

Calibration of flow-through detectors of ^{14}C in gas-liquid chromatographic effluents

ARTHUR KARMEN

Department of Radiological Science, The Johns Hopkins Medical Institutions, Baltimore, Maryland

SUMMARY A simple procedure for calibrating flow-through detectors of radioactivity in the effluent of a gas chromatography column is described.

KEY WORDS ^{14}C · gas-liquid chromatography · calibration · flow-through detectors

SEVERAL APPROACHES to measuring ^{14}C in the effluent of a GLC column have recently been reviewed (1, 2). One of the most straightforward and simple methods is to place a radiation detector directly in the gas stream, and to pass the column effluent, preferably after it has been subjected to combustion, through it. When this approach is used, it is often helpful to calibrate the system under actual operating conditions. The response can then be related to the absolute quantities of radioactivity in the sample. Also, since the response to known quantities of radioactivity can be predicted, the choice of appropriate scale settings and the decision concerning the quantity of sample to be injected are both facilitated.

When a radioactive sample passes through any flow-through proportional counter, ionization chamber, or scintillation counter, a number of counts (or in the case of an ionization chamber an electrical charge) is recorded. This response is determined by the product of: (a) the activity of the sample; (b) the efficiency of the detector; and (c) the time the sample is in the active volume of the detector, which is determined by the ratio of the mixing volume of the detector to the flow rate of the gas through it. The system can be calibrated on the basis of this relationship by passing a metered flow of gas through the detector, injecting a known quantity of radioactive gas, and recording the response of the detector. The product of the mixing volume of the detector and its efficiency can be calculated from this response.

Suppose, for example, that a sample containing 10,000 dpm is injected into a gas stream flowing at 100 ml/min and passes through a detector with a geometrical volume of 10 ml which records 500 counts. Substituting these values in the equation:

$$\begin{aligned} \text{Response of detector (counts)} \\ &= \text{activity (dpm)} \times \frac{\text{efficiency (cpm/dpm)} \times \text{mixing volume (ml)}}{\text{flow (ml/min)}} \\ 500 \text{ counts} &= 10,000 \text{ dpm} \times \frac{\text{efficiency} \times \text{volume}}{100 \text{ ml/min}}, \end{aligned}$$

Abbreviation: GLC, gas-liquid chromatography.

we obtain: efficiency \times volume = 5 ml, which may be considered the "effective volume" of the detector. When this value is known the response of the system to the passage of a known quantity of radioactive gas through the detector can be predicted. If we substitute the geometric volume of the detector, 10 ml, in this example, for the "mixing volume," the efficiency can be estimated to be 50%. On the other hand, since the mixing volume may be appreciably more than the geometric volume if the sample is adsorbed temporarily on the walls of the detector, or less than the geometric volume if mixing is incomplete, a better estimate of efficiency can be obtained if the mixing volume is measured under the conditions of the experiment. This measurement can be performed by introducing a quantity of radioactive gas into the detector and measuring its rate of disappearance. The time taken to decrease the activity to one half its previous value, the "half time," is given by the relationship, $t_{1/2} = 0.693 V/F$, where F is the flow rate and V the mixing volume. In the example cited, if the half time at 100 ml/min were 0.05 min, the mixing volume would be calculated to be 7.2 ml and the efficiency of the detector estimated to be 69.5%.

