J. Lipid Research, July, 1962
Volume 3, Number 3

A flow-through method for scintillation counting of carbon-14 and tritium in gas-liquid chromatographic effluents

ARTHUR KARMEN,
IRMGARDE MCCAFFREY, and
ROBERT L. BOWMAN

National Heart Institute,
Bethesda 14, Maryland

[Received for publication April 5, 1962]

SUMMARY

A method for radioassay of gas-liquid chromatographic effluents by scintillation counting has been developed. A cartridge filled with anthracene crystals is used as a "flow-through" radiation detector. Upon leaving the column, the effluent is passed over hot copper oxide, which converts the organic materials to carbon dioxide and water. If tritium is to be counted, the water is reacted with hot iron to release hydrogen and tritium gases. The effluent is then passed through the anthracene cartridge. The counting rate of the cartridge is monitored continuously during the analysis by a highly efficient, low-background, scintillation counter to yield a record of eluted radioactivity that resembles the conventional mass detector record.

The detector is simply constructed, easily maintained, insensitive to change in gas composition but highly sensitive to radiation. High efficiency (more than 70% for carbon-14 and 20% for tritium) is coupled with low background counting rates (ca. 15 cpm at carbon-14 settings, 50 cpm at tritium settings). The counting rate returns to this low background rate following the detection of a labeled compound.

Two kinds of methods have been used for measuring radioactivity in effluents of gas-liquid chromatography columns. In the first of these, the effluent gas is fractionated and the radioactive components are condensed out of the gas stream for subsequent radioassay; in the second, the analysis and radioassay are performed simultaneously. The first kind of method offers higher sensitivity since the radioactivity in each fraction can be assayed for whatever time is necessary for an accurate measurement. In many analyses, however, there is sufficient radioactivity in the individual components to permit the faster and more convenient simultaneous procedure to be used.

Simultaneous radioassay and analysis of high-boiling materials using ionization chambers and proportional counters has been accomplished either by constructing these detectors so that they can be operated at high temperature (1, 2) or, alternatively, by subjecting effluent materials to combustion to carbon dioxide and water (3, 4, 5). The detector can then be operated at ambient temperature. There have also been several publications describing the use of scintillation counting for the measurement of radioactivity in gases. Plastic scintillator at room temperature has been used for the radioassay of carbon-14-labeled carbon dioxide (6). Scintillation crystals have also been used at high temperatures in connection with gas-chromatographic effluents (7). In another method of applying scintillation counting (8), the hot effluent of a gas column is bubbled through liquid scintillator solution while the counting rate of the scintillation is monitored continuously. High-boiling materials in the column effluent condense in the scintillator as they emerge from the column. The result is a cumulative record of radioactivity eluted from the column. The elution of a radioactive compound is indicated by a stepwise increase in the counting rate.

A method has been described previously (9) by which the effluent is automatically passed through a succession of cartridges containing anthracene crystals coated with silicone oil. High-boiling compounds condense in these cartridges in close proximity to the highly efficient scintillator, and the counting rate of each cartridge is then measured. We have also reported monitoring the scintillation counting rate of a single anthracene cartridge while the high-boiling materials of the column effluent successively condense in it (10).

The high efficiencies obtained with these methods suggested exploring the use of anthracene crystals in a 'flow-through' detector. Anthracene crystals are being used this way to detect carbon-14, tritium, and other beta emitters in flowing, aqueous effluents of liquid-liquid chromatographic columns (Packard Instrument Co., Inc., P. O. Box 428, LaGrange, Ill.) This application is based on the work of Steinberg, who demonstrated highly efficient detection of carbon-14 in aqueous solution using anthracene crystals (11).

A detector system was therefore constructed consisting of a tube filled with anthracene crystals mounted between the opposing faces of two photomultiplier tubes for coincidence scintillation counting. High-boiling materials were converted to carbon dioxide and water by combustion over heated copper oxide to prevent their condensation in the anthracene. When tritium was to be assayed, the combusted effluent was passed over heated iron filings to produce tritium gas from the water of combustion. The effect of geometry of the detector on efficiency of detection was studied, and an efficient detector was developed This was then integrated into an entire gas-chromatographic system, which was then applied to the analysis of samples of biological origin containing low levels of radioactivity to determine the sensitivity and resolution of the method.

