet containing accumulations (droplets) of dirt, was not important under the experimental conditions.

A study on the effect of dirt was carried out with rape seed oil, analysing the sterol fraction and using the triglycerides as a model for dirt. For various columns the maximum amount of oil that could be injected without distorting the solute peaks was determined. The analysis of milk fat in chocolate was an analogous sample.

The distribution of the dirt (triglycerides) in the column inlet was of little importance (long and thin *versus* thick and short layer). The column must contain an uncoated inlet (retention gap), which may be partly filled in with dirt. The capacity of the retention gap for dirt increases with increasing film thickness of the stationary phase in the coated column. A column coated with a very thin (0.06 μm) film of an apolar stationary phase, with a retention gap, tolerated about 1 μg of triglycerides without broadening the sterol peaks, and a column coated with a 0.6 μm film about 20–30 μg.

The retention gap should be regarded as a disposable inlet. The use of fused-silica pre-columns is recommended (several metres long), attached to the separation column (glass or fused silica) by butt connectors or similar unions. From such pre-columns dirty sections (with a length according to that of the flooded zone, *e.g.*, 50 cm) may be cut away after every few injections if necessary.

INTRODUCTION

The undesirable byproducts of most "real" samples are a major source of problems in gas chromatography (GC), in particular capillary GC. If an analyst

0021-9673/84/$03.00

wants to inject a sample on to someone else's column, the "dirt" is the major concern of the column owner. Some column owners even require that only distilled samples are analysed on their columns.

"Dirt" as present in many extracts from environmental sources, biological fluids, foods or other complex matrices frighten many chromatographers more than is justified, possibly because they feel helpless if some defects become visible. They might have an unclear idea of where the dirt may be deposited in the column and what the dirt might affect. Many columns that produce broadened peaks or adsorb polar solutes are thrown away, although they are only locally damaged. If the first 40–100 cm of the column is removed, the problem is often solved[1]. In vapourizing injection some effects on quantitative analysis are to be expected, which should be known also.

If packed column and capillary column GC are compared, there is a paradox. On the one hand the high separation efficiency of the capillary column allows the peaks of interest to be isolated in a far more complex matrix than if packed columns are used. Thus less clean-up is required to establish that the important peaks only contain the solute materials of interest and no interfering "dirt". On the other hand, it is well known that capillary columns are far more rapidly deteriorated than packed columns by dirt. This serves as an argument that more clean-up is needed before the analysis by capillary GC than by packed column GC.

This paper deals with the question of whether it would be possible to load more dirt on to capillary columns than is common practice, provided the chromatographer pragmatically uses the technical possibilities, knows the expected symptoms caused by dirt and is able to repair the damage.

The injection of dirty samples causes certain costs in terms of consumed pre-column or, if no pre-column is used, in terms of column length, sometimes even column life. However, these costs of materials and time are low. They should be pragmatically estimated and compared with those of alternative solutions.

The costs of clean-up steps are relatively high. The development and periodic control of clean-up procedures may require several days. Clean-up procedures involve losses of the material of interest, which must be determined and periodically checked (unless using internal standards or surrogates, which are of similar structure to the material of interest). There is a permanent danger of cross-contamination (memory effects) or even of confusing samples. If an unexpected result is obtained, the sample must be run again to exclude accidental errors.

Even if it is often impossible to work without clean-up methods, there is a strong interest in reducing the procedure to a minimum. Purposeful "dirt management" in the GC system is thus an important factor.

Dirt is a particularly severe problem in trace analysis. The injection techniques are of the splitless, on-column or moving needle types. Each of these injection techniques has its advantages and suffers from dirt in different ways. With on-column injection the peaks are broadened or tail, the transfer from the injector to the column becomes insufficient and discriminative in splitless injection and in the moving needle technique, and there may be memory effects with moving needle injection. Thus each of these injection techniques has a particular range of optimal applications.

In this paper we only consider the effect of dirt on on-column injection, because it is the simplest system to understand. For a broad range of components the splitless

injection system tolerates far more dirt than on-column injection, a subject we shall report on separately.

EFFECT OF DIRT INTRODUCED BY ON-COLUMN INJECTION

Volatile by-products of a sample pass through the column rapidly and seldom affect the performance of the column. However, the major proportion of the dirt is practically involatile and cannot migrate through evaporation. It is spread and remains within the zone where the sample flows in the liquid phase. Hence the contaminated inlet section of the column is identical with the flooded zone[2–4], of length about 30 cm per microlitre of injected sample volume.

