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IMPROVED SYNCHRONIZED ACCUMULATING RADIOISOTOPE DETECTOR FOR GAS CHROMATOGRAPHY

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SUMMARY

A synchronized accumulating radioisotope detector for radio gas chromatography was developed. It comprised seven gas-flow proportional counters each with an inner volume of 10 ml. Every counter tube was connected by a mutual anti-coincidence circuit to reduce the background. The transit time of gas particles in one counter tube could be set to an optimal value between 1 and 4 s by regulating the flow-rate of the counting gas, according to analytical requirements. The improved detector maintained high chromatographic resolution, which suggested the applicability of the apparatus to capillary gas chromatography.

INTRODUCTION

Recently, picogram amounts of biologically active substances with similar chemical structures, for example steroids and prostanoids, have often been analysed. Radio gas chromatography (GC) is one of the most effective techniques in biological studies, using ^{14}C- or ^{3}H-labelled substances as tracers. Developments in radio GC methodology that yields both high resolution and high sensitivity are considered to be of great importance.

Radio GC in general has the inherent drawback that enhancement of the sensitivity results in a decrease of the chromatographic resolution. We previously developed the synchronized accumulating radioisotope detector (SARD) for radio GC, which overcame this drawback to an appreciable extent [1,2]. This paper describes extensive improvements to the SARD by miniaturization and an increase in the number of counter tubes.

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EXPERIMENTAL

Radioactive samples and reagents

n-[1-^{14}C]Hexadecane (specific activity 61 mCi/mmol; radiochemical purity > 97%) was purchased from the Radiochemical Centre (Amersham, U.K.). It was diluted by mixing with it non-labelled hexadecane to give a specified specific activity and used as a cyclohexane solution. All reagents were purchased from Wako (Tokyo, Japan) and were of analytical-reagent grade.

Radio GC system

A Shimadzu GC-6AM gas chromatograph equipped with a flame ionization detector (FID) was used. Unless otherwise stated, column effluents were led to the SARD only and subjected to combustion in an oxidation–reduction furnace. The resulting gas was dried over magnesium perchlorate and mixed with counting gas before the detector assembly, as described in the previous paper [1].

Seven gas-flow proportional counters, each with an inner volume of 10 ml (10 mm I.D.; effective length of anode wire 128 mm) were used, which were connected longitudinally by tubing of 0.75 mm I.D. The counter tubes were arranged as a close-packed structure and placed in a 30 mm thick cylindrical lead housing (Fig. 1). The electric circuit was made up so that signals from each counter tube were accumulated in synchronization with the travelling speed of the radioactive gas after passing through a mutual anti-coincidence circuit [2]. The gate width of the mutual anti-coincidence circuit was 3 μs. The output signals from each counter tube or the sum of the output signals from the seven counter tubes could be obtained by a rate meter.

Operating conditions

A glass column (1 m × 3 mm I.D.) packed with 1.5% OV-1 on Shimalite W (80–100 mesh) was used. Unless otherwise stated, the column oven and injection port temperatures were 180 and 200 °C, respectively. The oxidation–reduction tube

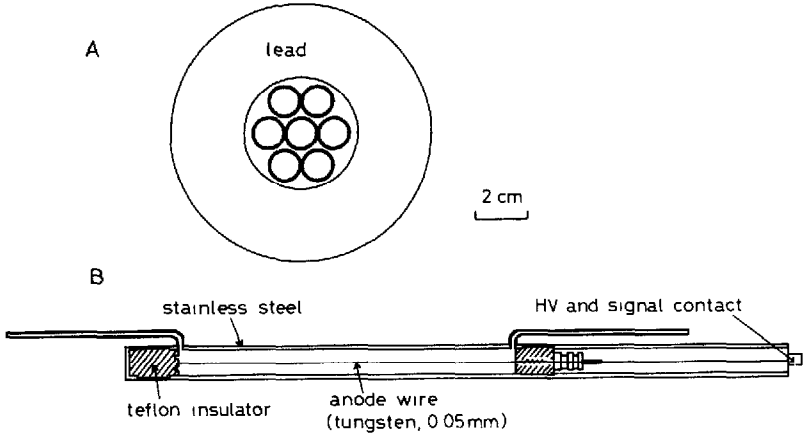

Fig. 1. Cross-section of detector assembly (A) and gas-flow proportional counter (B) of the SARD.

