Notes

CHROM. 4571

Efficient trapping and transfer of microgram gas chromatographic fractions for infrared analysis

Methods of collecting gas chromatographic eluents for instrumental analysis have been reviewed by LITTLEWOOD[1]. Few of these methods, however, are effective for microgram amounts of compounds of widely differing volatilities. Metal capillaries have a high trapping efficiency, but there is a risk of compounds being irreversibly adsorbed or converted. Cooled glass tubes filled with a solid support offer the best prospects for trapping microgram amounts, especially when there is the chance of aerosol formation.

The transfer of samples from the glass tube to, for example, an infrared cell poses some problems. HOWLETT AND WELTI[2] describe the cocondensation of the trapped sample with argon. This method gives good results, but it is time-consuming and the recovery of low boiling compounds is poor due to losses during the subsequent evaporation of the argon.

We have had very satisfactory results with a technique by which fractions are collected in cooled glass tubes containing solid support and transferred into an infrared cell by cocondensation with carbon tetrachloride[3]. The technique has proved relatively rapid and equally suitable for high and low boiling compounds. We have now determined optimal conditions for the trapping and transfer operations by measuring the recovery after each stage with ^{14}C-labelled model compounds. The design of the glass trapping tubes has been modified accordingly.

Materials and methods

Acetone (b.p. 58°), benzaldehyde (b.p. 178°), and 1-dodecanol (b.p. 256°) were chosen as model compounds in order to cover a reasonably wide range of volatility. [1,3-^{14}C]Acetone, [*carbonyl*-^{14}C]benzaldehyde, and [1-^{14}C]1-dodecanol were purchased from the Radiochemical Centre (Amersham, Great Britain) and diluted with inactive compound to 1.0, 1.0, and 0.04 μCi/mg, respectively. In all experiments radioactivity was determined by liquid scintillation counting in a toluene solution of 5.0 g/l PPO and 0.3 g/l POPOP with a Packard Tri-Carb Model 3380 spectrometer.

The gas chromatograph was an F&M Model 5750; all parts of instrument which come into contact with the injected sample were made of glass[4]. Solutions of acetone and benzaldehyde were chromatographed on a 3-m-long glass column of 2 mm I.D. packed with 10% Carbowax on Diatoport S (60–80 mesh); a dodecanol solution was chromatographed on a 2-m-long glass column of 2 mm I.D. packed with 1% Carbowax and 10% Apiezon L on Diatoport S. The carrier gas was hydrogen; the flow rate was 40 ml/min. An all-glass splitter led 12% of the effluent to the flame ionization detector, the remaining 88% being passed through a 10-cm-long glass

tube containing solid support. The I.D. of the tube was 1, 2 or 3 mm. Both limbs of the splitter were supplied with capillary restrictions to reduce the effect of the trapping tube on the split ratio. The tube was connected directly to the effluent port of the column only when the flame ionization detector gave a signal. During trapping, the tube was cooled over part of its length by immersion in powdered Dry Ice supported in a perspex trough.

The prototype glass tube had a conical joint at each end, one male and the other female. The latter fitted onto a male joint at the outlet of the chromatograph. After collection of a fraction, the tube was disconnected and a small capillary vessel (Fig. 1) was linked to its female joint. Its male joint was sealed with a conical glass stopper fitted with a silicone rubber cap.

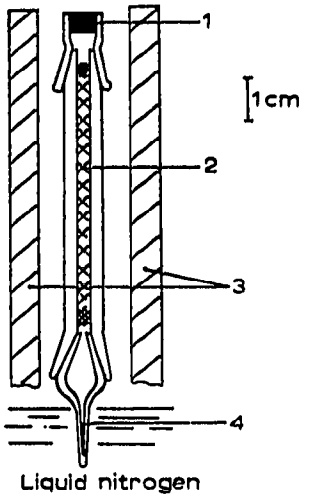

Fig. 1. Cocondensation of trapped material into a capillary. 1 = silicone rubber cap; 2 = prototype trapping tube; 3 = heater element; 4 = capillary vessel.

