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ALL-GLASS SYSTEM FOR PREPARATIVE GLASS CAPILLARY GAS CHROMATOGRAPHY

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SUMMARY

An all-glass effluent splitter of virtually zero dead-volume and a peak collecting device were developed for the recovery of pure samples from the effluent of glass capillary columns for field testing, organoleptic evaluation or spectral identification.

INTRODUCTION

In work on the identification of the semiochemicals involved in insect and mammalian chemical communication, the isolation of impure samples for bioassay may result in erroneous or contradictory results, as even traces of a pheromone in an otherwise inactive fraction may lead to a positive response in the test animal or insect. The same type of problem is often encountered in flavour research where pure compounds have to be isolated for organoleptic evaluation.

From our previous experience with preparative packed and capillary column chromatography, using different splitting and collection techniques such as multiple zone heating and cooling of the collection tube, there seem to be several major causes for the collection of samples whose purities do not meet expectations based on the apparent separating efficiency of the column. Firstly, in the case of packed columns, the presence of preceding component impurities in collected samples may be due to the tailing of preceding peaks that may not seem to be appreciable at the attenuation at which the preparative separation is carried out, but may nevertheless contribute to the composition of the baseline effluent during the collection of a component. Secondly, the presence of preceding components in a collected sample is often due to the presence of one or more cold zones in the line conducting the effluent from the splitter to the collection device. If, for example, metal is used in the construction of the splitter, and compounds susceptible to catalytic decomposition on hot metal surfaces have to be collected, it may not be feasible to prevent precipitation at the tip of the effluent delivery line by increasing the temperature of the splitter assembly. Specifically at this point in the system the problem will be aggravated if fractions are collected in devices with a high heat capacity at their inlet end, such as Luer-Lock needles or 5 mm O.D. ground glass joints. On starting the collection of a fraction the tip will immediately be cooled down and if the peak is sharp, *i.e.* if an efficient column

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is used, it may not reach the correct operating temperature before the collection of that particular peak has to be terminated. Therefore, if a capillary column is used and closely spaced peaks have to be collected, the temperature of the effluent line tip will almost invariably be below the temperature of the splitter assembly, resulting in serious contamination of fractions with preceding components.

In a recent publication on preparative capillary gas chromatography, Williams and Vinson[1] have reviewed previous systems[2–4] in which *inter alia* heat-shrink PTFE and platinum-iridium have been used. Using materials such as silicone rubber and PTFE to effect a gas-tight connection at the inlet end of the collection device will have a disastrous effect on the purity of the collected material, due not only to the heat capacity and insulating properties of these materials, but also to solubility and/or adsorption effects. As far as the use of platinum-iridium is concerned, the arguments advanced by Grob[5] against the use of noble metals in the construction of these devices, were confirmed by results that we have obtained with a platinum-iridium capillary as a gas chromatography–mass spectrometry interface. It was found that although the platinum–iridium surface can be deactivated by heating the capillary at red heat with oxygen flowing through it[4], the inert surface is gradually destroyed when it comes into contact with halogen-containing solvents at elevated temperatures, resulting in the formation of dehydrogenated and even doubly dehydrogenated compounds. Although the design described by Williams and Vinson apparently solves some of the shortcomings of previous systems, our research required a system that would also allow highly volatile substances to be collected in a small volume of solvent. A system was therefore devised which meets the following requirements:

(1) Minimal dead volume in the column-to-splitter and splitter-to-collector couplings;

(2) Minimal contact of the effluent with hot metal surfaces;

(3) Independent and variable heating of the splitter tip and inlet end of the collection tube;

(4) Thin-walled glass tubes of low heat capacity for the collection of samples;

(5) Elimination of ferrules or other seals in the splitter-to-collector coupling;

(6) Provision for the collection of samples in pure form or in small volumes (1–6 μl) of solvent;

(7) Efficient precipitation of material in the glass collection tubes.

This paper describes such a system which has been giving trouble-free service in our laboratory for more than two years.

EXPERIMENTAL

Splitter construction

The dimensions of the splitter shown in Fig. 1 are similar to those of the Varian splitter (Varian, Walnut Creek, CA, U.S.A.) used for dual-detector operation of the Varian 3700 gas chromatograph. For preparative purposes one detector (flame ionization, FID) is used and the splitter is therefore installed with the second arm extending through the second detector port. However, the basic design can be adapted to any gas chromatograph, provided the collection tube can be connected vertically to the splitter.

