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SYNCHRONIZED ACCUMULATING RADIOISOTOPE DETECTOR EQUIPPED WITH A MUTUAL ANTI-COINCIDENCE GUARD FOR RADIO-GAS CHROMATOGRAPHY

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SUMMARY

Further examination was made of the performance of the previously reported synchronized accumulating radioisotope detector (SARD) for radio-gas chromatography (radio-GC). The precision of the measurement was found to exceed that of a conventional detector by a factor of $\sqrt{5}$ without any decrease in chromatographic resolution. Application of the mutual anti-coincidence method to the SARD caused the background to decrease by approximately half. The detection limit of the low-background SARD for [^{14}C]hexadecane was 15—18 pCi as injection radioactivity.

INTRODUCTION

Radio-gas chromatography (radio-GC) detects only radioactive substances in multi-component samples and is one of the most effective techniques in biochemical studies, using ^{14}C- or ^{3}H-labelled substances as tracers. In most radio-GC systems, substances in the column effluents undergo combustion to CO_2 and H_2O and the H_2O is reduced to H_2; the radioactive gases ($^{14}CO_2$ and/or $^{3}H_2$) are measured by a single gas-flow proportional counter. However, the system has the serious disadvantage that enhancement of the detection efficiency results in decreased chromatographic resolution.

We previously developed a synchronized accumulating radioisotope detector (SARD) for radio-GC (see Fig. 2), which overcame this disadvantage to an appreciable extent [1]. This paper describes the results of a further examination of the performance of the detector and the reduction of its background by a mutual anti-coincidence method.

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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

The apparatus previously reported (Shimazu GC-6AM gas chromatograph combined with an Aloka RGC-212) was equipped with a mutual anti-coincidence circuit. In the SARD, five identical gas-flow proportional counters are connected longitudinally in series. The cathode of each counter is a cylinder of stainless steel with an effective volume of 30 ml. Each counter is arranged so that its outer wall is in contact with the next. All the counters are placed in a 25 mm thick cylindrical lead housing as shown in Fig. 2. The output signal of each counter and the sum of the output signals from the five counters are obtained by a rate meter (time constant 10 s). The first counter was used both as part of the SARD and as an independent conventional detector. Its signal was obtained at the same time as the SARD signal in the experiments on reproducibility and detection limits.

Operating conditions

The carrier gas (nitrogen) and counting gas (methane) flow-rates were 50 and 250 ml/min, respectively. The sampling time [= one counter volume/(carrier gas flow-rate + counting gas flow-rate)] was thus set to 6 s [2]. A glass column (1 m × 3 mm I.D.) was packed with 1.5% OV-17 on Shimalite W (80—100 mesh). The column oven and injection port temperatures were 160 and 250°C, respectively. The combustion tube was packed with 10 g of copper oxide wire (dimensions 5 × 1 mm), as only a ^{14}C-labelled substance was used. It was heated to about 800°C. The sample radioactivity was measured in 10 ml of toluene base scintillation cocktail with a liquid scintillation counter (Aloka LSC 903).

Mutual anti-coincidence circuit

In this circuit, when pulses occur within 4 μs (resolution time) from more than two counters constituting the SARD, the gate of the mutual anti-coincidence circuit is open, and these and other pulses within the gate width are removed. The circuit was switched off in the reproducibility experiments and switched on at a gate width of 100 μs in the detection limit experiments.

Reproducibility

A 3-μl volume of a 0.14 nCi/μl solution containing [^{14}C]hexadecane (1.10 mCi/mmol) was injected into the radio-GC—SARD system. The radioactivity was measured by the above method and the sum of the output signals of the five counters was also obtained at the same time.

Mutual anti-coincidence method for background reduction

The background was measured for 10 min with the circuit switched off. The circuit was then switched on at a gate width of 8 μs and the background was measured for 10 min. The same procedure was also conducted at gate widths of 100 and 1000 μs.

Detection limits

A cyclohexane solution (13.1 pCi/μl) of [^{14}C]hexadecane (45.6 μCi/mmol) was injected into the radio-GC—SARD system in amounts of 0.5, 1, 2, 4, 6 and 8 μl at about 4-min intervals, followed by measurement of the radioactivity. This procedure was performed three times.

RESULTS AND DISCUSSION

In the radio-GC system in which the column effluent is monitored continuously by a gas-flow proportional counter, the counter volume should be as small as possible to maintain the chromatographic resolution. However, any reduction in the counter volume is accompanied by a corresponding decrease in the detection efficiency, as the response of the counter is a function of the total radioactivity in the counter at any time. Hence in the radio-GC system using a single counter (conventional method), enhancement of the detection efficiency is not possible without a reduction in chromatographic resolution. A compromise must be made according to the analytical requirements. Hitherto, no effective method for overcoming this problem has been available.

In the radio-GC—SARD system, n identical counters are connected longitudinally in series, and signals from each counter accumulate in synchronization with the travelling speed of the radioactive gas. In the present method, n-fold counts may thus be obtained and then the precision of measurement theoretically exceeds that of the conventional method by a factor of $\sqrt{n}$ without a reduction in chromatographic resolution.

