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SEPTUMLESS LIQUID INJECTION FOR HIGH-TEMPERATURE GAS CHROMATOGRAPHY ON PACKED COLUMNS*

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SUMMARY

A pre-column arrangement permitting septumless injection of dilute solutions for high-temperature gas chromatography is described and evaluated. The sample is dropped, at atmospheric pressure, on to the top of a two-zone packed pre-column. An appropriate temperature cycle permits the successive vaporization of the solvent, which is partly eliminated through the injection channel, and insertion of the less volatile solutes into an isothermal column. The initial feed volume is eliminated by means of a cold trap. Some observations could be made concerning initial band spreading when a coloured volatile solute was used.

INTRODUCTION

In high-temperature chromatographs equipped for injection by microsyringe, damage to the inlet septum due to repeated puncture, and, in some designs, contact of the septum with hot gas, lead to the release of interfering silicone material into the hot flash heater. Another drawback of closed injection is a tendency of the usual microsyringes to develop leakage when they are discharged against a positive pressure.

JAMES AND MARTIN[1], in early experimental work on gas–liquid chromatography, devised the method of simply dropping a liquid sample, at atmospheric pressure, on to the top of the column packing. Although now obsolete in commercial equipment, this method is potentially advantageous over closed injection in permitting free expansion of the solvent vapour from dilute samples. The latter feature, however, is not directly exploitable in the high-temperature analysis of low-volatility mixtures on more recent[2] thin-film columns. Indeed, flash vaporization requires that the temperature at the injection point considerably exceeds the boiling point of any volatile solvent commonly used as the sample carrier. Consequently, a rapidly formed vapour plug would tend to eject the sample outside an unlocked injection chamber.

The present paper deals with a combination of expedients whereby the early procedure of open-column sampling is adapted for high-temperature analysis. The

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initial temperature of a packed pre-column is adjusted at a value suitable for smooth vaporization of the solvent. The medium- and low-volatility solutes are further vaporized and displaced to a cold storage area. The chromatogram is finally developed from the latter spot.

EXPERIMENTAL

Apparatus

This is shown schematically in Fig. 1. A Pye (Cambridge, Great Britain) Series 104 isothermal gas chromatograph, with flame ionization detection, is used as the basic instrument. The inlet of a standard 5 ft. × 3.5 mm I.D. glass column extends vertically for a distance of 30 cm outside the oven. Heat is conducted from the oven up to the point of emergence of the column by means of a 12 mm O.D. × 6.5 mm I.D. aluminium tube (A in Fig. 1).

The column, including its external extension (pre-column), is uniformly packed with the desired partitioning phase, up to 8–9 cm from the top of the pre-column. Packing is completed with a plug of glass-wool (B), and finally a 3-cm layer of 0.2-mm glass beads (C).

The top of the pre-column is equipped with a 1/4 × 1/16 in. swagelock reducing union (D) with a lateral inlet for the carrier gas. The 1/16-in. side of the union, at the top, features a channel approximately 1 mm in diameter, for injection by a micro-syringe, which is normally closed by a screw-cap. Carrier gas (nitrogen) is supplied from a flow controller (E) via a shutting valve (F).

The pre-column, as in a previous study[3], is tightly wound, without a gap, with a metal coil. The coil is formed from No. 19 (s.w.g.) Nichrome wire. The air gap