MATERIALS AND METHODS

The detector cell in all experiments consisted simply of a transparent tube fitted with a gas inlet and outlet and filled with anthracene crystals (blue-violet fluorescent grade, Eastman Chemical Co.). This detector cell was placed between the opposing faces of two photomultiplier tubes so that the maximum surface area of the detector faced the tubes. In most of these experiments, the detector was a U-shaped tube drilled in a rectangular block of clear plastic "Lucite". The parallel limbs of the U were 5 cm long, the connecting limb 2 cm long All were drilled with a $^{15}/_{32}$-inch drill. Gas-tight connections at the top of the U were made by pushing stainless-steel hypodermic tubing through rubber stoppers. In this particular series of experiments, no light pipe was used. The two photomultipliers and associated electronics for coincidence scintillation counting were part of the Tri-Carb liquid scintillation spectrometer (Packard Instrument Co.,Inc., LaGrange, Ill.) A rate meter (Packard Instrument Co., Inc., Model 280A) was connected to provide an analogue output. This was placed on one channel of a two-channel recorder. The output of the mass detector was put on the other channel.

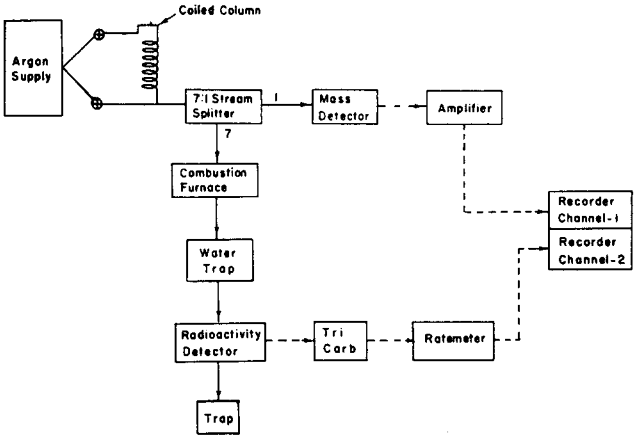

FIG. 1. Schematic diagram of integrated gas chromatograph-scintillation counter.

A flow-diagram of the entire system is shown in Figure 1. The gas chromatography column was coiled glass, 6 ft long, 4 mm inside diameter. Gas was supplied to this column through $^{1}/_{16}$-in.-diameter hypodermic tubing connected to the column with a silicone rubber seal. Samples were injected into the column through a solid silicone rubber stopper with a microsyringe.

The column effluent was led to a T connection. A supplementary gas flow was added through the sidearm of the T. The combined flow was then led to a second T connection constructed of two unequal lengths of 0.010-in. inside-diameter stainless-steel capillary so that the flow was divided approximately 1:7.[1] The smaller fraction was conducted to an argon ionization detector (13) containing a tritium source. The detector cell had an inside diameter of $^{3}/_{8}$ in., a total

---

[1] The design of a stream splitter is based on the assumption that components being chromatographed divide between the two arms of the stream splitter in the same ratio as does the carrier gas. Since the viscosity of the gas containing organic vapor can be shown to be different than that of the carrier gas alone, the division may not be as constant throughout the course of the analysis as could be desired. For accurate quantitative work, the division must be independent of the concentration of component in carrier gas as well since the concentration may vary markedly from one component to the next. However, because of the splitting ratio used, any effect produced in the radioactivity analysis as a result of a change in the splitting ratio is relatively small, compared to the possible effects on the accuracy of the mass analysis. The mass detector should be calibrated for quantitative accuracy with the stream-splitting device in place. A detailed discussion of the design and performance of a stream-splitter has been published by Ettre and Averill (12).

TABLE 1. Effect of Detector Diameter on Efficiency*

Diameter (mm)	Efficiency (%)	
	Carbon-14†	Tritium‡
4	68	23
8	68	14
9.3	64	12
11	62	11

* Detectors were straight sections of glass tubing 5-cm long stoppered at both ends with "one hole" rubber stoppers.

