BAND BROADENING IN SPACE IN SPLITLESS INJECTION

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SUMMARY

Band broadening in space occurs with splitless injection if the sample solvent recondenses in the column inlet (used to produce solvent effects). The recondensed solvent flows in the column inlet, spreading the solute material dissolved within the flooded zone. The resulting peak broadening is important for columns less than 25 m long if the sample solvent wets the surface of the stationary phase. Severe peak distortion is observed if the sample solvent is repelled by the stationary phase surface. The most effective means of overcoming such problems is to use uncoated column inlets (retention gaps), which may also serve as disposable inlets for the analysis of dirty samples.

INTRODUCTION

Splitless injection is more than 15 years old and is perhaps the most frequently used injection technique in capillary gas chromatography (GC). Nevertheless, a number of fundamental aspects are still not recognized or are new to most users of splitless injection. New manuals from instrument manufacturers and recent papers dealing with the performance of splitless injection show that the problem of the transfer of vapour from the injector into the column[1] has not been adequately considered: carrier gas flow-rates applied are often too low and syringe needles too short. The reduced transfer efficiency due to non-volatile sample by-products is also a new discovery[2,3]. Is splitless injection still underdeveloped at a time when many replace it by on-column or programmed temperature vaporizing (PTV) injection[4,5]?

Band broadening in space in on-column injection has been described by a number of workers[6–9]. It is caused by the flow of sample liquid in the column inlet, spreading the solute material throughout the flooded zone. The broadening effect is called "in space" because all solute bands are spread over the same length of the column inlet, and to distinguish it from broadening "in time", where solute bands have the same width in terms of chromatographic time[10].

In splitless injection there are both types of band broadening effects: band broadening in time due to the slow introduction of the sample from the injector into the column (with a band width corresponding to the transfer time or the splitless period), and band broadening in space if recondensation of the sample solvent causes

a flow of liquid in the column inlet. Solvent recondensation is used to achieve solvent effects. Hence, band broadening in space is a side-effect of a reconcentration technique effective for bands broadened in time.

The first description of band broadening in space in splitless injection appeared in 1981[6]. However, the extent of the resulting peak broadening and the practical implications were not recognized at that time. This paper summarizes the source of the band broadening in space in splitless injection, points out the cases where peak broadening becomes significant or even drastic (peak distortion) and lists the possibilities for overcoming the problem.

SOURCES OF THE BAND BROADENING IN SPACE

The sample solvent recondensing in the column inlet if the column temperature is at least 20–25°C below the solvent boiling point forms a thick layer of liquid just below the attachment of the column to the injector. Hardly a second after the injection this liquid starts to flow along the column wall further into the column, driven by the carrier gas. This flow continues either until the layer of liquid is reduced to a thickness that renders it mechanically stable or until the solvent is evaporated. The length of the flooded zone is of the order of 15–25 cm per microlitre of recondensed sample liquid if the latter wets the surface of the stationary phase[11]. If the sample does not wet the stationary phase, it cannot form a film. The flowing liquid leaves only a few droplets behind and flows correspondingly far, easily 5 m per microlitre of liquid. This data obtained by on-column injection require some adjustment to splitless injection: owing to the slow sample introduction and the simultaneous evaporation, at no time is the full amount of solvent recondensed in the column inlet. Therefore, the higher the column temperature, the less far this liquid will penetrate into the column. However, as solvent effects are to be obtained, a minimum of about half of the sample (solvent) volume must be recondensed in the column inlet.

Solute material dissolved in the flowing liquid is spread throughout the flooded zone, forming initial bands of corresponding length.

Another possible source of band broadening closely related to band broadening in space is not discussed here: thermal defocusing due to a cold spot in the bottom part of the injector. The injector part reaching into the GC oven, including the ferrule and screw of the column attachment, are cooled when the oven is at low temperature. During rapid temperature programming or ballistic heating the temperature of the bottom part of the injector may lag behind the oven temperature.

SHAPE OF THE INITIAL SOLUTE BAND

First it should be recalled that only solute bands of peaks eluted at least about 50°C above the injection temperature are affected by band broadening in space because the more volatile solutes are reconcentrated in terms of time and space by solvent trapping[6,12].