A glass capillary column is connected to a mixing chamber A of zero dead-

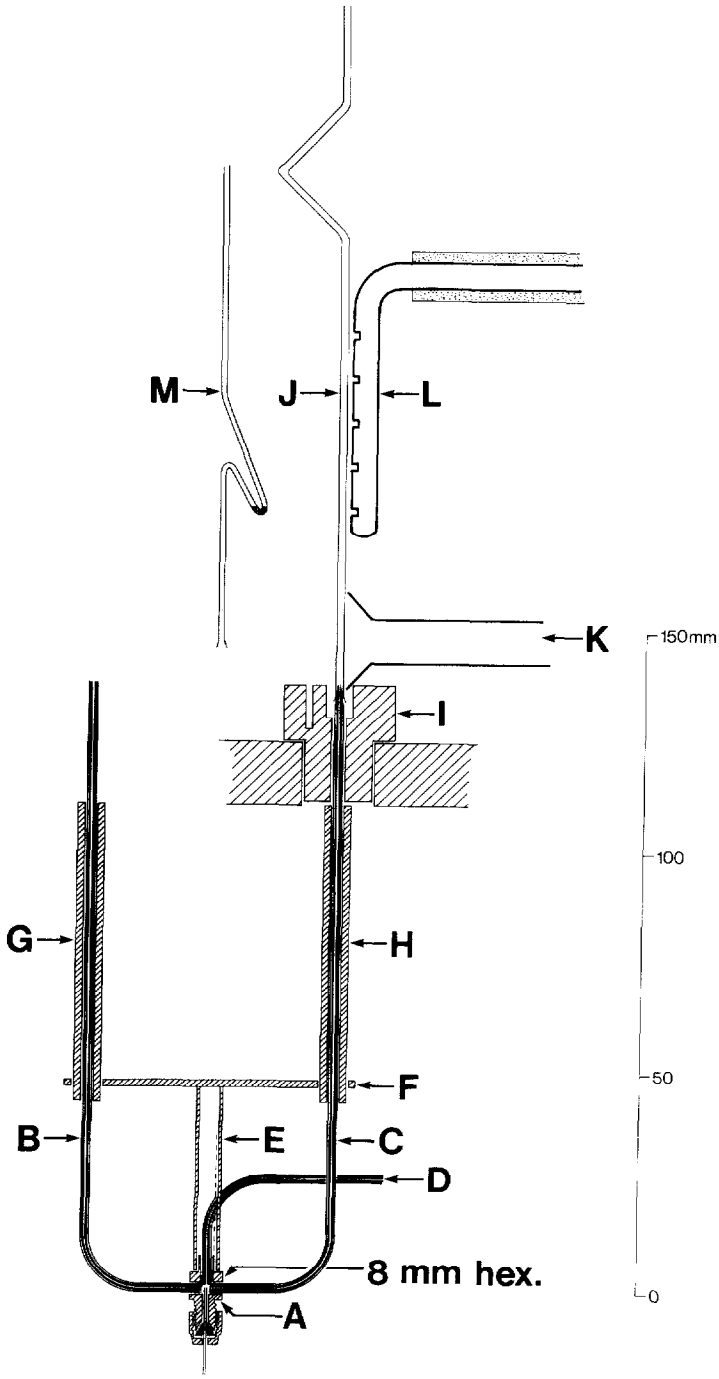

Fig. 1. Diagram of the splitter and trapping system. A = mixing chamber; B = glass-lined stainless-steel line to the detector; C = glass-lined stainless-steel line to the collection tube; D = column make-up gas line; E = vertical tubular support; F = horizontal support; G and H = stainless-steel sleeves; I = cylindrical aluminium heating block with thermocouple well; J = thin-walled glass collection tube; K = heating gas line; L = cooling gas line with horizontal exit slits; M = alternative collection tube with solvent plug shown in the elbow; hex. = hexagonal.