The most frequently seen phenomenon caused by dirt is peak broadening (loss of separation efficiency) and peak deformation (tailing, sometimes splitting). It is commonly caused by a "hill" of accumulated dirt in the column inlet which behaves as a (irregular) stationary phase, retaining the solutes. Polar dirt (*e.g.*, sugars, proteins, detergents) primarily affects polar solutes because it retains them far more than apolar sample components. Polar dirt creates what is usually called "increased adsorptivity" of the column —a poor description because it is a chromatographic phenomenon. Apolar dirt such as waxes, certain plastics and lubricating oils tend to affect the apolar solutes and the column tolerates more injections of dirty samples until the polar solutes produce broadened and distorted peaks also.

The retention effect of the dirt often becomes apparent first by an increase in the retention times, commonly associated with a "changed polarity of the column" owing to the selective retention of the solutes by the dirt layer in the inlet section. When the column is further loaded with dirt, the peak shapes start to suffer until certain peaks "disappear" —the solutes are released from the dirt layer during such an extended period of time that the peaks are too flat to be visible.

In addition to the retention effect, the dirt may cause a number of other problems, which are not further discussed in this paper. Dirt may be condensed or adsorbed on the support underneath the stationary phase and change the wettability of the surface. Further, small amounts of medium to very polar materials dissolved, *e.g.*, in silicone stationary phases, destabilize the film. Both factors lead to contraction of the film of stationary phase on the contaminated inlet section to form droplets. Peak broadening and distortion is the visible result. Immobilization of the stationary phase has essentially solved this problem.

Other effects are due to the chemical activity of the dirt. If agressive products such as hydroxides are injected (*e.g.*, with ether extracts of alkaline solutions), silicone stationary phases (not polyglycols such as Carbowaxes) are degraded. The degradation proceeds with increasing velocity if the column temperature is programmed. The column shows strong bleeding, which is due, however, to material produced in the column inlet.

Dirt may chemically affect the sample material. A trivial example are acid–base effects. After a few injections of hydroxide-containing ether solutions, the inlet section no longer allows the passage of acidic solutes. However, the same inlet is likely to degrade also, *e.g.*, esters with a high elution temperature such as triglycerides and wax esters. Degradation of solutes or cleavage of silyl derivatives (see column test according to Donike[5]) by dirt becomes a severe problem at high column tem-

peratures, especially during long temperature programmes, because the solutes are "grilled" for a long time on the dirty surface of the inlet before the column temperature is sufficiently high to allow their migration. This kind of problem suggests that the sample types run on a column should be listed, together with an indication of when the inlet section was removed or replaced.

Sterols in oils and fats

We have studied the effects of dirt on analyses carried out by on-column injection using triglycerides as a model. The analysis of the sterol fraction by direct injection of diluted oils and fats provided a realistic "dirty" sample with well defined characteristics.

The sterol fraction of edible fats and oils is analysed to determine the nature and the origin of the material. Often the composition of the sterol fraction is more characteristic of an oil than that of the fatty acids or the molecular weight distribution of the triglycerides (*e.g.*, refs. 6 and 7). If compounds other than the sterols, *e.g.*, triterpene alcohols and acids, are taken into consideration, such analyses also allow one to distinguish between the different qualities of olive oils such as the oil of the first pressing, the virgin oil and the extracted, heat-pressed or refined oil[8].

The components in the sterol fraction are present in the oils and fats at concentrations between about 10 and 1000 ppm. Hence direct injection introduces 1000 to 100,000 times more dirt (triglycerides) into the column than material of a solute of interest.

Common methods of analysing the sterol fraction include the saponification of the esters, usually carried out under conditions that cause complete hydrolysis of the triglycerides but only partial cleavage of the sterol esters, present in the oils and fats in a minor proportion compared with the free sterols. The major task of the saponification is the removal of the hindering triglycerides.

The analysis of the sterol fraction may serve as an example of what was discussed above: the saponification of the triglycerides facilitates the chromatographic analysis. However, the procedure is time consuming and tiring because of the formation of emulsions, and not very accurate because of low yields in the extraction step (important if absolute concentrations are determined). A direct analysis, injecting the complete sample, produces more reliable results with much less work, but it is more demanding for the chromatographic analysis.

Desbordes *et al.*[9] carried out direct injections of oils by the moving needle method to detect brassicasterol (from rape seed oil) in sunflower oils. High sensitivities were obtained, but no data were given concerning the quantitative aspects of the moving needle injection technique (it was not important for the work cited).