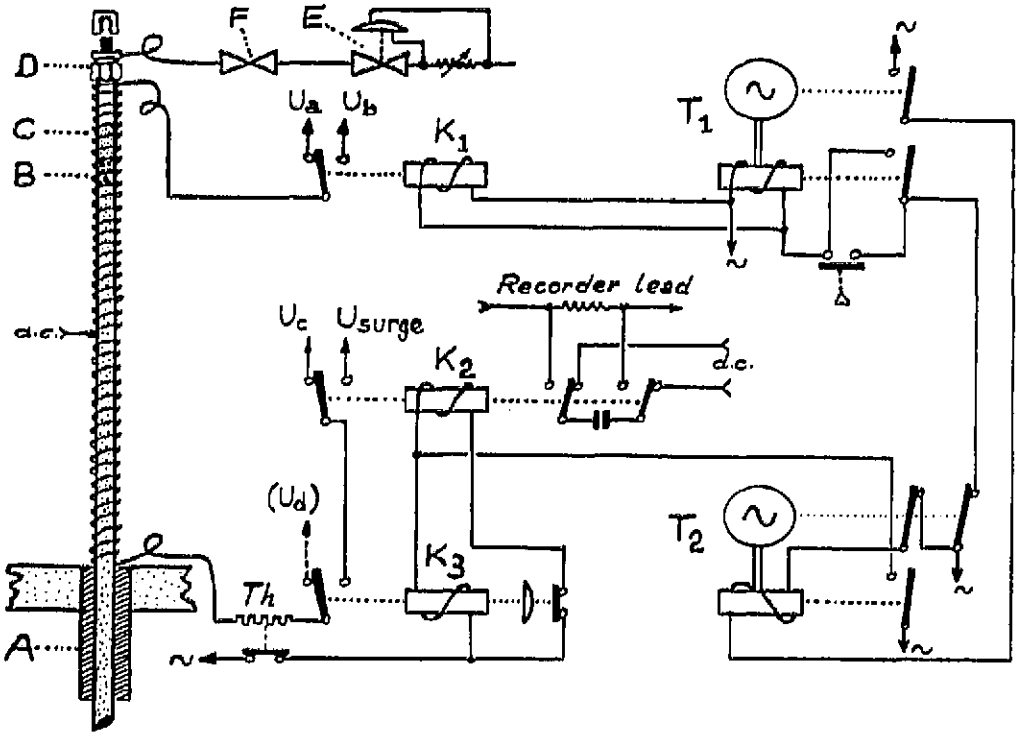


Fig. 1. Schematic diagram of the open pre-column injection system. For description see text.

between adjacent turns of the coil is adjusted to 0.1 mm. The coil is tapped, at its centre, with welded No. 22 (s.w.g.) silver wire, which determines the boundary between electrically distinct heaters. The upper heater, as seen in Fig. 1, covers the injection chamber and part of the packed volume of the pre-column. This segment can be heated at either of the two pre-set temperatures by means of terminals (U_a, U_b) on a low-voltage transformer, through the contacts of relay K_1. The circuit of the lower coil is normally interrupted by relay K_3, the latter being de-energized. K_3 has a pneumatic delay attachment (range 5–15 sec) which de-energizes another relay, K_2, after a pre-set time. This delay is used for supplying the lower heater with a high-current surge (up to 15 A) through a contact of excited K_2. This ensures the rapid warm-up of the glass tube; the heating current is finally reduced to the maintenance value when K_2 is de-excited. A thermal breaker, Th, rated 3–4 A, protects the pre-column against accidental overheating.

The heating periods for both heaters are interlocked and are independently adjustable on timers T_1 and T_2 (Fig. 1) of the synchronous motor-type (Model 7PN-6051, Siemens, G.F.R.). Timer T_1, started by a push-button, is wired in parallel with power relay K_1, which controls the upper heater. The fast lower heater, through relays K_2 and K_3, is started by timer T_2, while the latter timer is retarded by the pre-set time on T_1. The initial condition is restored with re-setting of both timers from T_2.

For the indirect estimation of temperatures, a 30-cm packed glass tube, B, identical to the pre-column A (Fig. 2) and located in the same environment, is equipped with a pair of similar heaters, which are wired in series with the corresponding coils of the pre-column A. Thermocouples, Tc, of negligible thermal inertia are imbedded in the packing of the auxiliary tube B. The presence of the latter devices should be considered implicit in the general drawing of Fig. 1, from which they have been omitted for clarity.

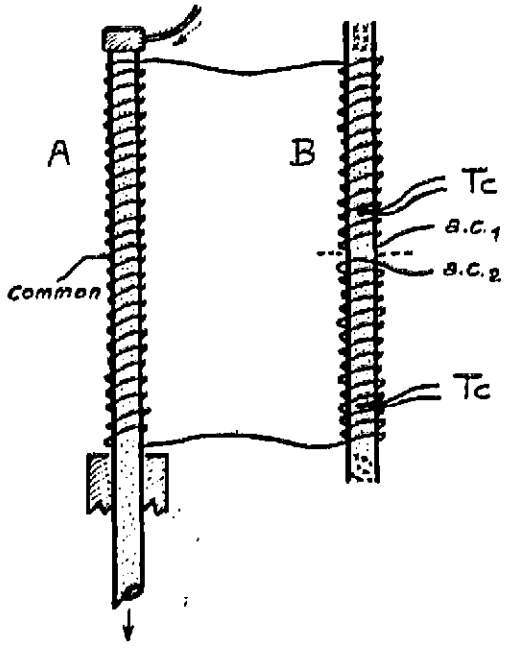


Fig. 2. Schematic representation of the auxiliary tube used for temperature estimations. For description, see text.