† Counted integrally at 880 volts.

‡ Counted integrally at 1040 volts.

volume of 1.2 cc, and was constructed as described previously (14) to prevent diffusion of air into the active volume of the cell and to avoid leakage. The larger fraction of the gas was conducted to the combustion furnace.

Combustion of the effluent organic materials was accomplished by passing the column effluent through heated copper oxide (cupric oxide wire, Fisher Scientific Corp., Fair Lawn, N. J.). The combustion furnace used was a Von Czoernig-Alber electric furnace (Cat. No. 5679-A, Fisher Scientific Corp.), 8 in. long. When the heating element of this furnace was supplied with 110 v, the temperature of the interior of the furnace reached a steady state with the 25° laboratory ambient at approximately 730°. The combustion tube was "Vycor" brand (Corning Glassworks, Corning, N.Y.) glass tubing, 6 mm inside diameter, 35 cm long. Both ends were modified to accept silicone rubber seals. The inlet end of the tube was connected to the column outlet within the detector oven of the gas chromatograph. The length of "Vycor" tubing between that connection and the beginning of the combustion oven was filled with glass beads to rule out the possibility that copper oxide at 200° would adsorb or otherwise retain labeled materials. This length of tubing was heated and insulated throughout its length. The remainder of the tube was filled with copper oxide. When tritium was to be counted, the combustion tube was followed by an identical tube filled with steel wool.

A water trap consisting of a 2-in. length of glass tubing filled with magnesium perchlorate was placed in the gas line, close to its entrance into the photomultiplier compartment. The gas was carried from the combustion tube to the water trap and from the water trap to the anthracene cartridge in $^1/_{16}$-in.-diam teflon tubing.

In analyses involving $C^{14}O_2$, the gases were vented into a carbon dioxide trap filled with "Ascarite" (Arthur H. Thomas Co., Philadelphia, Pa.). When tritiated compounds were analyzed, the gases leaving the anthracene were led through a second 2-in.-long combustion tube filled with heated copper oxide followed by a water trap.

Assay of all materials for total carbon-14 and tritium radioactivity was performed prior to gas chromatography by liquid scintillation counting using the Tri-Carb liquid scintillation spectrometer (Packard Instrument Co., Inc.). The scintillator used was diphenyloxazole in toluene, 500 mg per 100 ml. The total radioactivity was computed from the counting rate of the unknown sample and the counting rate of the sealed standard (supplied by the Packard Instrument Company) at the same photomultiplier voltage and discriminator settings.

In some of this work, the performance of the system was evaluated in analyses of methyl esters of fatty acids using polyester gas chromatography columns. The conditions and columns used in our laboratory have been described previously (9). In other parts of the work, the sensitivity was evaluated by injecting radioactive fatty acid esters into a miniature gas chromatography column, 2 in. long, filled with glass wool and heated to 250°.

RESULTS

Completeness of Combustion. Completeness of combustion was evaluated in a series of experiments utilizing the properties of a gas discharge to distinguish carbon dioxide from organic vapors. A self-sustained discharge detector (15) was placed in the gas stream following the combustion furnace. When "organic" molecules pass through this detector, the electrical conductivity of the gas is increased. Carbon dioxide and water decrease the conductivity. The sensitivity to intact organic vapors is greater than the sensitivity to carbon dioxide. Power to the combustion furnace was turned on and, as its temperature increased, repetitive samples of methyl esters in isooctane were injected into the column. The emerging isooctane peak as well as the methyl esters produced increases in the electrical conductivity of the argon. As the temperature of the combustion furnace increased, the sensitivity to the methyl esters decreased and the peaks became "negative" (the electrical conductivity was diminished), indicating a preponderance of carbon dioxide and water over intact esters. The solvent peak was last to be so affected, presumably because of the high concentration of isooctane in the argon during the peak. The minimum temperature required to effect complete reversal of the solvent peak was determined to be approximately 720°. Completeness of combustion under these conditions was confirmed in previous experiments (5) by placing a cartridge filled with coated anthracene crystals in the gas stream after the combustion furnace and injecting labeled methyl esters into the furnace. The effluent of this cartridge was then bubbled through 25 cc DPO-toluene:Hyamine solution 10:1. Failure of the coated anthracene to trap significant quantities of radioactivity and recovery of the radioactivity in the DPO-toluene-hyamine was taken as further evidence for complete combustion.