The on-column injection technique is not necessarily the method of choice for the direct analysis of the sterol fraction. Vapourizing injection techniques allow a certain pre-separation of the sterol fraction from the triglycerides in the injector with the consequence that more concentrated solutions may be analysed and higher sensitivities obtained. On the other hand, on-column injection assures accurate and reliable results[10].

Our experiments were primarily aimed at finding the maximum amount of fat or oil that could be injected before the sterol peaks started to be broadened. These determinations were of interest for optimizing the sensitivity of the method (which

is low for the particular analysis), but it also gives a more generally valid idea about the amount of dirt a certain type of column tolerates. The determinations also give some data on the mechanism involved in the deterioration of the peaks and on the parameters that can be optimized to reduce the effect of a certain amount of dirt on the peak shapes.

Determination of milk fat in chocolate

The phenomenon of peak broadening due to dirt is basically the same as that observed if the solutes of interest elute before an overloading bulk of the sample. If this bulk of the material is the solvent, the phenomenon is called "partial solvent trapping"[11]. In some other instances this bulk material is neither the solvent nor the dirt, which does not show up on the chromatogram, but part of the sample itself. The determination of milk fat in chocolate may serve as an example. The triglycerides of the milk fat elute before the bulk of the sample, the triglycerides of the cocoa butter.

EXPERIMENTAL AND RESULTS

Rape seed oil was diluted in *n*-hexane to give concentrations varying by a factor of two in the range between 1:200 and 1:12.5. A number of columns with various film thicknesses and lengths were tested on the maximum amount of oil that did not broaden the solute peaks noticeably.

Fig. 1 shows the results obtained using a 15 m × 0.32 mm I.D. glass capillary, coated with SE-52 of 0.6 μm film thickness. A 1 m × 0.32 mm I.D. retention gap[12], a persilylated but uncoated pre-column of fused silica (Carlo Erba, Milan, Italy), was attached to the inlet by means of a butt connector (Carlo Erba).

Volumes of 1 μl of the rape seed oil solutions were injected at a column temperature of *ca.* 50°C and 30 sec after the injection the oven temperature was ballistically raised to 240°C, where a temperature programme of 3°C/min was started. Attenuations were doubled if solutions of doubled concentrations were injected. After the elution of the sterols, the column was heated to 350°C and the triglycerides eluted from the column with a drastically increased carrier gas flow-rate.

The peak shapes in the chromatograms of the 1:100 and 1:50 diluted oil solutions were identical with those obtained from the unsaponifiable matter of the same oil, thus from the "clean" sample without triglycerides. However, there was a considerable difference in the baseline drift. The apparent bleeding seen in Fig. 1 is primarily due to degradation products of the triglyceride material, mainly free fatty acids[13], which are formed in increasing amounts if the column temperature is raised. The chromatogram of the 1:25 solution shows significant peak broadening. The height of the sitosterol peak is reduced by about 30% (and the peak broadened accordingly). If the amount of injected triglycerides was doubled again, the peaks deteriorated drastically. It was concluded that the column tested tolerated a single injection of a 1 μl volume of a sample containing 2% of dirt, *i.e.*, 20 μg.

Analogous determinations were carried out on 15 m × 0.32 mm I.D. columns coated with 0.15 and 0.06 μm films of SE-54, again containing a retention gap in the inlet. The column with the 0.15 μm film thickness tolerated about 5 μg of dirt, an amount reduced proportionally to the film thickness of the stationary phase if com-