Operation

The temperature of the upper zone of the pre-column is adjusted, in the initial condition, to a value slightly in excess (by 10–20°) of the boiling point of the sample solvent to be injected. The heater of the lower zone is normally cut off, but it is possible to heat this segment slightly by supplying a low voltage through U_d (Fig. 1, dashed line, and resting contact of relay K_3). The temperature of the isothermal column is kept at the value required for analysis.

Prior to injection, the gas supply is shut off. After the release of pressure at the top of the pre-column, the cap on the injection aperture is removed. The gas supply is turned on again, which scavenges the injection channel and reduces the diffusion of air[4]. The sample is discharged, from a microsyringe, on to the top of the layer of glass beads. The solvent vaporizes smoothly, while any vapour in excess of the hold-up of the injection chamber is swept to the atmosphere through the injection channel. Closure of the chamber re-establishes the normal flow of carrier gas through the pre-column and column. Trapped solvent vapour and eventually very volatile contaminants are swept through the packing, while the medium- and low-volatility components of interest remain as a residue at the top. At this stage, some heating of the lower pre-column segment is sometimes necessary to prevent condensation of a high-boiling solvent at the inlet of the latter zone.

The automatic heater cycle can then be started. Closure of relay K_1 (Fig. 1) initiates superheating of the upper half of the pre-column to a temperature appropriate for sample vaporization. This step is progressive; *e.g.*, equilibration to 250° starting from 100°, with the pre-column size as specified, requires about 5–7 min. The various sample components are displaced down to the inlet of the relatively cold lower zone, which acts as a cold trap. The duration of the heating period is adjustable on timer T_1, according to sample requirements.

The last step, which starts the chromatogram, consists in rapidly raising the temperature of the lower half of the pre-column. With the circuit described, under the control of timer T_2, a rise in temperature of 200° requires 20–25 sec, with some overshooting (see warm-up curve on Fig. 4).

Chromatographic records

As development of the chromatogram is delayed until the temperature of the cold trap is raised, the various detector signals produced at the preceding stages (from the solvent and from any volatile contaminants) are useless. A fair approximation of the zero time of the chromatogram is given with closure of relay K_2 (Fig. 1), which produces a mark on the recorder chart. Therefore, auxiliary contacts of K_2 (Fig. 1) discharge a condenser across a low-value resistor in series with one of the recorder input leads.

Pre-column conditioning. The necessity for thermally pre-conditioning a fresh packing before use, according to standard practice, applies to the pre-column segments as well as to the main column. When a sufficiently large oven is available, the entire glass system is best pre-conditioned in bulk. Alternatively, both coils of the pre-column are heated in series at a temperature close to that of the main oven.

Evaluation of the pre-column system

Temperature distribution — Validity of practical temperature measurements.

The temperature distribution along the pre-column specified above was scanned by means of small thermocouples. The test conditions were: carrier gas flow-rate, 60 ml/min at S.T.P.; and temperature of the upper segment (at a point remote from the top), 250 ± 5°. The lower segment (unheated) was in equilibrium with the ambient air.

The temperature of the carrier gas above the top of the packing, owing to the omission of a pre-heater, was 20–30° below the equilibrium value observed at about 1 cm inside the packing. Further on, the temperature of the upper segment was uniform to within 2°. Starting from the point of electrical branching of the heating coil, half-way up the pre-column, there was a gradual decrease in temperature along the cold trap, spread over about 4 cm at a flow-rate of 60 ml/min. Downstream to the latter area of heat exchange, the temperature of the cold trap was comparable with that of ambient air.

Thus, for specification of pre-column parameters in the usual terms, temperature readings made at a point remote from a zone interface are the most satisfactory, being independent of the flow-rate. Internal thermocouples placed accordingly, with glass-sealed terminals protruding from the pre-column wall, however, were found to be impractical, rendering installation of the heating coil around the pre-column difficult.

Instead, the feasibility of displacing the sensing points to an auxiliary glass tube, thermally identical with the pre-column, was investigated. Differential thermocouple measurements were made in paired glass tubes with series-wired heating coils as shown in Fig. 2. The terminals for the thermocouple leads were accessible at both ends of the tubes (details not shown in Fig. 2). With reasonable care in duplication of the tubes and heaters, discrepancies between readings did not exceed 3% over the temperature range 50–300°.

Pre-column processes — Observations with a coloured solute. Samples (10–100 μg) of azobenzene, a thermally stable coloured compound of medium volatility (b.p., 293° at 760 mm), dissolved in up to 50 μl of *n*-heptane (b.p., 98.4° at 760 mm), were injected with a microsyringe on to the top of a pre-column as specified above under *Apparatus*. The packing was 3% of XE-60 on Gas-Chrom Q, 100–120 mesh (Applied Science Laboratories, State College, Pa., U.S.A.). There was provision for easy withdrawal of the heating coil, when required, for visual inspection of the glass tube.