[1] was packed with ca. 10 g of copper oxide wire for elemental analysis (dimensions ca. 5×1 mm) and heated to ca. 800°C. The carrier gas (helium) flow-rate was 50 ml/min. The total flow-rate of the counting gas (methane) and the carrier gas in the counter tubes was measured at the outlet of the detector assembly by a soap-film flow-meter and set at 150 (condition A), 200 (condition B), 300 (condition C) or 600 (condition D) ml/min, using a constant-flow regulator. Accordingly the sampling times (one counter tube volume/the total flow-rate of carrier gas and counting gas) in conditions A, B, C and D were set at 4, 3, 2 and 1 s, respectively. Thus the sampling time was equal to the transit time of gas in one counter tube. The plateau characteristics of the SARD for the above conditions were examined with an external source of radiation (^{137}Cs). Each optimal high voltage (HV) is discussed in the text. Samples were injected with a 10-μl Hamilton microsyringe. The time constant of the rate meters was set at 1 s. Sample radioactivities were measured in 10 ml of toluene base scintillation cocktail, with a liquid scintillation counter (Aloka LSC 903 or 1000).

RESULTS AND DISCUSSION

Radiochromatography has been one of the most useful techniques in metabolic studies. In the field, there are few cases of analyses by radio GC, although radio thin-layer chromatography and radio high-performance liquid chromatography are often used. The reason is mainly the extreme loss of high resolution power, which is one of the most important features in GC [3–8].

In usual radio GC systems, the transit time of gas particles in the counter tube should be as short as possible to maintain the chromatographic resolution. However, any reduction in the transit time is accompanied by a corresponding drop in the counting time, viz. detection sensitivity, which is based on the integral intensity of peak. The transit time depends on the inner volume of counter tube and on the flow-rate in the tube. Consequently, a compromise between maintenance of chromatographic resolution and improvement of detection sensitivity must be made according to the analytical requirements, by selecting the appropriate tube volume and flow-rate.

In GC with an SARD consisting of n counter tubes, the transit time in one counter tube can be reduced to $1/n$ in order to improve the maintenance of chromatographic resolution without any reduction in detection sensitivity, compared with the conventional detection method by a single counter tube.

The detector assembly of the new SARD is illustrated in Fig. 1. In order to improve the maintenance of chromatographic resolution, the number of counter tubes was increased to seven, and the inner volume of the tube reduced to one third: the tubes were also made slenderer than those of the previous SARD. The counter tubes were placed in a close-packed arrangement, for two reasons. One is to reduce the volume required for housing the detector assembly, which makes it possible to increase the thickness of lead shielding by 5 mm. The other is that the mutual anti-coincidence circuit is expected to work more efficiently than in the previous SARD, because the possibility that external radioactive rays will penetrate only one counter tube is reduced.

It is desirable to be able to select easily the compromise between maintenance of chromatographic resolution and improvement of detecting sensitivity in a given radio GC system according to the analytical requirements. Such selection was not generally performed in conventional radio GC. With the new SARD, an attempt was made to change the sampling time by regulating the flow-rate of the counting gas with the flow-rate of the carrier gas fixed. In such cases, the voltage of the counter tubes must be adjusted in accord with the sampling time, since the plateau characteristics are dependent on the gas composition in the tube. The plateau characteristics were examined under various sampling times. Then, as shown in Fig. 2, the plateau was obtained under the sampling time between 4 (curve A: counting gas, 100 ml/min; counting time, 7×4 s) and 1 s (curve D: counting gas, 550 ml/min; counting time, 7×1 s) at the fixed flow-rate (50 ml/min) of the carrier gas. The plateau shifted to the higher voltage region and became longer as the content of counting gas increased. The optimal voltages were decided according to these plateau curves. The various parameters for each counting condition are shown in Table I. The performance of the apparatus was examined under four standard conditions in the following experiments.

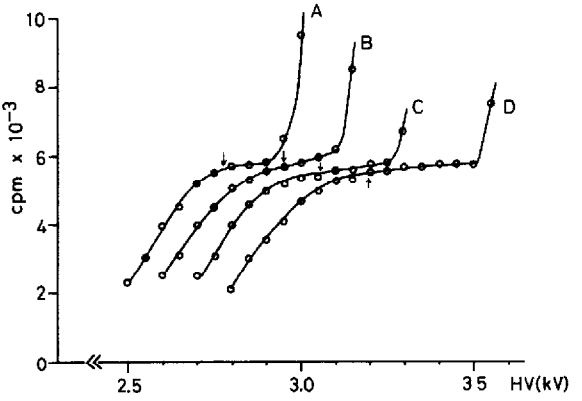

Fig. 2. Proportional counting plateau of the SARD for various ratios of helium (50 ml/min) to methane, obtained with an external source of radiation (^{137}Cs). Arrows show the HV to be set. A, B, C and D correspond to A, B, C and D in Table I.