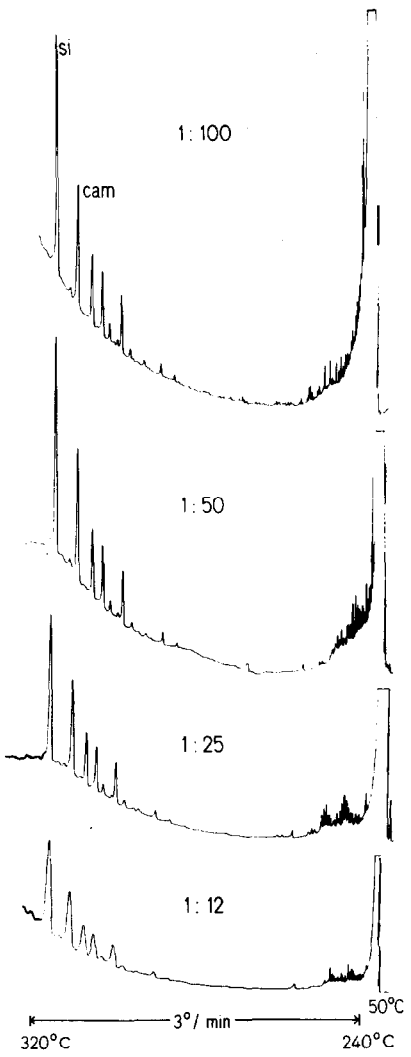

Fig. 1. Chromatograms of sterol fraction of rape seed oil. On-column injections of the oil diluted to various concentrations with *n*-hexane to determine the maximum amount of oil that can be injected without causing peak broadening. Glass capillary column (15 m × 0.32 mm I.D.) coated with a 0.6 μm film of SE-52, equipped with a retention gap of length 1 m. Sample volume, 1 μl; attenuations adjusted to give same peak areas. Si, sitosterol; cam, campesterol. With the injection of the 1:25 diluted oil an amount of triglycerides (40 μg) entered the column that disturbed the chromatography of the sterols. As the triglycerides are considered as a model for dirt, this value gives an idea of the tolerance of this column towards dirt.

pared with the column of Fig. 1. The column with the very thin film (0.06 μm) accepted only about 1 μg of triglycerides without showing broadening of the sterol peaks.

Sample volume and concentration could be traded against each other. The chromatogram in Fig. 2A shows the result obtained with a 4 μl injection of a 1:100

diluted solution (column as in Fig. 1 but with a retention gap of length 2 m). The observed peak broadening is nearly identical with that obtained by the 1 μl injection of the 1:25 diluted solution. This result indicates that the peak broadening depends on the amount of dirt injected and not on the length within the retention gap on to which it was spread. The 4 μl injection produced a liquid layer (flooded zone) of four times the length of that of the 1 μl injection. However, as the same amount of dirt was injected, the resulting film of rape seed oil was four times thinner.

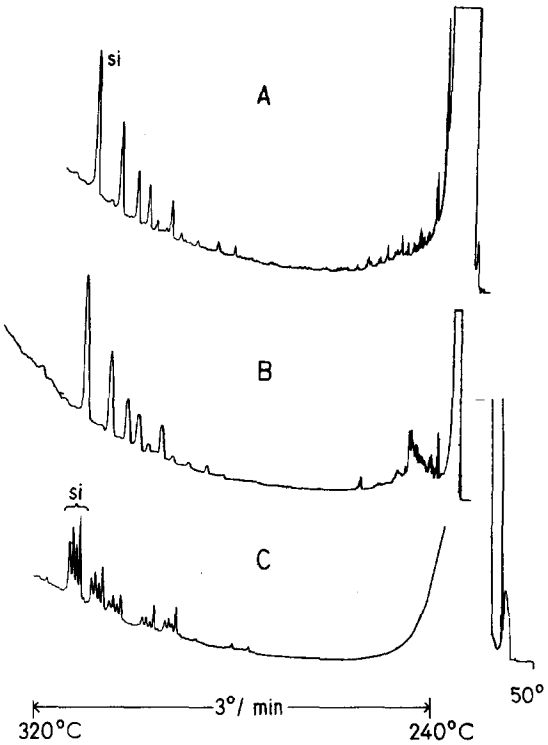

Fig. 2. (A) 4 μl of a 1:100 diluted rape seed oil injected on to the same column as in Fig. 1 except that a 2 m retention gap was used. The observed peak broadening is very similar to that observed in Fig. 1 for the 1 μl injection of the 1:25 diluted oil. The amounts of oil injected were identical, but the length of the flooded zone (in which the sample is spread) varied proportionally with the injected solvent volume. This comparison shows that the distribution of the dirt in the column inlet is not important. (B) Result of a 1 μl injection of the 1:50 diluted oil on to the same column as in Fig. 1 but without a retention gap. The peaks are much more strongly broadened than in Fig. 1, where a retention gap was used. (C) Column as in (B), but the oil was injected in acetic anhydride–pyridine 1:1 (to acetylate the free hydroxy groups of the components in the sterol fraction). Owing to the poor wettability of the internal column surface for this liquid, the flooded zone became very long.

If a sample is injected into a column kept about 10°C above the boiling point of the solvent, the sample is deposited as a relatively narrow band in the column inlet. This is expected on the basis of the fact that under such conditions the band broadening in space is strongly reduced[4]. To see whether an accordingly modified distribution of the dirt in the column inlet would affect the peak broadening of the

sterols, a 1 μl volume of the 1:25 solution was injected at 78°C on to the column of Fig. 1. We found that the peaks were slightly, but not significantly, more broadened than in Fig. 1 (with a column temperature of 50°C during the injection). This is another indication that the distribution of the dirt in the column inlet is of minor importance.