Other than in on-column injection, not all solute material passes into the flooding sample liquid. In contrast to what has been claimed by several workers, solvent and solute vapours enter the column simultaneously. However, not all solute material enters far enough to reach the oven-thermostated part of the column where the solvent recondenses.
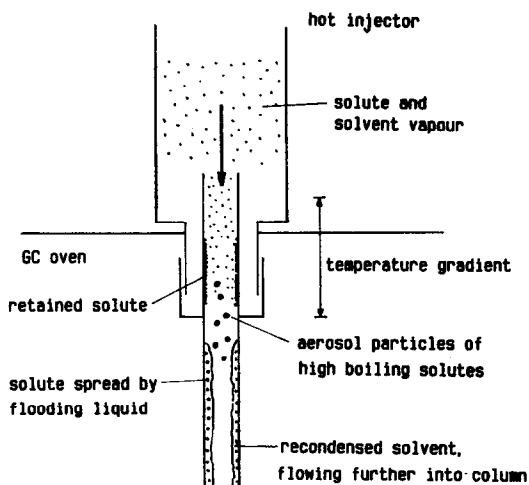
Fig. 1. Pre-separation of the higher boiling solute material into a part remaining in the capillary section kept in the bottom part of the injector and a part being spread within the flooded zone. The bottom part of the injector is at an intermediate temperature: too high to cause solvent recondensation but low enough to create a zone of high retention power in the capillary column enclosed. However, surprisingly large proportions of solute material pass through this section, possibly via formation of an aerosol, and become re-dissolved in the recondensed solvent and spread within the flooded zone.

The temperature in the column section kept inside the injector drops from the thermostated injector temperature to the oven temperature in an irregular temperature gradient (Fig. 1). One might expect that most solutes (except the most volatile ones) would be retained at a particular point within this temperature gradient, the temperature of which would render the migration speed negligible. However, experimental results show that surprisingly large proportions of solute material pass this barrier and reach far enough into the column to come into contact with recondensed solvent. As was shown earlier[6], nearly half of the C_{20} n-alkane and even 10–15% of the C_{30} n-alkane were spread in the flooded zone. As the point of solvent recondensation is usually 2–4 mm below the column attachment to the injector, this material must pass through a considerable length of column of low (in this instance almost ambient) temperature. The rapid cooling of the solute vapour (within a time of the order of 10 msec) might cause the formation of an aerosol, agglomerating molecules in the gas phase owing to the lack of contact with the column wall.

The shape of the initial solute bands may be determined by visual examination of the inlet section of a glass capillary column after injection of a fluorescent solute[6,13]. A typical shape is shown in Fig. 2 in the form of a hypothetical chromatogram as "seen" by a detector situated just beyond the front end of the flooded zone, and hence recorded before diffusion processes during the passage through the column have taken away some of the clearness of the band shape. The band is assumed to correspond to a solute of intermediate volatility, as shown by the fact that about half of the material penetrated into the flooded zone. First, a broad and distorted part of the band is eluted, representing the material spread within the flooded zone. Its shape is poorly reproducible because of the complexity of the factors determining how much sample liquid is evaporated (and deposits its solute material) at a given location
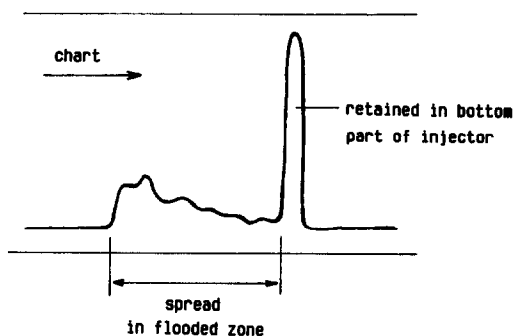
Fig. 2. Shape of the initial band of a solute eluted at least about 50°C above the injection temperature after splitless injection with solvent recondensation, shown as a peak eluted before diffusion processes during chromatography "washed" away some of the initial clearness. As the distribution of the solute material in the flooded zone is poorly reproducible, the shape of the first eluted part of the peak may look very different.

in the column inlet. Eluted last is a sharp band of solute material retained in the capillary section kept inside the injector. The ratio of the widths of the two band sections corresponds to that of the lengths of the band sections in the column inlet. A typical length of the flooded zone is 25 cm and that of the sharp band in the rear is 1–2 cm.