Since the mixing volume of the detector, as defined by the rate of disappearance of a sample, may vary with flow rate, it ought therefore to be determined at several flow rates. The mixing volume of the detector may also be different for different gases if one is adsorbed on the surface of the detector to a greater extent and is therefore retained in the detector longer. If adsorption of a sample is appreciable, the mixing volume of the detector for that sample may be appreciably larger than its geometrical volume, just as the retention volume of a compound in a GLC column may be appreciably more than the geometric volume of the column. By this reasoning, it is preferable to convert all the compounds in a mixture to the same chemical form prior to passing them through the detector. It also follows that when a detector is calibrated, it is preferable to inject a radioactive sample that is not appreciably adsorbed. For this reason, calibrating a GLC system by injecting an organic compound either directly into the detector or into the GLC column may yield results that are either falsely high or low depending on where the adsorption occurs. One of the most accurate methods is to deliver a metered stream of $^{14}\text{CO}_2$ into the gas stream. Unfortunately, this requires special equipment that is often not readily available as well as a source of gas in which the ^{14}C concentration is accurately known. It was therefore of interest to us to study the possibility of developing an equally reliable but simpler procedure for delivering $^{14}\text{CO}_2$ to the detector.

$\text{NaH}^{14}\text{CO}_3$ is readily available in solid form commercially and is relatively inexpensive. A stable aqueous solution can be prepared by dissolving it in 0.1 M NaOH.

Microliter quantities of this solution can then be assayed by liquid scintillation counting in conventional PPO (2,5-diphenyloxazole)-toluene solutions to which a small quantity of an organic base such as Hyamine [*p*-(diisobutylcresoxy-ethoxyethyl)dimethyl benzylamine] has been added. CO₂ can readily be released from similar aliquots by injecting them into an acid solution. The sodium bicarbonate solution can thus serve as a convenient source of ¹⁴CO₂.

A simple CO₂ generator was constructed from a test tube 15 mm o.d. × 50 mm, fitted with a rubber stopper (Fig. 1). A metered flow of carrier CO₂ was bubbled through the acid solution in the tube, 3 ml of 1.0 M NaH₂PO₄. The outlet tube was connected to a moisture trap consisting of a 4 inch length of 5 mm i.d. tubing filled with magnesium perchlorate crystals, the effluent of which was delivered either directly to the detector or to some point in the gas line leading to the detector. Several microliters of bicarbonate solution could be injected directly into the acid through the puncture-type rubber stopper with the use of a microsyringe, just as samples are introduced into a GLC column.

The time required to sweep the CO₂ quantitatively from this generator to the detector depended on the flow rate. When 50 ml/min of gas was bubbled through 3 ml of solution, the counting rate returned to background level after approximately 3 min.

The reproducibility of the system was tested by recording the total number of radioactive events (detected

by a flow-through proportional counter) in the first 5 min after injection of each of a series of samples. The standard deviations from the means of repeated determinations were not appreciably greater than the standard deviations predicted from the random nature of the radioactive emission (Table 1). This system was also used in a study of the effect of flow rate on the detection of ¹⁴C by a 5 ml, flow-through anthracene crystal scintillation detector and a 30 ml flow-through proportional counter attached in series (Fig. 2). The gas flowed through the scintillation counter first and then through

TABLE 1 REPRODUCIBILITY OF ¹⁴CO₂ GENERATOR

No. of Samples	Average of Counts Recorded	SD Observed	SD Predicted
6	10,071	203.2	100
4	5,522	119	74
3	4,229	84	65
6	2,752	102	53
6	9,654	105	97
3	5,382	119	73
4	15,852	123	125

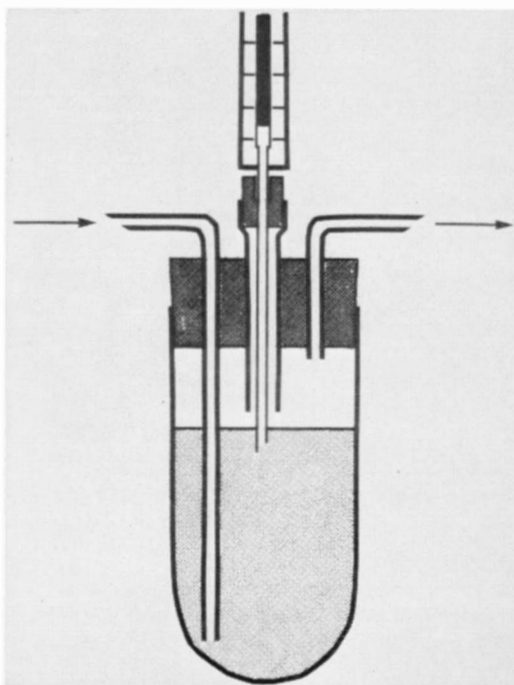


FIG. 1. Schematic diagram of an apparatus for generating ¹⁴CO₂ from NaH¹⁴CO₃. The tube contained 3 ml of 1.0 M NaH₂PO₄.