On the other hand, the use of a retention gap is very important. Fig. 2B shows the result of a 1 μl injection of the 1:50 diluted oil on to the column of Fig. 1, but without a retention gap. Without the retention gap the peaks were strongly broadened (nearly as much as the 1:12.5 diluted solution with a retention gap), whereas the peaks were not significantly broadened if the sample was deposited into a retention gap (Fig. 1).

The results were disastrous if a 1 μl volume of a 1:25 solution of the oil in acetic anhydride–pyridine (1:1) (to acetylate the hydroxy groups of the components in the sterol fraction) was injected without a retention gap (Fig. 2C). Owing to wettability problems, this liquid flows far into the column[3] and causes strong band broadening in space.

Determination of milk fat in chocolate

Fig. 3 shows possible problems in the determination of butter fat in chocolate fat. A 15 m × 0.33 mm I.D. glass capillary was used, coated with a 0.09-μm film of SE-52. The first 3 m of the column were left uncoated during the preparation of the column to serve as a retention gap.

The chromatogram of the pure milk fat serves as a reference. When 150 ng of milk fat were injected together with 150 ng of cocoa butter, no serious peak deformations were noted. When 850 ng of cocoa butter were added to give the milk fat a proportion of 15% of the mixture, as is typical for chocolate, the peaks were significantly broadened. The addition of 1.4 μg of cocoa butter caused strong peak deformation (Fig. 3), whereas 2.8 μg of cocoa butter destroyed the efficiency of the column. The tolerance of this column for cocoa fat was clearly below 1 μg, the tolerance determined for rape seed oil of the column with the 0.06 μm film during the analysis of sterols.

DISCUSSION

The deformation of, *e.g.*, the sterol peaks by the dirt, the triglycerides, may be discussed on the basis of the band broadening in space[2,14] or "flooding effect". If the column is coated with stationary phase, there is a band broadening in space due to the flow of the liquid sample from the injection point further into the column, independent of whether the sample and/or the column inlet are dirty or not. The broadening effect is primarily dependent on the length of the flooded zone (which is strongly influenced by the wettability of the column surface by the solvent[3]) and on the length of the column (accentuated for short columns). We have shown previously that peaks of sterols are broadened even if the triglycerides are eliminated by the isolation of the unsaponifiable part[12].

If dirty samples are injected, the band broadening in space is accentuated because the layer of dirt (on the film of the stationary phase) forms a retention "hill" (the reverse of a retention gap). The molecules deposited in the rear of the flooded