volume, using a Vespel ferrule. The column tip must extend *ca.* 5 mm into the mixing chamber to allow proper mixing of the column effluent with the make-up gas. Three glass-lined stainless-steel tubes B, C and D (SGE, 0.3 mm I.D., 1.6 mm O.D.) are silver-soldered into the mixing chamber, the exposed tip of one of these tubes (C) having been machined to a 6° taper, using a fine file, and polished with emery-paper (00 grade). Using a cutting tool instead of a file for this purpose will result in fracturing of the glass lining of the tube. A tubular support E (58 × 6.4 mm O.D.) with a slit in its side is soldered to the mixing chamber and to a horizontal support F (69 × 14 × 1.1 mm). Make-up gas (helium) is introduced into the mixing chamber through tube D. The mixture of column effluent and make-up gas leaves the mixing chamber through the glass-lined tubes B and C, which are further silver-soldered to the stainless-steel sleeves G and H (64 × 6.3 mm) with the tips of the glass-lined tubing protruding 27 mm from the upper ends of the sleeves. The flow through tube B leading into the detector is restricted by insertion of a quartz fibre with a diameter of typically 0.23 mm until a slight resistance is encountered when the fibre reaches the bend in this arm of the splitter. To facilitate its eventual removal the quartz fibre is cut off *ca.* 1 mm above the tip of the tube. The two sleeves are silver-soldered to the horizontal support F. The stainless-steel sleeve G is secured to the FID with a Swagelok ferrule and nut in the usual manner. The upper part of tube C is heated by a cylindrical aluminium block I which fits snugly into the second detector port and around tube C.

Collection tubes

Fractions are collected in glass tubes (180 × 0.9 mm I.D., 1.4 mm O.D.) produced on a capillary drawing machine. The tubes are flared at one end on a lathe or a laboratory stirrer motor (Heidolph, Kelheim, G.F.R.) using a micro-burner and a sharp brass reamer, and are then bent as shown in Fig. 1 (J). To bend these tubes in batches they are attached with adhesive tape at 10-mm intervals along the edge of a metal plate, leaving *ca.* 40% of the length of each tube free for the three bends. Positioning the tubes at the correct intervals and at right angles to the edge of the support is facilitated by shallow grooves at 10-mm intervals in the plate. The first bend is then made with the metal plate clamped at an angle of 45°, the glass tubes pointing downwards, whereafter the next two bends are made with the plate clamped at the appropriate angle, such that after each bend the tubes point vertically downwards.

Collection tubes were used without prior cleaning with cleaning solutions, as they were made from tubing that was sealed off in a flame after it had been produced on the capillary drawing machine.

Hot air or nitrogen is directed onto the tip of splitter arm C and the inlet end of the collection tube through a quartz tube K (10 mm I.D.) containing a constantan heating spiral, the temperature of which is regulated with a voltage regulator (2.5 kVA).

The collection tube is cooled at five points between 40 and 90 mm from its inlet end. Cooling gas is generated, and its flow and temperature are regulated by boiling liquid nitrogen in a Dewar with an immersion heater (50 watt) connected to a voltage regulator (1 kVA). The cold nitrogen conducted to the collection tube through an insulated Pyrex tube (L) incorporating five horizontal exit slits. The temperatures of the heating and cooling gas streams and the aluminium block I are monitored with thermocouples.

Operation

The collection of components from a canine urine extract provides an example of the isolation of components from a complex mixture (Fig. 2). A wall-coated open tubular capillary column [free fatty acid phase (FFAP), 40 m × 0.3 mm I.D., film thickness 0.2 μm] was employed for this purpose. Helium was used as carrier gas (1.2 ml/min at 40°C) and column make-up gas (2.3 ml/min). A 0.23-mm quartz restriction in line B gave a splitting ratio of 9:1. Thus, at a column temperature of 40°C, a total