Effect of Cell Diameter. When a number of cylindrical cartridges was tested, the efficiency was found to decrease with increase in the diameter of the cartridge. As may be seen in Table 1, the effect was more pronounced in the detection of tritium than in the detection of carbon-14.

Background was subtracted from each reading in this table. The carbon-14 efficiency usually obtained using anthracene was equal to or slightly greater than that obtained using DPO-toluene (62% at 820 v). The efficiency for tritium was lower (11% compared to 16% at 1,050 v). The efficiency for tritium was higher (see Table 1) when a smaller-diameter tube was used.

A number of different geometries was tested. As a result of these experiments, the effect of cell diameter was attributed to incomplete light transmission by the anthracene. Therefore, when an increase in detector-cell volume was desired, the length of tubing was increased rather than its diameter.

Efficiency. The efficiency of this system of radioassay was determined on the basis of several assumptions. The number of radioactive disintegrations that occur within the detector cell when a radioactive peak passes through is a function of the total radioactivity of the peak (dpm) and the time the peak spends in the detector cell. The time in minutes that an average molecule spends within the cell is given by the fraction: cell volume (cc)/flow rate (cc/min).

Because of several factors, not all of the disintegrations that occur in the detector are recorded as counts. The fraction of the number of events that is counted may be considered the "efficiency" of the method of detection and may be taken as one measure of the sensitivity of the radioassay.

The efficiency of the method of radioassay using the U-shaped detector was determined by injecting accurately known amounts of radioactivity in a system in which the gas flow through the detector was accurately determined. The free volume of the detector cell was determined with anthracene in place by measuring how much water, with added detergent, was required to fill it. The number of counts recorded compared with the number calculated to have occurred in the detector following the injection of samples of methyl palmitate labeled with carbon-14 or with tritium is shown in Table 2.

TABLE 2. EFFICIENCY OF U-SHAPED DETECTOR FOR $C^{14}O_2$ AND TRITIUM IN FLOWING GAS STREAM

Radioactivity injected (dpm)	Disintegrations in Detector (Calculated)*	Photomultiplier Voltage	Total Counts (Observed)†	Efficiency‡ (%)
		Carbon-14		
22,400	2,690	800	1,746	65
22,400	2,690	800	1,661	62
35,670	4,280	1,040	3,555	83
51,790	4,660	1,040	4,030	86
51,790	4,660	1,040	3,881	84
		Tritium		
950,000	114,000	1,040	12,951	11.4
950,000	114,000	1,040	12,756	11.2
950,000	114,000	1,040	12,184	10.7
950,000	114,000	1,040	13,337	11.7

* Volume of detector = 7.5 cc. Flow rate = 60 cc/min.

† Pulses counted integrally from 10 volts to infinity.

‡ C^{14} in DPO-toluene, in 1-in.-diam glass vial, counted in the same photomultiplier compartment, integrally at 800 volts with 62% efficiency and at 1,040 volts with 85% efficiency. Tritium in DPO-toluene counted at 1,040 volts with 16% efficiency.

The background readings obtained with the prototype detector ranged between 30–50 cpm at the voltage used for carbon-14 (62% efficiency) and approximately 100 cpm at the setting for tritium (10% efficiency). These high backgrounds were attributed to our failure to shield the preamplifier electronics adequately in this prototype. The backgrounds obtained using similar but well-shielded equipment were approximately 15 cpm for carbon and 50 cpm for tritium under the same conditions.

Sensitivity. The preceding section contains a discussion of efficiency defined as the fraction of radioactivity in the cell that is counted. A more practical consideration is the least amount of radioactivity detectable in a given peak and the least amount necessary to get a measurement reliable to some chosen accuracy.