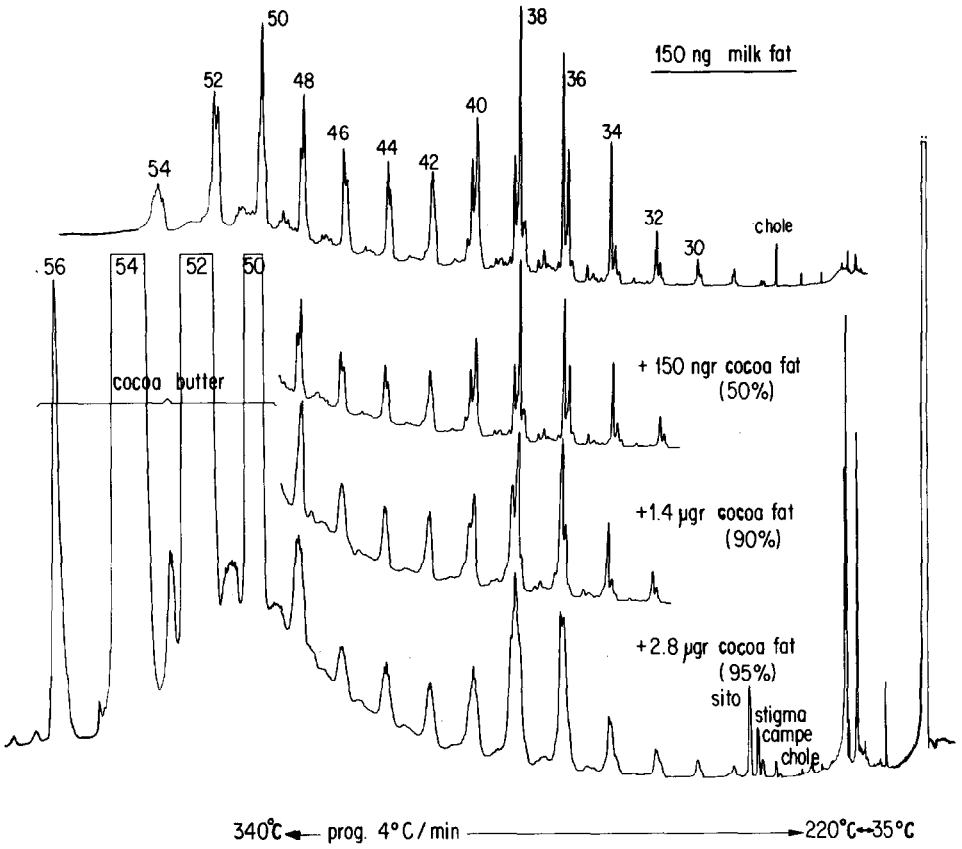

Fig. 3. Analysis of chocolate fat to determine the proportion of milk fat[20] and to detect possible additions of cocoa butter substitutes[21,22] by the triglycerides. Depending on the importance of analysing the cocoa butter (triglycerides 50–56), a thin-filmed column should be used to obtain minimal elution temperatures and to minimize degradation especially of the high-molecular-weight triglycerides (the "hump" underneath the triglyceride 50 is due to degradation material of triglycerides 50–56). On the other hand, the cocoa butter may hinder the chromatography of the milk fat eluted before. The chromatograms shown were obtained on a 15 m × 0.33 mm I.D. column coated with a 0.09-µm film of SE-52, equipped with a retention gap of length 3 m, built into the column inlet. The more cocoa butter must be injected, *e.g.*, because of a small milk fat content, the more the peaks of the milk fat triglycerides are broadened. The peak broadening may be reduced by the use of a thicker filmed column to obtain a deeper retention gap, or by higher sensitivity, which allows a smaller amount of fat to be injected (the deterioration of the milk fat peaks depends on the absolute amount of cocoa butter injected and not on its porportion of the total of the sample). Chole = cholesterol; campe = campesterol; stigma = stigmasterol; sito = sitosterol.

zone become even further away from the molecules in front because their migration through the dirty inlet section is retarded by the retention power of the dirt. If the dirt doubles the retention power of the inlet and the migration speed in this section is halved, the distance between the first and the last molecules is doubled.

The band broadening in space may be avoided by choosing a column temperature during the injection about 10°C above the boiling point of the solvent[15]. However, this has little effect with dirty samples because it piles up the dirt in a very short inlet section, creating a correspondingly thick layer.

The retention gap reconcentrates bands broadened in space by accelerated migration of the spread solutes in the inlet[12,16]. The reconcentration of the solute bands corresponds to the reduction of the retention power in the retention gap compared with the coated column[17].

The layer of dirt (*e.g.*, triglycerides) tends to fill in the retention gap, as shown in Fig. 4. In situation 1 the amount of dirt in the retention gap is relatively small. Its small retention power compared with the coated column causes the solutes to migrate more rapidly in the dirty inlet than in the coated column. Thus there is still a reconcentration effect. In situation 2 the retention power of the dirt layer exceeds that of the stationary phase, possibly owing to a thick film or to strong interaction between the dirt and the solutes. The already broadened initial bands of the solutes are further broadened by the slow migration of the rear material through the dirty inlet.