Once the tube had been sealed, 3 μl of carbon tetrachloride were injected through the cap onto the solid support. The tube was wrapped in cotton wool, onto which liquid nitrogen was slowly dripped. In this way the temperature of the trapping tube and its contents readily dropped to about $-120°$. To avoid pressure build-up during the subsequent heating, the tube was evacuated to 5 cm Hg in 20 sec with a hypodermic needle inserted through the cap. After removal of the cotton wool the tube was placed upright and surrounded by a cylindrical heater, while the capillary was dipped into liquid nitrogen. The tube was then warmed in 3 min to 10° above the boiling point of the trapped material and maintained at this temperature for about 5 min. Slight variations in time or temperature had no influence on the recovery.

The tube was allowed to cool to room temperature and was removed from the heater. The capillary vessel was detached and rinsed with 1–2 μl of carbon tetrachloride to flush any condensate in the neck into the tip. The resulting solution was transferred with a microsyringe to a Barnes microcell which had an optical path length of 0.1 mm and a volume of 3 μl. In some cases, carbon disulfide was used instead of carbon tetrachloride as a cocondensation solvent.

Results

Trapping efficiency. A solution of labelled benzaldehyde in pentane (3.3 g/l) was prepared, and 3-μl aliquots (containing 10 μg benzaldehyde) were injected into the gas chromatograph. The operating temperature was 105°. The distribution of benzaldehyde in a trapping tube was determined by breaking it into 1-cm sections and measuring the radioactivity of the contents of each section. Irrespective of the solid support (Diatoport S or quartz wool), 96–99 % of the trapped benzaldehyde was found in the first of the quartz wool plugs that keep the solid support in place. If the tube was not cooled in powdered Dry Ice, however, only about 5 % of the eluted benzaldehyde was trapped, the majority being found in a second, cooled trapping tube connected in tandem. The overall recovery of benzaldehyde in all cases was 80–85 %*. Further experiments showed that another 10 % of the injected material could be recovered if the effluent following the benzaldehyde peak was collected as well.

A solution of labelled acetone in *n*-propanol (4 g/l) was prepared, and 2-μl aliquots (containing 8 μg acetone) were injected into the gas chromatograph. The operating temperature was 50°. The acetone was trapped on various solid supports; the recoveries are summarized in Table I. Acetone was almost completely recovered when tightly compressed quartz wool was used as the solid support. Since the recovery was not appreciably influenced by the I.D. of the trapping tube, tubes with an I.D. of 2 mm were used from this point on. These have a small "dead volume", which makes for efficient transfer of the fraction into an infrared cell (or a mass spectrometer), they are easy to fill, and they enable the quartz wool to be compressed without difficulty during the filling procedure.

TABLE I

RECOVERIES OF ACETONE IN TRAPPING TUBES PACKED WITH VARIOUS SOLID SUPPORTS

Run no.	*Solid support*	*Internal diameter of tube (mm)*	*Recovery (%)*
1–4	Diatoport S	3	15–50
5–8	Diatoport S with 10 % Apiezon + 1 % Carbowax	3	12–60
9–12	Quartz wool	3	50–90
13–15	Tightly compressed quartz wool	3	85–100[a]
16	Tightly compressed quartz wool	1	80

[a] 95 % of the trapped acetone was found in the first half of the tube.

Such tubes, filled with tightly compressed quartz wool, satisfactorily trapped larger amounts of acetone; the recovery for a single injection of 200 μg of acetone was 75 %. When three successive amounts of 10 μg of acetone were injected into the chromatograph and trapped in one and the same tube, recovery was 66 %. In the latter case the trap was connected with the outlet of the gas chromatograph for 6 min in all. Apparently, acetone is washed from the trapping tube by the carrier gas if the connection with the outlet is maintained for a period exceeding the elution time (~30 sec). Trapping recoveries were not affected if the quartz wool was silanized or if the temperature of the effluent liner was raised up to 250°. No acetone was lost from

* All recoveries are corrected for the split ratio.

sealed trapping tubes when these were stored for several hours in powdered Dry Ice.