TABLE I

VARIOUS PARAMETERS IN EACH COUNTING CONDITION

The sampling time equals to the transit time of gas particles in one counter tube.

Counting condition	Flow-rate (ml/min)		Sampling time (s)	Counting time (s)	HV (kV)
	Carrier gas	Counting gas			
A	50	100	4	28	2.78
B	50	150	3	21	2.95
C	50	250	2	14	3.06
D	50	550	1	7	3.20

[^{14}C]Hexadecane was measured under the various counting conditions, in order to verify the relationship between the maintenance of chromatographic resolution and the improvement of detection sensitivity. Typical chromatograms are shown in Fig. 3, and it is evident that the detecting sensitivity in Fig. 3A is superior to that in Fig. 3C but the resolution (half-width) is sacrificed, and the reverse is also true. Thus it is desirable to select any counting conditions according to analytical requirements.

Both the background and the counting efficiency for [^{14}C]hexadecane were measured under the counting conditions of A, B, C and D. The counting efficiency was ca. 100% in all the counting conditions, and its constancy was good (for example in A, 95.4 ± 1.42%). The background was ca. 40 cpm in all the counting conditions, which was ca. 50% of that of the previous SARD. The mutual anti-coincidence guard of the new SARD could eliminate 55–60% of the origin background, and its efficiency was slightly greater than that of the previous SARD. The background of counter tube is dependent on the type of construction and the extent of shielding, but commonly that of the tube with the 10-ml inner volume is in the region 25–50 cpm [9]. In spite of the large tube volume (7×10 ml) of the present apparatus, the background proved to be comparable with the values.

Simpson [10] developed a radio GC system equipped with tubes of 10-ml inner volume, anti-coincidence guard counter and graded gamma shield, and reported the background to be ca. 1 cpm. Strong et al. [11] reported that the background

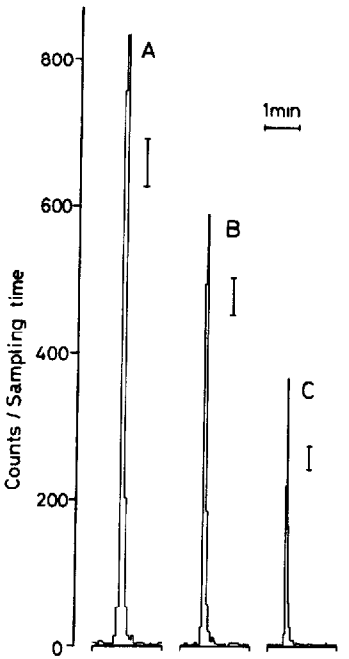

Fig. 3. Relationship between the maintenance of chromatographic resolution and the improvement of detection sensitivity. Sample, [^{14}C]hexadecane (2.68 mCi/mmol), ca. 1.5 nCi. The column oven and injection port temperatures were 250 and 270 °C, respectively. A, B and C correspond to A, B and C in Table I. The vertical lines next to each chromatogram represent 1000 cpm.

of the same type of radio GC is ca. 3–4 cpm in practical use. Our background per counter tube (inner volume 10 ml) was calculated to be ca. 6 cpm and proved to be closer to that of the counter tube reported by Simpson [10] and Strong et al. [11], in spite of the simple shielding. The above experimental results showed that the improvements resulted in the appreciable low background. The background reduction resulted from the decrease of external radiation due to the miniaturization of detector assembly and the slight increase in the thickness of the lead shielding, but mainly to the increased efficiency of the mutual anti-coincidence guard due to the close-packed structure. This can contribute to the improvement of the detection limit [12].

The feasibility of using the SARD as a detector for GC is largely dependent on how much peak-broadening there is due to the prolonged transit time of the radioactive gas in the multiple counter tubes. [^{14}C]Hexadecane (3–5 nCi, 6–8 mCi/mmol) was measured under the various counting conditions described in Table I, and the signals from the first and seventh counter tubes were simultaneously recorded through two rate meters. The width of the peak obtained by the first counter tube was compared with that obtained by the seventh tube. The results are shown in Table II. The peak-broadening proved to be slight under conditions B, C and D, but under condition A it was a little larger due to the low flow-rate. However, this condition did not matter in practical application, as shown in Fig. 3.