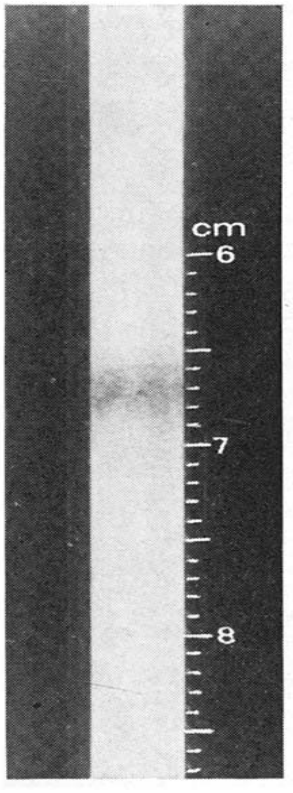
The top of the packing was kept at 110–120° at the time of injection, so that the vaporization of *n*-heptane from the larger samples (50 μl) proceeded smoothly, without bursts, over about 30 sec. Condensation was not encountered during the exhausting of *n*-heptane vapour through the injection channel swept by carrier gas (the nitrogen flow-rate was usually 50 ml/min at S.T.P.), although the metallic inlet fitting (D in Fig. 1) was not equipped with a special heater. Even with the larger samples, there was only a small degree of penetration of liquid into the packing composed of glass beads at the top (C in Fig. 1); after complete vaporization, a stain of azobenzene was found to be concentrated at the top of the packing, with very little spreading.

The azobenzene spot at the top was further displaced to the inlet of the cold-trapping segment half-way up the pre-column (see under *Operation*). Flow-rates within the range 10–100 ml/min at S.T.P. and final temperatures of the upper zone within the range 150–250° were used in these experiments. In each instance, build-up of

a narrow yellow-orange azobenzene band was observed, freezing at a short distance downstream to the boundary of the upper heating coil. A photograph of a typical spot obtained with 20 μg is shown in Fig. 3. Over the range of conditions tested, the visible width of the coloured band varied between 1.5 and 3 mm, being a function of increasing flow-rates. At a given flow-rate, increasing the final temperature of the upper heater caused the spot to penetrate further into the cold-trapping zone. The greatest penetration (by 8 mm, counted from the boundary of the upper coil) was observed with a final temperature of the upper segment of about 250° with a flow-rate of 100 ml/min. This finding correlates with the existence of a temperature gradient at the inlet of the cold trap (see under *Temperature distribution*).

A frozen band of comparable width (a few millimetres), although less easily observed, was recorded in similar experiments with estradiol, a less volatile compound revealed by its fluorescence under ultraviolet light.

Mobilization of the frozen sample for chromatography. By means of special circuitry (see under *Apparatus* and *Operation*), the rate of warm-up of the trapping segment of the pre-column is increased, whereby an approximation of isothermal insertion of the frozen sample on to the main column can be obtained. In Fig. 4 is reproduced a typical thermocouple reading inside a 6 mm O.D. × 3.5 mm I.D. cold trap. The graph shows that for a final temperature of 300° during the warming-up period the temperature increases from 40° to 250° in 22 sec. This result was obtained with a surge current of 12 A applied during 11 sec, followed by the appropriate



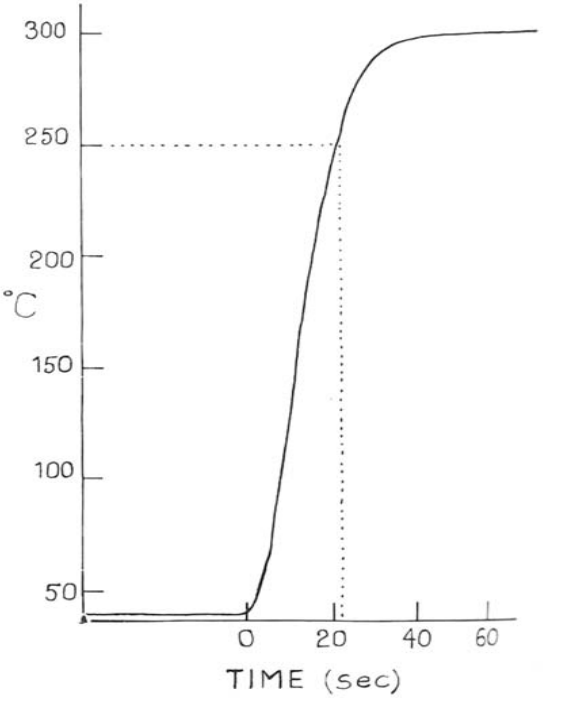


Fig. 3. Photograph of the central portion of a 3.5 mm I.D. pre-column showing a trapped sample of azobenzene. Conditions are given in text.