EXTENT OF PEAK BROADENING

Theoretical calculations

The length of the flooded zone in splitless injection ranges between 15 and 50 cm, with a typical average of 25 cm, provided that the sample liquid (solvent) wets the surface of the stationary phase. This initial band length must be compared with the terminal band length, the length of the solute band as it leaves the column. The initial band length should not contribute significantly to the terminal band length. Saxton[14] suggested the use of the terminal band length, b, corresponding to four times the standard deviation of the solute concentration profile. Related to the column length, L, and the height equivalent to a theoretical plate (HETP), h, of a column this terminal band length is[14].

$$b = 4 \sqrt{Lh}$$

Using band widths at half-height and assuming that h corresponds to the internal column diameter, the terminal band length of a solute eluted from a 10 m × 0.30 mm I.D. column is 18 cm, that from a 20 m × 0.30 mm I.D. column 25 cm and for a 50 m × 0.30 m I.D. column it is still only 40 cm.

The contributions to the final peak width are added as squares[15], with the effect that the band broadening due to the initial band length is not equal to the initial band length itself. If the initial band length is equal to the band length due to

chromatography, the peak is only broadened by $\sqrt{2}$ or about 40%.

A calculation of the peak broadening due to band broadening in space is complex, primarily owing to the distorted shape of the initial band, and not useful. How-

ever, a rapid comparison of the initial and the terminal band lengths shows immediately that band broadening in space is an important contribution to the final peak width. The peak broadening is significant even if long capillary columns (with long terminal band lengths) are used. For a 25-m column used under conditions providing optimal separation efficiency (minimal terminal band length) and for an initial band length of 25 cm, a peak broadening of several tens percent is expected.

Experimentally determined broadening effects

Peak broadening effects were determined on a 12 m × 0.32 mm I.D. glass capillary, fully coated with immobilized PS-255 (a methylsilicone) of 0.6 μm film thickness, using an average carrier gas (H_2) velocity of 70 cm/sec and with C_6–C_{18} fatty acid methyl esters. In Fig. 3 a chromatogram obtained by split injection (chromatogram C, excluding band broadening effects) is compared with two chromatograms produced using splitless injection. In chromatogram B the column temperature during the sample transfer was 25°C, leading to nearly complete recondensation of the solvent (*n*-hexane). In chromatogram A injection was carried out at 60°C, precluding solvent recondensation. Solvent recondensation and the resulting spreading of solute material in the flooded zone did not cause visible peak distortion (chromatogram B). However, all peaks except of that of the C_6 ester were broadened by 25–35% compared with chromatogram C. In chromatogram A the first peaks are broadened owing to missing reconcentration of the bands broadened in time (no solvent effects). However, peaks eluted at elevated column temperature (reconcentrated by cold trapping) are as sharp as in chromatogram C, because band broadening in space is precluded.

Fig. 4 shows chromatograms obtained with the same system and conditions as used for chromatogram B in Fig. 3, but using acetone and methanol as the sample solvent instead of *n*-hexane. As the solute concentrations are identical with those in the *n*-hexane solution, the peak areas in Fig. 4 are the same as those in Fig. 3B. Acetone wets the surface of methylsilicones poorly, resulting in a flooded zone about 50 cm long. Peak distortion (splitting of peaks 8 and 10) becomes obvious. Methanol does not wet apolar stationary phases and flows very far into the column. The resulting peak distortion resembles that shown in Fig. 2. The later eluted sharp peaks are as narrow as those obtained by split injection (Fig. 3C). The shape of the peaks representing the spread material changes within the chromatogram, which is not typical for band broadening in space. It is probably due to extraction of the esters into the stationary phase during the flooding of the methanol. This extraction, particularly pronounced for the less methanol-soluble high-boiling esters, causes the solute concentration in the flooding methanol phase to decrease towards the front of the flooded zone.

Conclusions

The peak broadening in Fig. 3B is not very obvious and may therefore be considered to be irrelevant. However, it should be remembered that peak broadening by 25–35% reduces the separation efficiency of the column in terms of resolution (measured as separation number, TZ) correspondingly and the number of theoretical plates by a factor of nearly two. This nearly corresponds to the loss in separation efficiency if the column length is reduced to half. Hence, if the separation efficiency

