Collection of gas-liquid chromatographic effluents*

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» Substances leaving gas-liquid chromatographic columns operating at high temperature tend to condense in the form of a very finely dispersed fog. While a fair fraction of such effluents can be trapped quite simply, it is important for work with radioactive substances to be able to condense each substance quantitatively. By measuring the area under each chromatographic recording, one can calculate the specific activity of each substance. Several devices have been described (1 to 7) for the trapping of such fogs, but in our experience, using particularly high temperatures and gas flow rates to obtain hexacosanoic acid, the simpler of the methods proved inadequate. Trapping in wetted cotton or glass wool plugs proved incomplete, and bubbling through solvents produced deflections on the recorder and occasional fogs. We have found that efficient collection can be obtained with a Millipore® filter,[1] which is a plastic filter having extremely uniform small holes. The porosity of the filter is so high that good gas flow rates can be readily maintained. Holders for the filters are commercially available and require only a simple modification for attachment to the gas-liquid chromatograph.

METHOD

The filter holder is a Swinny hypodermic adapter,[2] a metal device ordinarily used for attachment to hypodermic syringes for sterilization by filtration. The adapter consists mainly of two metal parts, held together by a screw thread (Fig. 1, A and B). Part A has a small standard taper joint, for attaching to a hypodermic syringe. Part B has a Luer-Lok® standard taper joint, for attaching to a hypodermic needle. The outer part of the Luer-Lok® connection is sawed off and a glass tube is cemented on the tapered joint with epoxy cement, obtained from a local hardware store. The glass tube is made from a length of 5-mm i.d. tubing by heating a short section and pulling slightly. This operation gives the tube a slight taper that improves the seal with the metal. The other end of the tube is pulled off to give a tapered tip for insertion into the hole of the exit gasket of the chromatograph.

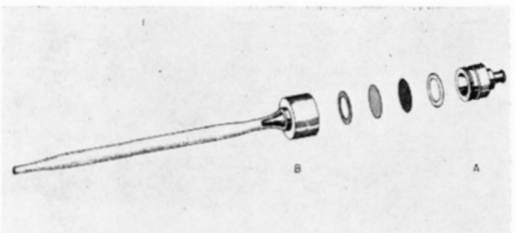

FIG. 1. Collector for gas-liquid chromatographic effluents.

The Millipore® filter disk (13 mm diam, 0.8 μ pore size) is held between the two metal parts by a fine metal screen, a Teflon® gasket, and a Teflon® O-ring, furnished with the adapter. The filter is inserted next to the metal screen so that the gas stream presses it against the screen. Whenever a sample is to be collected, the entire adapter is inserted into the end of the chromatograph and withdrawn when needed. The change can be made rapidly for trapping closely eluting components. A convenient marker is produced in the chromatographic trace by the short pip produced as the collector is inserted or withdrawn.

Much of the sample is condensed within the glass tube and the Millipore® filter serves to collect only the fog. For radioactivity determinations, the contents of the collector tubes are washed out directly into a counting vial using scintillator solution. The collector tube is clamped in a vertical position with the glass end down (above the counting vial), and a 10-ml glass syringe is attached to the top. Five milliliters of 0.8% diphenyloxazole in toluene is placed in the counting vial and the solution is sucked in and out of the glass tube by means of the syringe. Care must be taken that no solution reaches the filter disk at this point. Next the syringe plunger is removed, 5 ml of toluene is placed in the syringe, and the toluene is allowed to drain slowly by gravity through the filter and the glass tube into the vial. When the flow stops, the upper part of the adapter is dismantled and the O-ring, screen, and filter are taken out. The glass tube is then rinsed with 1 ml of toluene.

The sample may be washed out with other hydrocarbon solvents, chlorinated solvents, glycols, isopropanol, and higher alcohols, but not with esters or ketones, which dissolve the filter. For economy, each filter disk can be reused a number of times, but they tend to tear after a while.

EXPERIMENTAL PROCEDURES

The method was tested with methyl esters of radio-

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[1] Millipore Filter Corporation, Bedford, Massachusetts.
[2] See footnote 1.

