Journal of Chromatography, 333 (1985) 156–160
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 17 944

Note

Rejection of the sample plug after on-column injection into capillaries at high temperatures

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(Received June 7th, 1985)

The classical rules of on-column injection require that the injection temperature is below the boiling point of the sample solvent[1], the boiling point at the column inlet pressure being slightly above the boiling point under standard conditions[2]. Injections at higher column temperature were shown to create losses of sample material, as the vapour pressure of the sample at the front of the sample plug in the column inlet pushes at least part of the sample backwards out of the column inlet into the cold injector. However, injection below the solvent boiling point often necessitates cooling of the column merely for the injection, which is of course awkward and time consuming. Furthermore, it may cause problems with the reproducibility of absolute retention times and the stability of the baseline.

Several attempts have been made to overcome this inconvenience. Grob[3] suggested slow injection, introducing an amount of liquid per unit time that could be immediately volatilized without building up a zone of increased pressure. However, this technique causes losses of high-boiling solute material owing to evaporation of sample liquid on the tip of the syringe needle (non-evaporating high-boiling solute material is withdrawn from the column together with the syringe)[1].

Carlo Erba pioneered the development of systems that allow cooling of the column inlet to a temperature below the solvent boiling point for a limited period of time[4]. In fact, all the systems available at present are mentioned in their patent of 1978[5]. Originally, these systems were designed to keep the syringe needle cool enough to prevent solvent evaporation inside the syringe needle even at elevated oven temperatures, which is of interest with regard to slow injection. This concept was abandoned, and today secondary cooling, a movable on-column injector and an injector with a separately thermostated column inlet are used for different purposes. However, the change of the original concept caused confusion, which still exists to some extent.

It is a fairly old idea, adopted *e.g.* by Varian, to use the inlet cooling systems mentioned above to retain sample liquid being rejected from an excessively hot column, the injected sample plug being driven forwards by the carrier gas into the column at high temperature again. At the point where the column temperature exceeds the solvent boiling point at the given inlet pressure, the vapour pressure of the sample keeps the sample plug from penetrating further into the column. Ideally, the sample plug would be stopped there, allowing the solvent to evaporate from the front to the rear of the plug. The sample plug blocks the passage of the carrier gas, and

0021-9673/85/$03.30

solvent vapour completely replaces the carrier gas in the column. This picture, presented in a similar form by Yang[6], deviates from reality as far as establishment of the solvent vapour pressure presupposes evaporation of solvent, which does not occur as smoothly as might be assumed at first. The column surface, mostly coated with stationary phase, is poorly suited for sustaining gentle evaporation. As a result, the sample liquid penetrates beyond the point representing the critical temperature (at column temperatures not too far above the actual solvent boiling point, often for several tens of centimetres). Overheating of the liquid causes the delayed evaporation to be violent and, if evaporation occurs at the front of the sample plug (which is often but not always the case), the plug may be rejected explosively. When returned into the cool section of the column at the rear, the solvent vapour recondenses and loses its propellent activity. However, owing to its inertia, the sample plug does not stop immediately and often covers a considerable distance within the cool column section before being pushed backwards towards the heated column again.

The length of the path covered by the rejected sample liquid in the cool inlet section was determined experimentally to serve as a basis for designing instrumentation suitable for isothermal runs at column temperatures above the boiling point of the sample solvent. In fact, instrumentation fulfilling these requirement already exists. However, it must be noted that rejection of the sample plug is not the only problem encountered when carrying out on-column injection at high column temperatures.

EXPERIMENTAL

Determinations of the distance covered by the rejected sample liquid were carried out by observing the sample liquid in a capillary end-section protruding from a GC oven. An on-column injector was held by a stand about 20 cm above the oven, in the roof of which was a small hole through which the end-section of the glass capillary column passed to the on-column injector. The cover of the oven was 2 cm thick (insulation material). The column end-section above the insulation material was kept at ambient temperature by ventilation with a hair dryer. For most experiments a whitish glass capillary of 0.30 mm I.D., obtained by alkali etching of Durobax glass, was used, turning transparent when the inner surface was coated with condensed liquid. Pure solvents were injected rapidly using a 10-μl syringe with a long 0.17 mm O.D. fused-silica needle. The injection point was slightly below the

TABLE I

LENGTH OF THE BACKFLOW ZONE FOR DIFFERENT SOLVENTS

Injection volume, 2 μl; column temperature, 250°C; carrier gas (hydrogen) flow-rate, 3 ml/min.

Solvent	*Length of backflow zone (cm)*
n-Pentane	11
n-Hexane	8
n-Heptane	7
Dichloromethane	9
Methanol	7

TABLE II

LENGTH OF THE BACKFLOW ZONE AT DIFFERENT CARRIER GAS FLOW-RATES

Injection volume, 2 μl; solvent, *n*-hexane; column temperature, 250°C.

Flow-rate (ml/min)	*Length of backflow zone (cm)*
1	10
2	10
3	8

expected return point of the sample liquid, and the needle was withdrawn immediately after injection in order to prevent liquid returning to the tip of the syringe needle being pulled backwards up the column neck. The distance between the rear edge of the returned liquid and the insulation material was measured.