A solution of labelled dodecanol in pentane (10 g/l) was prepared, and 20-μl aliquots (containing 200 μg dodecanol) were injected into the gas chromatograph. The operating temperature was 150°. The trapping tubes (I.D. 2 mm) were filled with tightly compressed quartz wool. When the effluent liner was maintained at 180°, the trapping recovery was only 1–2 %, most of the dodecanol having condensed in the effluent liner of the gas chromatograph. When the temperature of the effluent liner was raised to 250°, 60 % of the dodecanol was recovered in the trap. Apparently some dodecanol still condensed on the protruding colder parts of the effluent liner. By fitting the latter with a female ball joint on the outside, losses due to premature condensation could be prevented. The recovery for a single injection of 200 μg of dodecanol was then 93–95 %.

The trapping tube thus developed is drawn in Fig. 2a. The conical joints of the prototype have been replaced by ball joints. The I.D. of the tube has been kept at 2 mm. Apart from improving the trapping efficiency, particularly for less volatile compounds, the new tubes are more versatile, since they can also be used as pre-columns for rechromatography[4].

Immediately after trapping, the tube is clamped in the device shown in Fig. 2b. This has been designed to permit rapid, waterproof sealing of the tubes. Before the transfer step, the tube is released, and the capillary vessel and the silicone rubber cap, which are both provided with ball joints, are joined to it with a similar type of clamp (Fig. 2c). The trapped compound is then ready to be transferred by cocondensation.

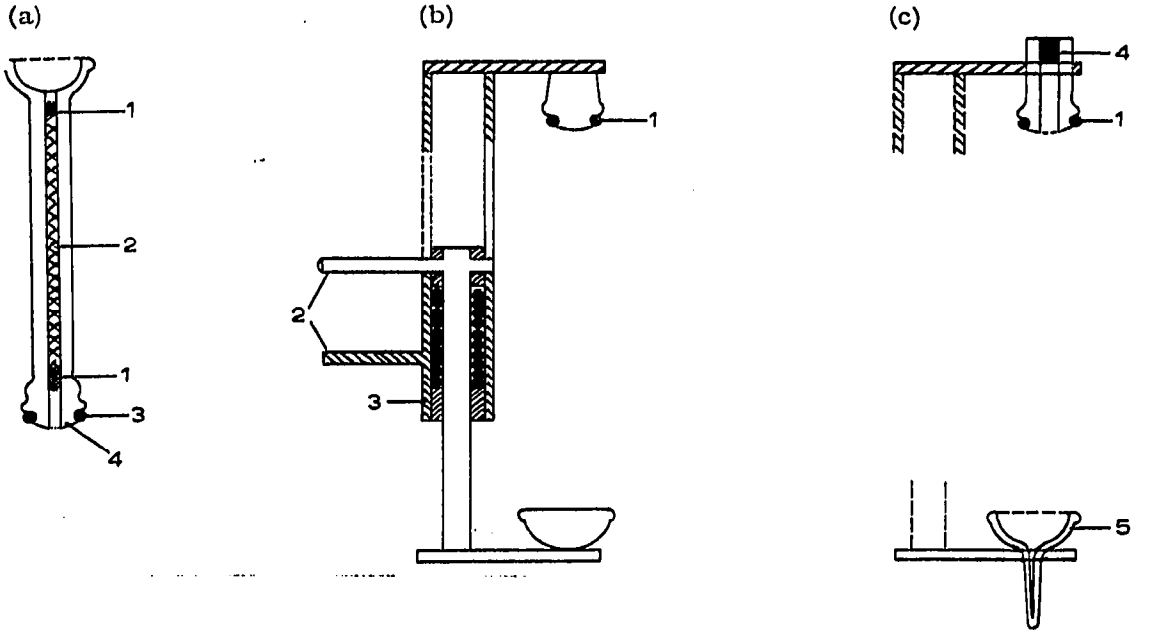

Fig. 2. (a) Modified tube for trapping microgram gas chromatographic fractions. During trapping and storage the tube is horizontal. 1 = quartz wool plug; 2 = tightly compressed, silanized quartz wool; 3 = silicone rubber o-ring; 4 = ball-joint connection to gas chromatograph or capillary vessel. (b) Device for rapid waterproof sealing of trapping tubes. (c) Similar device for subsequent cocondensation. Device, together with tube, fits closely into a specially designed oven. 1 = silicone rubber o-ring; 2 = handles; 3 = spring; 4 = silicone rubber cap; 5 = detachable capillary vessel.