TABLE 1. RECOVERY OF SYNTHETIC RADIOACTIVE METHYL ESTERS

Compound Injected	Run No.	Radioactivity in Fractions* (count/min) I	II	III	IV	V	Total Radioactivity Recovered (count/min)	Total Radioactivity Injected (count/min)	Radioactivity Recovered (per cent)
Methyl ester of stearic acid-1-C^{14}†	1	296	188,839	1,307	1,152		191,594	193,700	98.9
	2	737	341,201	6,556	4,310		352,804	360,520	97.8
Methyl ester of lignoceric-1-C^{14}‡	1	21	58	12,811	171	18	13,079	13,460	97.2
	2	44	121	12,998	148	13	13,324	13,721	97.1
Methyl ester of oleic-1-C^{14}§	1	574	21,280	168	622		22,644	23,290	97.2

* In collecting fractions, the collector tube was changed in the middle of the flat recorder trace following each peak.

†Fr. I, O-C_{17}; Fr. II, C_{18}; Fr. III, C_{19}; Fr. IV, C_{20}–C_{24}.

‡ Fr. I, O–C_{18}; Fr. II, C_{19}–C_{23}; Fr. III, C_{24}; Fr. IV, C_{25}; Fr. V, 30′ collection after C_{25}.

§ Fr. I, Collection before the oleate peak; Fr. II, oleate peak; Fr. III, 0–5′ after the oleate peak; Fr. IV, 5′ to 40′ after the oleate peak.

active fatty acids, synthetic and naturally occurring. The chromatograph (Aerograph®[3] Model A-90-ACS with an 80 x 1/4 inch o.d. stainless steel column containing General Electric SE 30 silicone gum (8) on Chromosorb® [60 to 80 mesh] in the ratio 1:10) gave excellent separation of methyl esters differing in molecular weight by one carbon atom. The column temperature was raised during each run from 190° to 260°. The injector and collector were maintained at 265°, helium was used as carrier gas at the rate of 100 to 120 ml per minute, and a thermal conductivity cell with filament current of 200 ma was used. Methyl esters up to hexacosanoic acid were separated and no condensate from the packing could be seen in the collectors.

Stearic acid-1-C^{14} and oleic acid-1-C^{14} were commercial samples. Lignoceric acid-1-C^{14} was prepared in our laboratory, and a mixture of saturated fatty acids was obtained from the brains of rats fed acetate-1-C^{14}. The free acids were converted to methyl esters with dimethoxypropane (9) and the unsaturated esters were removed by reaction with mercuric acetate (10). It was found that the commercial acids and the lignoceric acid contained radioactive impurities, apparently shorter and longer homologs. The stearate (initially 80% pure) and lignocerate were purified by gas-liquid chromatography and the peaks were collected and used for recovery studies. The oleate, however, was not purified.

The radioactive stearate and lignocerate were mixed with a liquid stock mixture of nonradioactive esters, ranging from laurate to lignocerate. Methyl oleate and the natural radioactive mixture were used without any carriers. The natural mixture of saturated fatty acid esters from rat brain (solid at room temperature) was dissolved in an equal volume of xylene for injection into the chromatograph. A Hamilton fixed-needle microliter syringe[4] was used for injection through a double silicone rubber disk. The sample was loaded into the syringe in such a way that a large air bubble was present on each side of the liquid. The bubble in the needle end minimized premature evaporation during insertion, and the distal bubble minimized leakage past the plunger due to the gas pressure. Only a single stroke of the plunger was made after insertion of the needle into the chromatograph. The amount of ester injected was found by weighing the syringe before and after injection, using a semimicro balance. About 5 to 20 μl of sample was injected in each run. The recoveries are shown in Table 1.

It is evident that almost complete recovery of the total radioactivity injected was obtained. A small amount of radioactivity appeared before the radioactive peak. Less than 2 % of the radioactivity was collected with the homologous ester with one carbon atom more. Trailing off over a longer period of time was an additional 1% or somewhat less of the total injected radioactivity. This trailing of radioactivity is not necessarily the result of a defect in the collection system. It may reflect in part the expected tailing due to the chromatographic process, made more evident by the greater sensitivity of the radio assay methods employed. In the case of the oleate, however, the relatively high level of radioactivity before and after the oleate peak is probably the result of homologous impurities.

The consequence of the trailing phenomenon is that a small correction must be made in the activities of esters following highly radioactive esters. This is illustrated in Table 2 where the results with a natural mixture are shown. The activity in the C_{17} ester that

[3] Wilkens Instrument and Research, Inc., Walnut Creek, California.