[^{14}C]Hexadecane (ca. 4.2 nCi, 537 μCi/mmol) was injected into the gas chromatograph with the SARD and the radioactivity was measured by three methods to verify the advantages of the new SARD (Fig. 4). Fig. 4I shows the output signals from the first counter tube constituting the SARD. Fig. 4II shows the sum of output signals from all seven counter tubes. Fig. 4III shows the SARD output signals. It may be all right to consider I and II to be the radiochromatograms from a single counter tube with an inner volume of 10 and 70 ml, respectively. The advantages of the SARD are clear when the three chromatograms are compared. The chromatographic resolution in I is superior to that in II, but the detection sensitivity is poorer. The maintenance of resolution in Fig. 4III is almost identical with that in I, and the detection sensitivity is comparable with that in II. Thus, the resolution of Fig.4I and the enhancement of detection sensitivity of Fig. 4II are both maintained with the SARD.

TABLE II

PEAK BROADENING IN THE COUNTER TUBES CONSTITUTING THE SARD

A, B, C and D correspond to A, B, C and D in Table I.

Counting condition	Ratio of half-width (seventh tube/first tube)[a]
A	1.29 ± 0.064
B	1.13 ± 0.037
C	1.10 ± 0.040
D	1.15 ± 0.076

[a] $n = 4$.

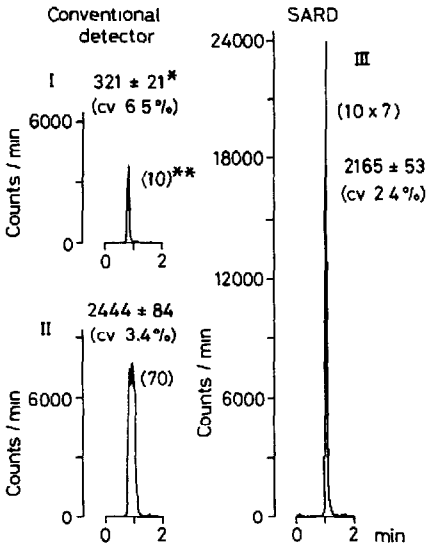

Fig. 4. Comparison of the chromatogram obtained with the SARD (III) with that of conventional detection method. (*) Counts (mean ± S.D., $n=13$); (**) detector volume (ml); counting condition C.

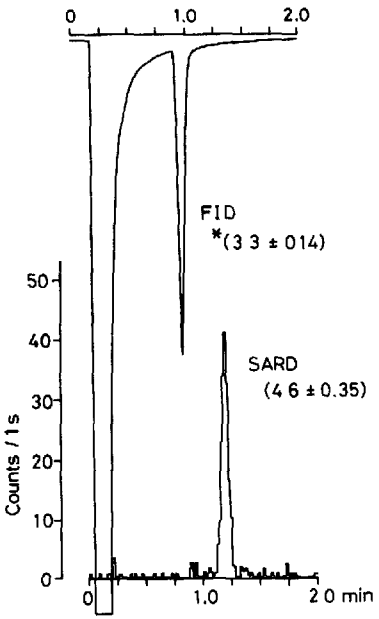

Fig. 5. Comparison of the SARD peak-width (lower trace) with the FID peak-width (upper trace). (*) Half-width (mean ± S.D., $n=3$); sample, [^{14}C]hexadecane (436 μCi/mmol), ca. 1.3 nCi; counting condition D.

[^{14}C]Hexadecane was injected in order to elucidate to what extent the resolution could be improved. Column effluents were split to the SARD and the FID (split ratio, ca. 1:1), and the radioactivity was measured under condition D. Typ-

ical chromatograms (Fig. 5) show that the width of the SARD peak was comparable to that of the FID peak under these conditions. With the SARD, the radioactivity of a gas particle is theoretically recorded at two sampling times on the chromatogram [13]. As a whole, the SARD peak is considered to become wider than the corresponding FID peak by two sampling times at the most. In this experiments, the half-width of the SARD peak was calculated to be less than 5.3 s, since that of the FID peak was 3.3 s. The half-width of the SARD peak was estimated to be 4.6 s, from the lines obtained by connecting the midpoints of each histogram constituting the SARD peak [14]. Thus the experimental results approximately agreed with the theory. However sharp the FID peaks are, the theory holds true. It is unnecessary to consider FID peaks narrower than these for GC on packed columns. From the above experimental results and discussion, the new SARD proved to be the apparatus that could mostly overcome the drawback of resolution sacrifice in conventional radio GC on packed columns. It was also suggested that the apparatus could be applied to capillary GC, and a later paper will describe this application.

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