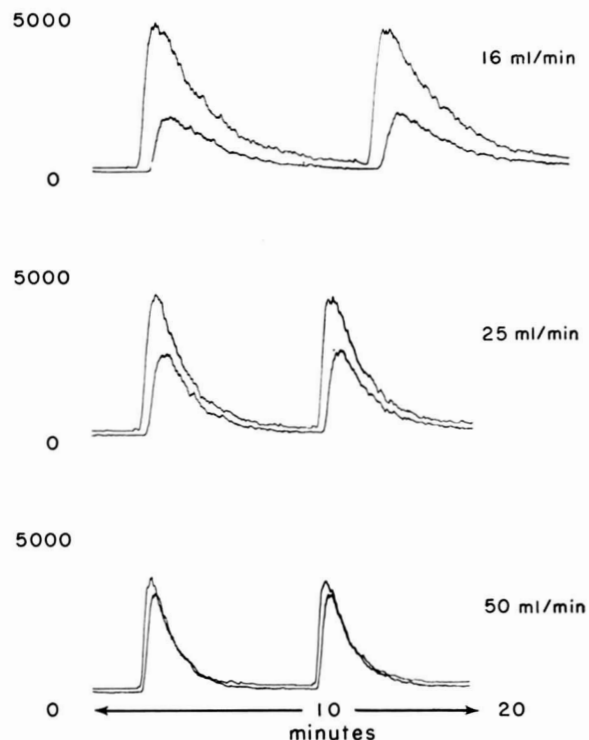


FIG. 2. The effect of gas flow rate on the responses of a crystalline anthracene flow-through scintillation counter (upper curves in each test) and a flow-through proportional counter (lower curves) connected in series, as determined by injecting samples of NaH-¹⁴CO₃ into the generator. The gas flow through the 5 ml scintillation counter was 16, 25, and 50 ml/min for the upper, middle, and lower records respectively. An additional 240 ml/min was introduced between the two detectors. The ordinate for each graph is from 0 to 5000 cpm.

the proportional counter. An auxiliary flow of 240 ml/min of argon was delivered to the gas stream between the scintillation detector and the proportional counter to reduce the response time of the proportional counter. Although increasing the flow through the generator from 16 to 50 ml/min tripled the flow rate through the scintillation detector, the flow through the proportional counter was increased only 1.13 times. The total number of counts recorded by the proportional counter (the areas of the lower curves) in each of the series of analyses was therefore not appreciably changed, although the increase in height and narrowing of the curve reflected the increased rate of delivery of the radioactive material to the detector. On the other hand, the total number of counts recorded by the scintillation detector decreased, as predicted, in direct proportion to the flow rate of gas through it.

This ability to introduce known samples of radioactive gas repeatedly and reproducibly was also particularly helpful in determining the voltage and discriminator settings for flow-through proportional counters and scintillation counters when these instruments were set up.

This study was supported by PHS Grant GM 11535 from National Institutes of Health, U.S. Public Health Service.

Manuscript received 10 June 1966; accepted 14 September 1966.

REFERENCES

1. James, A. T. 1964. In *New Biochemical Separations*, edited by A. T. James and L. J. Morris. Van Nostrand, London, pp. 1-24.
2. Karmen, A. 1964. *J. Assn. Off. Agric. Chem.* 47: 15.