(A) SPLITLESS WITHOUT SOLVENT RECONDENSATION

210°C ←————————— 7°/min ——————————→ 60°C

(B) SPLITLESS WITH SOLVENT RECONDENSATION

(C) SPLIT

18 16 15 14 12 10 8 6

|←——————————— 7°/min ———————————→| 25°C
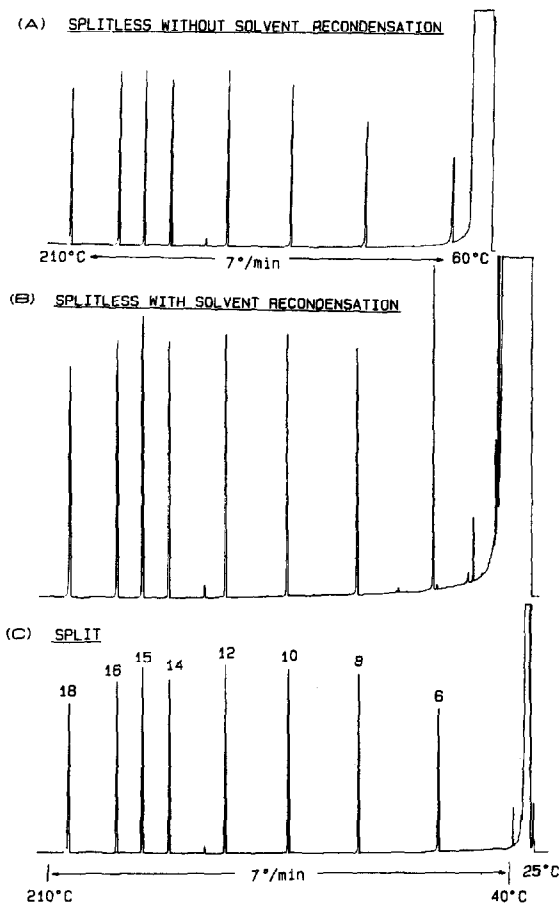210°C   40°C

Fig. 3. Band broadening in space in splitless injection involving a sample solvent (*n*-hexane) that wets the surface of the stationary phase (an immobilized methylsilicone). Methyl esters of C_6–C_{18} fatty acids (20 ppm). C, Split injection (splitting ratio, *ca.* 30:1; sample volume, 1.5 μl, including needle volume). Band broadening effects are practically excluded. B, Splitless injection (2 μl; splitless period, 50 sec) at 25°C, resulting in strong solvent recondensation. Solute material reaching the flooded zone is spread through a flooded zone about 25 cm long. Peaks (except of the C_6 ester) are broadened 25–35% compared with C. A, As B but injected at 60°C, precluding solvent recondensation; double attenuation. No band broadening in space occurs, but broad, early eluted peaks due to missing solvent effects for reconcentration of bands broadened in time are seen.

is so high that the peak broadening by band broadening in space is negligible, the analyst is better off using a column of half the length, saving analysis time and doubling the sensitivity.

   Band broadening in space in splitless injection is important if the column length is less than 20 m (although a reduction in separation efficiency is still measurable even for longer columns). Further, peak distortion becomes disastrous if the sample liquid does not wet the surface of the stationary phase, regardless of the column length.
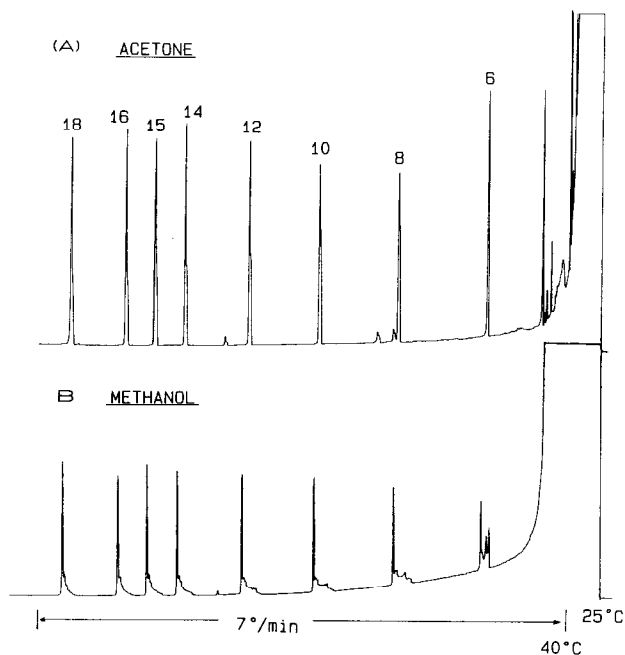
Fig. 4. Peak distortion due to band broadening in space in splitless injection involving solvents not wetting the surface of the stationary phase. Sample, column and conditions as in Fig. 3B. Acetone poorly wets methylsilicones such as PS-255, OV-1 or equivalent (but it does wet SE-54, SE-52 or similar). Methanol does not wet apolar stationary phases and floods the column for several metres.