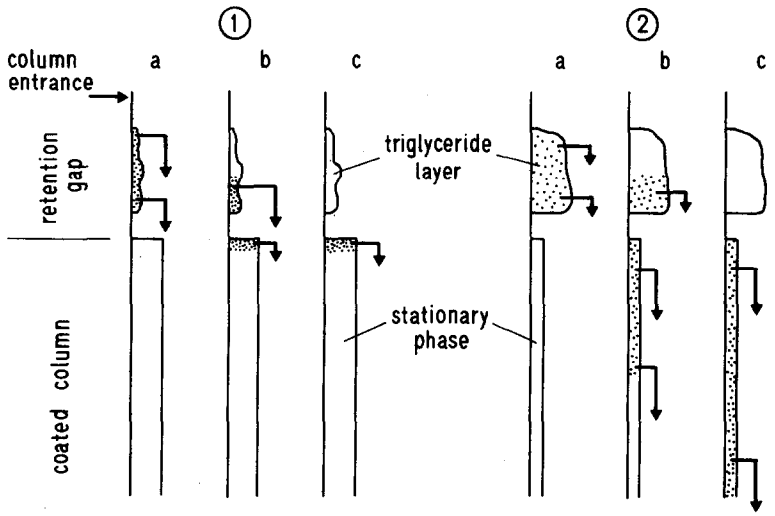

Fig. 4. Peak broadening by a layer of high-boiling or involatile byproducts (triglycerides for the samples discussed) in the retention gap. By on-column injection the solute material is spread in the dirt layer located in the flooded column inlet. All solute bands are broadened in space. In situation 1 the amount of dirt in the flooded zone is relatively small. Owing to the low retention power of the dirt layer, the solute material rapidly leaves the retention gap section and is reconcentrated on the beginning of the coated column. In situation 2, however, the retention gap is "overfilled" with dirt; the retention power of the dirt layer exceeds that of the coating with stationary phase owing to the thickness of this deposit and/or to a strong interaction between the dirt and the solute material. The solute migrates more slowly in the dirt layer than in the stationary phase, resulting in an increased broadening of the bands already broadened in space. If the flooded zone is 50 cm long and the (average) speed of the solute within the dirt layer is half of that in the stationary phase, the band width is doubled. The most advanced solute material migrates 1 m through the coated column within the time the rear material requires to cross the 50 cm long dirty inlet.

This explanation of the peak broadening by the dirt may be checked semi-quantitatively with the results obtained for the sterol/oil sample. The injection of a 1 μl volume of a hexane solution on to a silylated retention gap creates a liquid layer about 25 cm long. The first significant peak broadening was detected for the 1:25 solution, which introduced 40 μg of rape seed oil into the flooded zone. The average thickness of this layer is calculated to be 0.16 μm, *i.e.*, four times less than the film

thickness of the stationary phase in the coated column. At this point it must be considered that the triglycerides retain the sterols more strongly than the apolar silicone stationary phase (increased partition coefficient, K). If K were to be doubled, the inlet coated with an average thickness of 0.16 μm of dirt would already have a retention power corresponding to half of that of the column section coated with stationary phase. Hence the reconcentration effect would correspond to a factor of two, which is not suffcient to suppress fully the band broadening in space of a 1 μl injection on to a 15 m column.

This type of interpretation easily explains the observation that a thin but long layer of dirt produces the same peak deformation as a thicker and shorter layer. An injected volume of 4 μl of a 1:100 diluted oil produced a sample layer within the first 1 m of the column, and hence the band broadening in space increased by a factor of four. However, the average thickness of thin layer only reached 0.04 μm, providing a four times stronger reconcentration effect.

For practical work the finding that a thick-filmed column with a retention gap tolerates more dirt than a thin-filmed column is very important. If the film thickness of the stationary phase is doubled, twice as much dirt may be introduced into the retention gap to fill it up to the critical level. The linear relationship between film thickness and tolerance of dirt is no longer valid if very thin-filmed columns are considered, such as the column tested with the 0.06 μm film. This is probably due to the fact that the retention power of the deactivated internal surface of the retention gap can no longer be neglected[17].

As the film thickness of most stationary phases can be varied within a large range[18], the choice of the optimal thickness is the most important factor for a system that is relatively weakly sensitive to dirt.

The experiment and the explanations on the basis of the band broadening in space neglect a second mechanism leading to peak distortion. If a solute enters the column as a sharp band (in terms of time and space, *e.g.*, by split injection), it may still form a broadened peak owing to an excessively dirty column inlet. In this instance the peak broadening is analogous to that occurring in an unevenly coated column. It is the result of an inhomogenous retention power. In a simplified picture, one molecule of a solute "falls" on to a droplet of dirt and is strongly retained there because such droplets easily have a thickness of several micrometres. It will be delayed compared with another molecule that passes the droplet without touching it.

The peak broadening due to an inhomogenous retention power is important only if the dirt is accumulated at some points. However, this often occurs, either because the dirt does not wet the support surface and contracts to form droplets, or because of the washing effect by subsequent injections that introduce liquid sample. In on-column injection or splitless injection with recondensation of the solvent, the thickness of the dirt layer deposited during the first injection varies only by a factor of 5 (estimated visually by injections of perylene[2]), but the following injections tend to wash the dirt towards the front of the flooded zone. The condensed solvent at least partially re-dissolves the dirt in the column inlet and carries it further into the column. Finally, if the length of the flooded zone remain constant, the dirt may accumulate on a short segment of the column, forming one or several usually brown droplets.

The concentration of the dirt towards the front of the flooded zone may create

puzzling results, as follows. After a first injection of a very dirty sample the chromatographer retests the column to see whether the dirt has been harmful. The column performs perfectly. Then a number of clean standards are injected by the on-column technique or the splitless method with recondensation of the solvent. After a number of such innocuous injections the column starts to degrade. The chromatographer is puzzled because he does not relate the new problem to the dirty sample injected the day before, and overlooks the fact that the clean solvent rinsed the deposited dirt further into the column and made a droplet out of the original layer.