Given 50% efficiency for carbon-14, 50 cc/min gas flow, and a 10 cc chamber volume, 100 counts will be registered when a component containing 1,000 dpm (0.5 x 10^{-3} μc) passes through the detector. For this component to be detected, these counts must occur in sufficiently short time to produce a change in rate that can be distinguished from fluctuations in the background counting rate. If the background counting rate is a result of randomly occurring events, the fluctuations are proportional to the square root of the background rate.

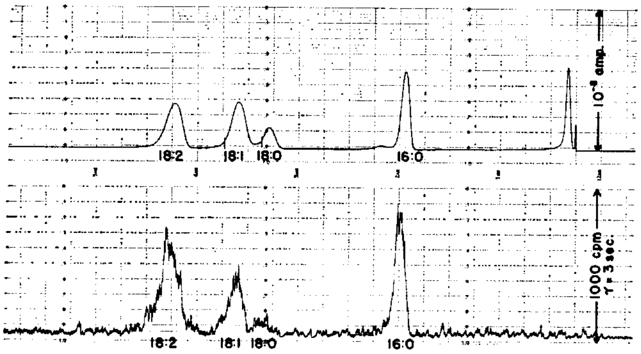

FIG. 2. Record of analysis of fatty acid methyl esters from chyle phospholipids of a rat fed a mixture of carbon-14-labeled fatty acids. The upper record is a record of the output of an argon ionization detector; the lower is the record of the scintillation counter rate meter. Chart speed: each vertical division (3/4″) equals 1 min. The quantity injected was approximately 1 mg containing 15,000 dpm.

During the passage of a radioactive component through the detector cell, the record of the counting-rate meter shows the wave-form characteristic of a chromatographic peak (showing the variation of the counting rate with time). The average counting rate during the peak is given by the total number of counts recorded, divided by the time between the beginning of the peak and its end. If the peak is reasonably symmetrical, the maximum counting rate approximates twice the average rate. Detecting the 0.5×10^{-3} μc in a peak emerging over 1 min, therefore, required that the square root of the background rate be small compared with the 200 cpm maximum counting rate. With backgrounds of 30 cpm, this peak may be detected with a 30:1 signal-to-noise ratio.

Figure 2 shows an analysis of methyl esters prepared from the chyle phospholipids of a rat fed a mixture of carbon-14-labeled fatty acids. The total radioactivity injected was approximately 15,000 dpm (6 mμc), $^7/_8$ of which reached the radioactivity detector. The area under the peaks written by the ratemeter corresponds to 1,200 counts. With a flow rate of 60 cc/min and a chamber volume of 7.5 cc, this corresponds to detection of $^7/_{10}$ of the total number of disintegrations estimated to have occurred in the detector, or 70% efficiency. The record shows the distinct resolution and detection of the radioactivity in the methyl stearate (18:0) peak. This component of the mixture emerged over a period of slightly more than 1 min and contained 4% of the radioactivity reaching the detector, or 500 dpm.

DISCUSSION

The detector cell was designed to have as large a volume as possible for maximum sensitivity and a maximum thickness of anthracene of about 1 cm. When the length was held constant, reducing the diameter of the cell below 1 cm resulted in a needless loss in sensitivity for carbon-14 since the increase in efficiency did not balance out the loss in volume. For assaying tritium, a smaller diameter was more effective.

Since it was desirable to minimize the dead volume (volume occupied by anthracene through which the gas did not flow but penetrated only by diffusion), narrow-diameter (1 cm) tubing was chosen rather than a short, wide cylinder so that the path of gas flow would be better defined.

The use of 2-in.-diam photomultiplier tubes limits the cell volume to 19 cc if a 1-cm thickness is maintained. A spiral tube can be constructed that will approximate this and, from the above considerations, should be ideal. In the experiments reported here, the simple U tube was used, although its volume was only 10 cc, primarily because of the ease with which it was constructed and cleaned and because the loss in sensitivity was only moderate.

Preliminary attempts to increase the effective volume of the detector cell by coating the anthracene, as solid support is coated for use in a gas chromatography column, with a liquid phase temporarily retentive for carbon dioxide, have not been successful. A two-inch length of anthracene crystals coated with hexamethyl phosphoramide did not sufficiently retain the carbon dioxide to increase the apparent effective volume an appreciable amount, and the peak passing through it "tailed" badly.