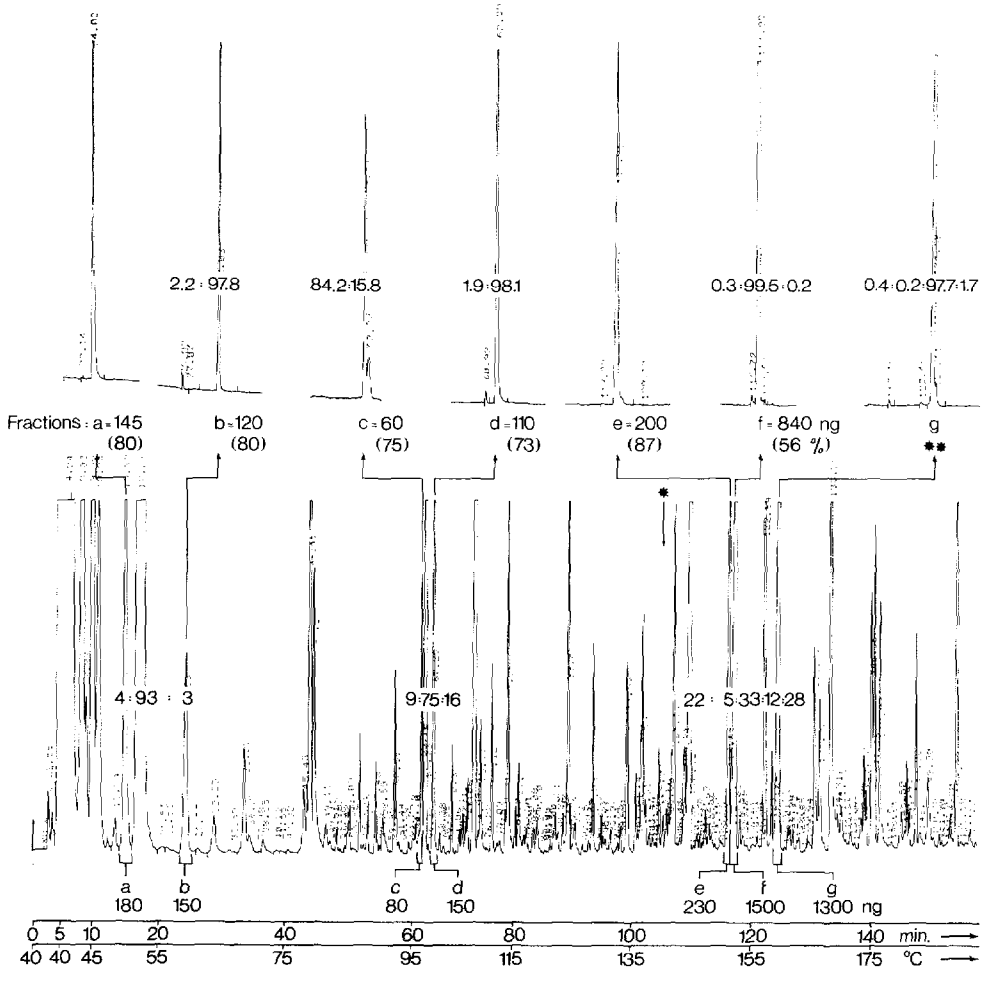

Fig. 2. Lower trace: Preparative gas chromatographic separation of a canine urine extract using splitless injection at an injector temperature of 100°C, whereafter the injector temperature was increased to 190°C. Temperature program: isothermal at 40°C for 5 min and then programmed to 220°C at 1°C/min. Column: 40 m × 0.3 mm I.D. FFAP on $BaCO_3$-treated Pyrex. The amounts (ng) of the components selected for collection are given at the bottom of the preparative gas chromatogram. The quantitative ratios of specific components in the urine extract are shown across the peaks to which these figures apply. Upper traces: Gas chromatographic analyses of the collected fractions on the same column and with gas chromatographic conditions similar to those used for the preparative separation. The amounts of material recovered (ng) and trapping yields (%) are given at the bottom of the analytical traces. Component ratios are given where applicable. *, Position of methyl dodecanoate. **, Sample tube fractured on insertion into the injector.

flow of 0.35 and 3.15 ml/min was obtained through lines B and C, respectively. Helium (100 ml/min) was used as detector make-up gas. Substituting the regular Varian detector insert with this splitter did not result in detectable peak broadening. The detector temperature was set at 240°C. Samples (1 μl, dichloromethane solvent) were injected in the splitless mode at an injector temperature of 100°C, whereafter the injector temperature was set at 190°C. This temperature was reached in *ca.* 7–8 min, whereupon the injector heater was switched off and the inlet splitter flow turned on. The temperature of the cooling gas was −50°C and that of the heating gas was increased stepwise from 200°C to 260°C during the initial 1.5 h of the separation and thereafter maintained at 260°C.

To collect components, a number of collection tubes are charged from their outlet ends with 4–6 μl of dichloromethane, or [^{2}H]chloroform if the samples are required for nuclear magnetic resonance (NMR) analysis. These traps are then suspended horizontally with the solvent plugs located in their elbows. Just before a component is to be collected, the trap is carefully brought towards the vertical position so that it can be connected to the splitter tip with the solvent plug almost at the inlet end of the trap. Depending on the gas flow through the trap and its diameter, a certain portion of the solvent will immediately be blown out of the trap, leaving the rest of the solvent as a film on the trap wall where it can be seen refluxing between the hot and cold zones. On completion of the collection of a component, the trap is removed and held horizontally and the two tips are immediately flame-sealed. With the flow conditions given above, components are estimated to reach the detector *ca.* 0.6 sec later than the trap. This delay has to be taken into consideration when very narrow peaks are obtained from an efficient column. It can, however, be reduced by inserting suitable quartz fibres into both arms of the splitter, whereby the internal volume of the system is restricted.