Fig. 4. Thermocouple record showing the warm-up profile of a 6 mm O.D. × 3.5 mm I.D. Pyrex cold trap. Conditions are given in text.

maintenance current (about 4 A). The profile of the curve in Fig. 4 suggests the advantage of significantly superheating the trapping segment, whereby a steeper temperature gradient can be obtained over a desired temperature range. The behaviour of Pyrex glass tubing under thermal shock was found to be satisfactory.

A pre-column of which the cold trap was operated under similar conditions was followed by an isothermal column of classical length (150 cm). With samples such as mixtures of C_{22}–C_{32} *n*-alkanes, chromatograms similar to classical isothermal ones were obtained; these samples approximate the volatility spectrum encountered, *e.g.*, in steroid analysis. It was found advantageous, keeping within the limits of the thermal stability of the stationary phase, to raise the cold trap to a temperature somewhat above that of the main column; when this was done, the retention data were insensitive to moderate fluctuations of the temperature profile at the cold trap.

For a still better approximation of isothermal insertion, the flow of carrier gas was interrupted during warm-up of the cold trap (for about 20 sec); the chromatogram thus starts after restoration of the carrier gas flow. The latter mode of operation, however, was found not to afford a definite advantage over the former method.

Detector compatibility — Influence of column bleeding. Most of our experience with open-column sampling was gained by using a hydrogen flame ionization detector (see under *Apparatus*). No attempt was made to modify the gas connections that are classical for packed columns, where hydrogen is admixed at the column outlet. As operation of the pre-column entailed interruption of the column effluent for 30–60 sec during each injection, combustion in the detector continued, during the same periods, with undiluted hydrogen. This unusual mode did not appear to affect detector condition in the long term.

An isothermal column with a relatively unstable liquid phase operated at high temperature caused, following restoration of the gas flow, an important rise of detector standing current slowly returning to the base-line. The latter classical effect, related to column bleeding, did not interfere on the chromatogram which in the present method normally starts only after a delay of 10–15 min.

DISCUSSION

The present experimental arrangement does not achieve complete removal of the solvent from the sample at the pre-column stage, but it affords, in addition to the septumless feature, a convenient means for limiting the volume of residual vapour from large samples to a relatively constant low value. Interestingly, these results were obtained with a completely all-glass system.

Even with large loads of solvent, there is evidence of satisfactory localization of the vaporization process to a narrow area at the top of the pre-column. This feature may be interpreted as follows.

Carrier gas at the top encounters the high pneumatic resistance of the column packing in downward direction, and the low, but non-negligible, resistance of the open injection channel in upward direction. The latter resistance develops a slight pressure in the injection chamber, which leads to penetration of the liquid sample into the heated layer of glass beads at the top of the pre-column. From this stage onwards, the solvent vaporizes progressively at a self-limiting rate, as the locally formed vapour plug, expanding upwards in the direction with the least resistance,

opposes a counter-pressure that tends to lift the liquid away from the packing.

It seemed worthwhile, in spite of some complications in design and operation, to introduce a special thermal cycle for the lower half of the pre-column. The latter "cold-trapping" stage, in the interest of isothermal chromatography of medium- and low-volatility components, provides a starting point for the chromatogram that is unaffected by either variations in sample distribution or the accumulation of deposits from impure samples at the top of the pre-column. The observations with a coloured spot confirm the well documented[5–7] superiority of insertion from a cold trap whenever on-line vaporization of the sample is precluded for some reason. Omission of the lower pre-column segment is conceivable, however, if wide-range temperature-programmed analysis is exclusively envisaged; of course, in the latter mode, the inlet of the analytical column is used as a cold trap.

Metallic heating coils for the pre-column may leave something to be desired in terms of the precision and accuracy of the temperature parameters, but are perhaps difficult to replace until elaborate small-sized ovens with a comparable flexibility become commercially available.

CONCLUSION

The present study seems to indicate a line along which adaptations of the early method of open-column sampling, adequate for high-temperature gas chromatography on packed columns, might develop. A system of relatively short pre-column segments with interlocked thermal cycles appears to afford maximum flexibility for such investigations.

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