It is difficult to quantitate inhomogeneity of the dirt layer to enable its importance for peak broadening to be ascertained compared with that of the band broadening in space. However, it appears that the peak broadening by band broadening in space completely dominates until the dirt is broken up into a few relatively large droplets.

CONCLUSIONS

The analysis of dirty samples using on-column injection requires that the column inlet is equipped with a retention gap, *i.e.*, with a section that is deactivated but not coated. This retention gap may be filled in with by-products that are involatile or less volatile than the solutes of interest.

Capillary columns with average film thicknesses tolerate about 10 μg of dirt, injected as one injection or as a total of many injections. If the dirt and the stationary phase are of similar polarity, more dirt is tolerated; on the other hand, polar dirt affects polar solutes in an apolar column even if present in a smaller amount. As most of the dirt is medium to very polar in nature, this explains partly the old rule that columns coated with polyglycols such as Emulphor, Ucones, Pluronics or Carbowaxes are preferable to apolar columns if very dirty samples must be analysed.

Thick-filmed columns (equipped with a retention gap) tolerate much more dirt than thin-filmed columns. However, even if the column is optimally designed to tolerate a maximum amount of dirt, techniques are needed that allow rapid repair of a damage. The routine analysis of dirty samples requires an inlet section that can be considered as a disposable part. It must be easy to remove or replace the inlet for a distance that corresponds to the length of the flooded zone, *i.e.*, in general about 25–40 cm per microlitre of sample volume injected at a time[3].

Retention gaps made of fused silica have the advantage that the dirty inlet section may be cut away (*e.g.*, with a pair of scissors) and the new inlet reconnected to the injector without the need to straighten the end section (which requires the analytical sequence to be interrupted because the column must be demounted and afterwards heated before being ready again). This manipulation is very rapid compared with the time needed for the clean-up of a number of samples.

The possibly rapid consumption of the retention gap (*e.g.*, sections 50 cm long twice a day) requires that the columns are equipped with long retention gaps. This is possible because even very long retention gaps do not cause peak broadening owing to their dead volume. Bands broadened due to the dead volume of the retention gap are reconcentrated on the beginning of the film of the stationary phase just as the bands broadened in space. Isothermal analyses carried out at the column temperature during the injection are an exception, but even there the broadening is not severe[12].

The retention gap may be prepared during the preparation of the column. A capillary tube of length, *e.g.*, 30 m is deactivated, but only 15 m of it are coated, leaving a retention gap of 15 m. This technique is less attractive for glass capillaries than for fused-silica tubes because of the need to re-straighten the inlet end section of the glass capillary whenever a dirty inlet is removed. Another drawback of an extremely long retention gap is the fact that the linear velocity of the carrier gas at a given inlet pressure is increased when the column is shortened.

As an alternative, already used in many laboratories, several metres of pre-column (preferably fused silica) are connected to the inlet of the separating column. There are a variety of connection techniques[16], but in most instances shrinkable PTFE tubing is used if the analysis temperatures do not exceed 220°C. "Butt connectors" (Supelco, Gland, Switzerland or Carlo Erba, Milan, Italy) or "zero dead volume" unions (various suppliers) allow one to work with column temperatures up to at least 350°C. With the exception of a few special cases no negative effects such as peak broadening (tailing) or loss of solute material were found. The most thermostable deactivation and the best inertness in the retention gap are obtained by silylation of the fused-silica tubing (available from Carlo Erba). However, for many purposes it is sufficient to deactivate raw fused silica with a Carbowax. The tubing is rinsed with a 0.1–1% solution of a Carbowax in dichloromethane, heated under an inert gas (carrier gas) at 280°C for 15–30 min and thoroughly washed with dichloromethane to remove excess of Carbowax.

For most samples the lifetime of the column beyond the flooded inlet is long. However, there are samples that contain aggressive volatile material that attacks the deactivation of the support and the stationary phase itself (*e.g.*, large amounts of water and alcohols). For columns coated with an immobilized film of a silicone stationary phase it is recommended that the column be resilylated[19], which is a very simple method that is successful in many, although not all, instances.

Finally, capillary columns should not be considered as "sacred objects". Their damage should be taken into pragmatic consideration if steps of a clean-up procedures may be avoided that are not essential to remove substances which interfere with the solutes of interest. The preparation of a column (working time excluding waiting time) is about as time consuming as the clean-up of a few samples.

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