Quantitation

A Fractovap 4160 gas chromatograph (Carlo Erba, Milan, Italy) equipped with an FID and a 3385A integrator (Hewlett-Packard, Palo Alto, U.S.A.) was used for the determination of percentage recovery and purity of the collected components. For analytical work the same capillary column and column oven temperature program that had been used for the preparative separation (Fig. 2) were employed. Samples were prepared for analysis by heating the lower tips of the vertically clamped and still sealed collection tubes in lukewarm water and cooling first the elbows and then the upper parts of the straight sections of the collection tubes with a piece of solid CO_2. This was done in order to rinse the collected material with refluxing solvent into the lower straight portions of the tubes, which were then sealed off in a small flame and introduced into the injector using a direct inlet procedure devised for those pheromone samples which cannot be applied with a syringe[6]. The same inlet technique was employed for samples of methyl dodecanoate, which was used as quantitative reference material. For the reference, however, shorter tubes that could be introduced directly into the injector and which therefore eliminated the time-consuming refluxing procedure, were employed.

RESULTS AND DISCUSSION

The isolation of components from a urine extract, serving as an example in this paper, was carried out using an FFAP-coated column whose efficiency had been greatly reduced by continued use. This column, which had started to develop dark patches over its entire length, was used intentionally in order to illustrate the performance of the system in the collection of incompletely separated peaks and peaks showing some tailing.

In order to subject the system to as rigorous a test as possible, as far as the purity of the collected material is concerned, peaks were collected over their entire width at a low attenuation (10^{-12} A/mV, $\times 4$). Furthermore, traps were changed as quickly as possible between closely spaced peaks (*e.g.* Fig. 2, e and f) so that, contrary to normal practice, the valleys between these peaks were not discarded. Gas chromatographic analyses of the collected components are shown in Fig. 2 (upper traces). When comparing the analytical and preparative gas chromatograms in Fig. 2, it has to be taken into consideration that different gas chromatographs and slightly different gas chromatographic conditions, as well as different volumes of solvent, were employed for preparative and analytical work and that retention times and peak shapes are therefore, especially in the lower temperature ranges, not directly comparable. From the analyses of the isolated components shown in Fig. 2 it is clear that components (*e.g.* a and e) that are not immediately preceded by major peaks were obtained in high purities. An exception is fraction b, which contains 2.2% of the major constituent of the urine extract, a thioether. The presence of this quite volatile impurity is most likely due to the fact that the gas chromatograph was operated in the splitless mode for more than 7 min after injection of the sample. In those cases (d, f and g) where components are immediately preceded by other major constituents, the isolated fractions contained only small amounts of the preceding constituents. The shoulder (1.7%) on the peak of the major component in fraction g is not unexpected as this shoulder is also present in the preparative gas chromatogram and was collected in the same fraction.

Although the recoveries, given in brackets in Fig. 2, may lack complete accuracy owing to the absence of an internal standard, it is clear that volatile material can be isolated effectively with this system. Nevertheless, the question arises as to why these figures are not in better agreement with the calculated splitting ratio of 9:1. To ascertain whether this may be due to incomplete trapping of components, samples of up to 1 μl of ethanethiol were injected and trapped. Using a glass tube held to the nose to smell the effluent, it was found that absolutely no trace of this compound could be detected at the outlet end of the collection tube, while only a faint smell of ethanethiol could be detected at the point where the collection tube is connected to the splitter tip. However, as the effluent is practically at atmospheric pressure in the collection tube, no appreciable loss is expected at this point if the splitter tip and the collection tubes are perfectly round and the tip is smoothly polished. The discrepancy between the calculated and experimental values is therefore most likely due to incomplete transportation of the collected material into the lower part of the collection tubes, which were sealed off for insertion into the injector. This factor does not come into play if the collected material is rinsed from the collection tubes with sufficient solvent or if samples are collected directly in micro-tubes for NMR analysis.

For initial bioassay, fractions were collected over periods of 40 min or longer in the same collection tube, without appreciable loss of the solvent (dichloromethane) in the tube, provided the cooling gas outlets are positioned within a few millimetres of the collection tube.

For the collection of relatively involatile components where the evaporation of larger volumes of solvent will not result in losing any materials, collection tubes bent as shown in Fig. 1 (M) may be used. In inexperienced hands these tubes are easier to connect to the splitter tip, as they do not require the correct positioning of the solvent plug at the inlet end of the tube before collection is started.

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