Transfer efficiency. The transfer efficiency was determined by injecting 10 μg of labelled model compound into the solid support in the tube and cocondensing it with carbon tetrachloride. Transfer efficiency was high, provided the solid support had been thoroughly silanized (three times with 5 % hexamethyldisilazane in benzene) in order to facilitate desorption. In this case recovery was the same with quartz wool or with Diatoport S. There are three critical stages in the transfer technique: firstly, the evacuation of the tube, during which about 5 % of the benzaldehyde or 20 % of the acetone was lost; secondly, the heating step, after which only about 1 % of sample remained on the (silanized) solid support; thirdly, the uptake of the condensed solution with a microsyringe and its transfer to the infrared cell, which could be accomplished almost without loss. Overall recovery of the transfer technique was about 80 % or better. These results are confirmed by determinations of recovery for a series of other compounds (Table II). Recovery was determined by comparing the infrared absorption after cocondensation with that of the same amount of compound injected directly into the infrared cell.

TABLE II

RECOVERY OF TEST COMPOUNDS IN INFRARED CELL AFTER TRANSFER FROM TRAPPING TUBES BY COCONDENSATION WITH CARBON TETRACHLORIDE

Recoveries are the mean of at least three determinations with 10–30 μg of compound.

Compound	*Boiling point* (°C)	*Mean recovery* (%)
Ethyl acetate	77	80
Methyl propyl ketone	102	96
2-Methylpyrazine	133	78
Methyl benzoate	195	81
2-Phenylethanol	219	92
Benzophenone	306	96

Similar recoveries were obtained with carbon disulfide as the cocondensation solvent. The tubes, together with the solid support, can be regenerated by heating them at 300–350° while a stream of nitrogen is passed through.

Conclusions

Microgram gas chromatographic fractions can best be trapped in glass tubes, packed with solid support and cooled in Dry Ice. To ensure consistently high recoveries, tightly compressed quartz wool should be used as the solid support. An internal tube diameter of 2 mm is the most expedient. To minimize premature condensation, a particular hazard with less volatile compounds, the effluent liner should be heated above the boiling point of the eluting compound and protrusion of the outlet should be kept as small as possible. We met the latter requirement by equipping the tubes with ball joints. With these tubes, recovery may be expected to exceed 80 % for compounds over a wide range of volatility. The tubes can be sealed with a specially designed device and stored in Dry Ice for several hours without loss of sample.

Amounts of 10–30 μg of trapped compound can be transferred from the tubes to an infrared cavity cell with an efficiency of more than 80 % by cocondensation with carbon tetrachloride.

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Combination of a gas chromatograph and a fraction collector using a small condenser

It is often necessary, either for rechromatography or for futher identification by such techniques as IR spectroscopy or nuclear magnetic resonance, to collect micro amounts of a resolved fraction of a complex mixture as they are eluted from the gas chromatograph. Conventional cold traps are inefficient for trapping high-boiling solutes which tend to form aerosols that are swept through the trap. A number of gas chromatographic traps of varying complexity, designed to prevent the formation of aerosols, have been described in the literature. Such devices may depend on the maintenance of a temperature differential between a heated inner wall and the cooled outer wall[1] or on the use of a cold trap and electrostatic precipitator[2]. A procedure for using argon[3] or carbon dioxide[4] as carrier gas and condensing a carrier gas along with the sample in a trap surrounded by liquid nitrogen has also been described.

The present paper describes a new collection method using simultaneous condensation of a sample and organic solvent vapor in a small Liebig-type condenser. This principle has also been applied to the combination of a gas chromatograph and IR spectrometer using a liquid flow cell[5].

Many fraction collectors are commercially available and various designs have been published[6,7]. However, only some of them can be built conveniently for the collection of a relatively large number of gas chromatographic fractions. The present paper also describes the combination of a gas chromatograph and fraction collector which is commercially available.

Experimental

The flow diagram is shown in Fig. 1. The gas lines which connected 1, 2 and 3 in Fig. 1 were heated with tape heaters. The generator of the organic solvent vapor consists of a 500-ml flask and 20-cm resistant glass tube packed with sand or firebrick C-22. They are maintained at 2–7 degrees above the boiling temperature of the solvent[5]. In this experiment tetrachloroethylene was used as an organic solvent.