The background of the entire system was found to be almost entirely a function of the photomultiplier tubes used when the system was operated in optimal fashion. However, several factors may contribute to an increase in background. It was our usual practice to operate the combustion oven only during working hours. Liquid phase and traces of carbon-14 radioactivity condensed in the cool combustion tube during the night. When the combustion tube was again put in operation, the temperature passed through a point that was insufficient to cause the compounds to be combusted but sufficient to cause them to volatilize and deposit radioactive material in the connecting tubing between the combustion oven and the anthracene. This effect was avoided by disconnecting the combustion furnace from the remainder of the system until it had reached combustion temperature. Connecting the anthracene directly to the combustion tube resulted in the deposition of water of combustion in the anthracene. When this occurred, the background counting rate of the anthracene increased with the passage of each radioactive peak. This was attributed to the solubility of carbon dioxide in the water since the background decreased with time, and the effect was not reduced by increasing the temperature of the combustion oven. The effect was eliminated by including the magnesium perchlorate water trap in the gas line.

The automatic liquid-scintillation spectrometer offers the possibility of using a printed digital output as well as a rate meter output. When the digital system was used, it became clear that it was not helpful to use a longer time interval for counting than the time of the width of the peak, since to do so was to include a needlessly large number of background counts. The same reasoning applied to the choice of rate meter time constant. In either system, use of a time constant less than the turnover time of the gas in the detector is necessary if resolution is to be preserved. With this as an upper limit, it was attempted to use as large a time constant as possible in order to facilitate integration. A 3-sec time constant was therefore used.

The operation of an ionization chamber at room temperature for measurement of radioactivity in an effluent similarly subjected to combustion has recently been extensively studied (5). Although each of the electric-discharge methods of radiation measurement is sensitive to change in the composition of the gas, this effect was minimized in our ionization-chamber experiments by combusting the effluent, using a large volume chamber with a brisk diluting flow of carrier gas, and by including a water trap in the line. Compared with this highly sensitive ionization-chamber method, the scintillation counting technique described in this paper permitted measure ment of moderately smaller quantities of radiation using shorter gas-turnover times in the detector and more rapid electrical-response times. In addition to this increase in sensitivity, there was a complete lack of sensitivity to changes in gas composition.

REFERENCES

1. Wölfgang, R., and F. S. Rowland. *Anal. Chem.*, **30:** 903, 1958.
2. Mason, L. H., H. J. Dutton, and L. R. Bair. *J. Chromatog.* **2:** 322, 1959.
3. Cacace, F., A. Guarino, and Inam-ul-Haq. *Ann. Chim.* (Rome) **50:** 915, 1960.
4. James, A. T., and E. A. Piper. *J. Chromatog.* **5:** 265, 1961.
5. Winkelman, J., and A. Karmen. Use of an Ionization Chamber for Measuring Radioactivity in Gas Chromatography Effluents (to be published).
6. Stranks, D. R. *J. Sci. Instr.* **33:** 1 (1956).
7. Evans, J. B., J. E. Quinlan, and J. E. Willard. *Ind. Eng. Chem.* **50:** 192, 1958.
8. Popják, G., A. E. Lowe, D. Moore, L. Brown, and F. A. Smith. *J. Lipid Research* **1:** 29, 1959.
9. Karmen, A., L. Giuffrida, and R. L. Bowman. *J. Lipid Research* **3:** 44, 1962.
10. Karmen, A., and H. R. Tritch. *Nature* **186:** 150, 1960.
11. Steinberg, D. *Nature* **183:** 1253, 1959.
12. Ettre, L. S., and W. Averill. *Anal. Chem.* **33:** 680, 1961.
13. Lovelock, J. E. *J. Chromatog.* **1:** 35, 1958.
14. Karmen, A., L. Giuffrida, and R. L. Bowman. *Nature* **191:** 906, 1961.
15. Karmen, A., and R. L. Bowman. *Gas Chromatography: Proceedings of Third International ISA Symposium,* Instrument Society of America, Pittsburgh, Pennsylvania, 1961, p. 129.