METHODS FOR AVOIDING PEAK BROADENING

Cold Trapping

Splitless injection relies on a technique for reconcentrating the broad initial bands due to slow sample introduction into the column (band broadening in time). If this can be achieved by the cold trapping method, keeping the column temperature not more than 15°C below the solvent boiling point, there is no spreading of the solute material in the column inlet. Band broadening in space is a side-effect of solvent recondensation to obtain solvent effects. However, for solutions, *e.g.*, in *n*-hexane, this means injection at a column temperature of at least 55°C. If a temperature difference of at least 80°C is required in order to achieve an efficient cold trapping effect, the minimal elution temperature for solutes of interest is about 135°C. Solvent recondensation cannot be circumvented for analyses at low temperatures.

Improved wettability

Peak distortion is strong if the sample solvent does not wet the surface of the stationary phase. This problem can be solved in most instances by the selection of a suitable combination of stationary phase and solvent. Methylsilicones are well wetted by hydrocarbons and chlorinated solvents, but not by oxygenated solvents even of intermediate polarity (acetone, ethyl acetate). Aged columns coated with methylsilicones may even repel benzene or dichloromethane. Slightly more polar silicone

stationary phases such as SE-54, SE-52 or OV-73 exhibit much better wettabilities. They are wetted by all solvents of intermediate polarity. More polar silicones such as OV-17 are even wetted by methanol[11]. On the other hand, polar stationary phases, e.g., Carbowaxes, are not wetted by apolar solvents.

Uncoated column inlets

If the flowing sample liquid spreads the solute material in an uncoated column inlet (retention gap[16]), the solute bands become reconcentrated at the beginning of the coated column owing to the accelerated movement of the solute material within the zone of reduced retention power. Reconcentration of bands broadened in space is very effective, often shortening the initial bands by more than a factor of 100[17], hence being abundant for splitless injection.

In the early days it was easy to prepare a retention gap by extraction of the stationary phase from the column inlet. Today, this is possible only for a minority of the columns with non-immobilized stationary phase films. Ironically, the immobilized stationary phase films were introduced primarily with the argument that phase stripping due to flow of sample liquid within the coated inlet would be excluded. However, the real solution to the problem of phase stripping, but also to the problem of band broadening in space and dirt effects, is the removal of the stationary phase. Hence, at least in this respect, immobilization of the stationary phase clearly is a step in the wrong direction.

The preparation of a retention gap during the column preparation procedure is easy. However, as only a few column manufacturers offer columns with a built-in retention gap, this would force many chromatographers to prepare their columns themselves, e.g. an 18-m capillary tube is deactivated, but only 15 m of it are coated.

Use of fully coated columns with immobilized stationary phases forcs one to connect a deactivated but uncoated pre-column to the separation column, the pre-column preferably consisting of fused silica, even if connected to glass columns. The quickest and easiest method of connecting the pre-column to the separation column involves the use of shrinkable PFTE tubing. However, thermal stability and diffusion problems mostly restrict the analysis temperatures to below about 200–230°C. In order to stabilize PFTE joints mechanically, a tube of suitable inner diameter is pushed over the connection, e.g., taken from the tip of a Pasteur pipette. For analyses involving higher column temperatures butt connectors are mostly used, which are thermostable up to above 360°C and usually provide good performance.

Recently, Etzweiler[18] proposed the preparation of column exit splitters by melting glass on to fused silica and strengthening the connections with polyimide glue. Applied to the preparation of joints between pre-columns and separation columns, this method provided excellent results.

Deactivation of the pre-column is often less critical than might be expected. The surface of the pre-column must be wettable with the sample solvent. Trimethylsilylated surfaces are not suitable as they are wetted only by alkanes and ethers[11]. Phenyldimethylsilylated surfaces, however, are reasonably wetted by nearly all solvents, including methanol but excluding water.

On the one hand, the use of pre-columns introduces a joint which is not really desirable. On the other hand, pre-columns are used successfully by many laboratories. They are convenient as disposable inlets for the analysis of dirty samples. Non-vol-

atile sample by-products accumulate in the column inlet section kept inside the injector. However as the solute material, part of it passes on into the column section, which is flooded by the recondensed solvent. The latter spreads it within the flooded zone, but owing to a lack of volatility it cannot penetrate further into the separation system. Hence the removal of a column inlet section corresponding to the length of the flooded zone usually solves the problem. If a pre-column several metres long is used, the inlet section can be removed several times until the pre-column must be replaced.

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