RESULTS

Table I shows the distances covered by the returning solvents in the cool column inlet when 2-μl volumes were injected at an oven temperature of 250°C and a carrier gas flow-rate of 3 ml/min, using the whitish, uncoated capillary (which was well wetted by all the solvents used). The distances were similar, but with a tendency for longer distances to be covered by the more volatile solvents.

Table II indicates that there is at best a slight dependence of the backflow on the carrier gas flow-rate.

Table III shows the relationship between the backflow and the column temperature in the oven when 2 μl of *n*-hexane (b.p. 69°C) were injected at a carrier gas flow-rate of 3 ml/min (0.15 atm inlet pressure). In general, the solvent returned further the higher was the oven temperature. However, the value obtained at 85°C did not follow this rule; it represents the mean of several, poorly reproduced experiments. Apparently the solvent enters far into the oven-thermostated column, resulting in a pronounced delay of evaporaton and a correspondingly violent (although not reproducible) backflow. At 70°C there is no backflow as the sample plug is not hindered in flowing into the oven-thermostated column.

The solvent returned further back into the cool column inlet the larger was the injection volume (Fig. 1). It is interesting that the length of column inlet through which the liquid returned increased more slowly than the length of the plug of injected

TABLE III

LENGTH OF THE BACKFLOW ZONE AT DIFFERENT COLUMN TEMPERATURES

Injection of 2 μl volumes of *n*-hexane (b.p. 69°C); carrier gas flow-rate, 3 ml/min.

Temperature (°C)	*Length of backflow zone (cm)*
85	5.5
100	4
150	6
200	7
250	8

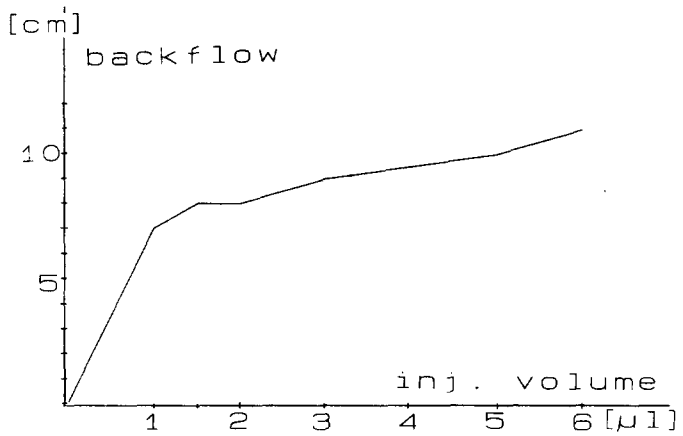

Fig. 1. Variation of the length of the backflow zone with injection volume. Injection of *n*-hexane at a column temperature of 250°C and a carrier gas flow-rate of 3 ml/min. On increasing the injection volume, the backflow zone is less elongated than the plug of the injected sample liquid.

solvents (a 1-μl volume corresponds to a plug length of about 14 mm). Apparently long sample plugs are not as easily rejected as short, light plugs.

Some experiments were repeated using a glass capillary with a smooth surface coated with OV-1. The length of the backflow zone was approximately equal to that measured with the internally roughened, uncoated capillary, except for methanol, which does not wet OV-1-coated surfaces. A 2-μl plug of methanol was rejected through an inlet section about 16 cm long (compared with 7 cm on the wetted capillary).

DISCUSSION

The experimentally determined lengths of the cooled column inlet section into which sample liquid is rejected on injection into capillaries kept above the solvent boiling point ranged between 4 and 11 cm. For sample liquids that do not wet the internal capillary surface the backflow zones are elongated.

An on-column injector suitable for injections at high column temperatures must be capable of handling rejected sample liquid. It must contain a length of capillary inlet cooled below the solvent boiling point that is at least equal to the length of the backflow zone (otherwise sample material is lost backwards into the cold injector). Further, it must ensure that the sample material deposited at the rear of the backflow zone is oven-thermostated after injection is completed; material deposited in a zone that remains cool is released with delay (forming a tailing peak) or remains there until the next injection brings liquid there, which (at least partly) flows back into the later oven-thermostated column section. Whether the backflow zone is oven-thermostated or heated to above the oven temperature is of secondary importance.

The length of the capillary section serving as the backflow zone should be of the order of 15 cm, including some safety margin considering that rejection is not a well controlled process and that unforseen factors or incidental processes could elongate the backflow zone.

The column inlet section representing the backflow zone must not be heated before the sample solvent is fully evaporated, otherwise the vapour volume generated overfills the available space and part of it flows backwards into the unheated capillary part in the rear or even into the injector.

The length of the syringe needle must be adjusted to release the sample liquid at the rear of the backflow zone. This helps to prevent sample liquid from returning to the tip of the syringe needle. If it touches the latter, liquid is pulled by capillary forces into the narrow space between the needle and the capillary wall, and when the needle is withdrawn this liquid is pulled further back up the column neck, causing losses of high-boiling sample material and memory effects[7].

As mentioned in the introduction, a system allowing a sufficient length of the column inlet to cool during injection does not ensure good performance of the system. Injection at column temperatures above the boiling point of the sample solvent causes the solvent effects to be inefficient or absent. The range of conditions providing sharp initial bands (avoiding peak deformation or splitting) will be discussed in a later paper.

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