[4] Hamilton Company, Whittier, California.

follows the palmitate may be assumed to be spurious on the basis of the data in Table 1. The activity in the C_{19} ester, however, is surely real, as it is 5% that of the stearate. Blank collections after each run showed that radioactivity did not accumulate in the chromatograph. The total recovery in this experiment was over 100%, the difference presumably reflecting weighing errors and statistical errors in counting the samples of lower activities. Recoveries in other runs with naturally derived samples were 103.0%, 103.0%, 96.7%, 98.9%, and 97.4%. Recoveries have not been determined for lower molecular weight, more volatile esters and it may be that these would not be quantitatively recovered, particularly if the same collector were left in place over a long time interval.

Various methods were tried for preventing the trailing effect. The injector and collector temperatures were changed, the injector site was packed with glass wool to increase the area of heating, and the tips of the collector tube were warmed before insertion into the collection zone. When the Swinny adapters were used without the glass tube, inserting the metal standard taper joint directly into the collection zone, a fog was seen to come out of the chromatograph just after the collector was withdrawn. It is possible that this is due to condensation of the esters on the outside of the metal joint (which would not be warmed up readily), the esters being wiped off by the silicone gasket and coming off the gasket as a fog. There is the possibility that a slight amount of sample is lost through the injection gasket as the syringe needle is withdrawn. We used xylene, where a solvent was needed, in the belief that a more volatile solvent would evaporate so explosively that a portion of the radioactive esters might splash back into the cool helium inlet tube and thereby be lost permanently.

The resistance to gas flow caused by increasing deposition of the esters on the Millipore® filter during collection rose slowly during a run. When the same tube was used to collect large amounts of esters or several peaks, clogging or total stoppage of gas flow occurred. For deposits up to 4 mg, however, only a 5% decrease in gas flow occurred and no disturbance in the recorder tracings was noticed. Larger Millipore® filters and holders are available and can be used when larger amounts of sample must be collected. The method used for washing the collector tubes was found to be satisfactory, as only 0.1% to 0.2% of the original activity was recovered by rewashing the tubes.

In early experiments, disks of facial tissue were used instead of Millipore® filters. In this method, a small glass tube about 5 cm long and 4 mm i.d. was used with one end tapered for insertion into the silicone rubber gasket at the exit of the chromatograph. About 4 to 5 mm before the other end of the tube, a constriction was made, against which was pushed a pack of 15 to 20 layers of the tissue paper disks. The fog of esters was trapped quite effectively by the paper disks. The radioactivity was determined by putting the tube in a counting vial, pushing out the paper plug from the tube with a thin wire, and leaving the glass tube and its contents in the vial. The vial was then filled up to the neck with 20 ml of scintillator solution and counted with the paper and the glass tube left inside. This method gave excellent recoveries also; the primary objections were that the manual packing of the tissue paper was tedious and not uniform from tube to tube, so that the gas flow rate changed as the collector tubes were changed, and that the small diameter of these filter disks restricted their use to small samples.

TABLE 2. RECOVERY OF RADIOACTIVITY FROM A MIXTURE OF METHYL ESTERS OF SATURATED ACIDS FROM BRAIN

Fatty Acid*	Radioactivity (counts/min)
0–12:0	8
13:0 plus 14:0	10
15:0	2
16:0	1376
17:0	15
18:0	422
19:0	22
20:0	19
21:0	11
22:0	11
23:0	9
24:0	22
25:0	12
30′ after 25:0	36
Total radioactivity recovered	1975
Total radioactivity injected	1887
Per cent recovered	104

* Tentative identification from the retention times.

The Millipore® filter method was also used to collect samples for infrared spectral analysis. Excellent spectra were obtained from single runs of the natural esters.

It should be mentioned that several microliter syringes[5] with double needles (one inside the other) rapidly developed leaks when used for injecting samples. Apparently the cement currently used between the needles is attacked by the esters or by xylene at high temperatures. We now use 50-μl syringes fitted with a sealed 24-gauge needle, prepared to our special order.[6]

The time lag between detection and exit of each sub-

[5] See footnote 4.
[6] See footnote 4.

stance was assumed to be negligible, although other workers have observed an appreciable interval. We use a somewhat higher gas flow rate and, moreover, the Aerograph® has a rather small volume in the region between detector and the collector gasket.

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