[^{14}C]Hexadecane was injected into the radio-GC—SARD system and the radioactivity was measured by both the conventional method and the SARD simultaneously. Examples of the chromatograms and reproducibility data are shown in Fig. 1 and Table I, respectively. In Fig. 1, the chromatogram in A is the output signal from the first of the five counters constituting the SARD. The chromatogram in B is the sum of output signals from all five counters. The chromatogram in C is the SARD output signal. B may be considered to result from a single counter whose volume is five times that of the counter of A. The advantages of the SARD are clearly evident by a comparison of the three chromatograms.

In general, a convenient compromise between improved detection efficiency and retention of chromatographic resolution is made by choosing a counter with a volume one third that of the narrowest chromatographic peak of interest [3]. Under the present experimental conditions, the [^{14}C]hexadecane peak emerged from the column within about 20 s, having a volume of about 100 ml. Most biochemical studies are rarely concerned with peaks narrower than that of [^{14}C]hexadecane. Although the counter volume (30 ml) in A is approximately compatible with the above compromise, that in B (150 ml) is

TABLE I

REPRODUCIBILITY

A, B and C correspond to A, B and C in Fig. 1.

Experiment No.	Integral intensity (cm^2)		
	A	B	C
1	0.76	3.38	3.45
2	0.59	3.29	3.67
3	0.67	3.16	3.47
4	0.64	3.15	3.57
5	0.50	3.54	3.28
6	0.68	3.10	3.29
7	0.62	3 38	3.57
8	0.61	3.25	3.36
9	0.66	3.46	3.67
10	0.73	3.62	3.42
Mean ± S.D.	0.65 ± 0.073	3.33 ± 0.174	3.48 ± 0.143
Coefficient of variation (%)	11.2	5.2	4.1

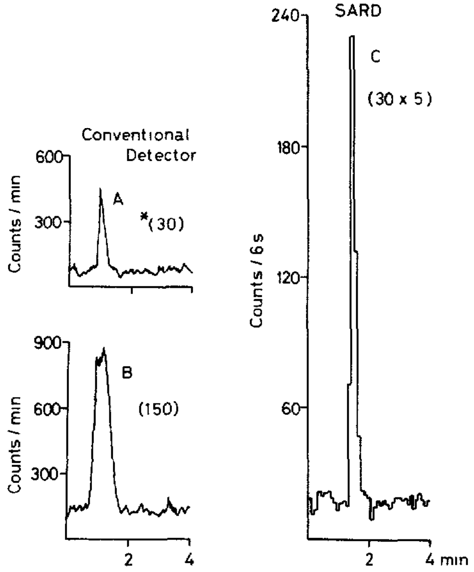


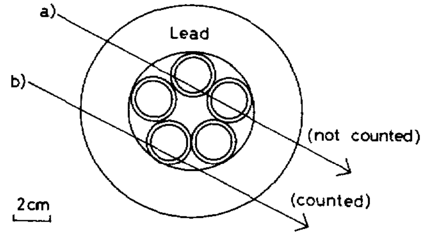
Fig. 1. Comparison of radio-gas chromatograms: * Detector volume (ml).

not. The resolution in B is thus sacrificed to give extreme peak broadening. However, the peak width in C is almost identical with that in A. As is evident from Table I, the integral intensity in C is comparable to that in B and about five times that in A. Although the coefficient of variation in B is improved $\sqrt{5}$ times that in A, that in C obviously improved even more. Although the areas of A and B are calculated by cutting and weighing, that of C is measured directly from the chromatograms and the corresponding improvement of precision is achieved. The above experimental results show that both the retention of resolution as in A and the enhancement of detection efficiency (response factor) as in B are realized simultaneously in the SARD, whereas both had to be selected in the conventional method.

Reduction of the background also contributes to the precise detection of peaks of low radioactivity. Low-background detectors for radio-GC have been discussed in a few papers [4—7]. In particular, an effective low-background detector was designed by Simpson [4], in which a plastic scintillator, an anti-coincidence guard counter and a gamma shield are used. Equipping the SARD with an anti-coincidence guard counter makes it a detector of large size, as the SARD initially consists of five counters arranged as shown in Fig. 2. An attempt was made to develop a relatively small and light low-background SARD by a mutual anti-coincidence method using the arrangement of the SARD.

A scheme to eliminate external radioactive rays in the mutual anti-coincidence method is shown in Fig. 2. In the usual anti-coincidence method, the main counter is surrounded by the guard counter, and pulses from the main counter (main pulse) are controlled by those from the guard counter (gate pulse), that is, the gate pulse determines whether the main pulse is counted or not. In the mutual anti-coincidence method, pulses from each counter serve as both main and gate pulses. Thus, at least two significant pulses are required for this circuit to operate. As the radioactive ray a which passes through the plural counters produces multiple pulses simultaneously, the mutual anti-coincidence circuit functions and pulses are eliminated. However, the radioactive ray b which passes through one counter is counted as background as it produces only one pulse. This is a defect not present in the usual anti-coincidence method. The cascade shower component due to cosmic radiation is a major source of background and reaches the ground within 1 μs. The resolution time of the mutual anti-coincidence circuit is set to 4 μs. Thus, the above defect is not encountered in the cascade shower component.

An examination of the effects of background reduction by the mutual anti-coincidence method at gate widths of 8, 100 and 1000 μs showed that 60—75 cpm (40—50% of the origin background) could be eliminated at any gate width, although with some fluctuation. As a typical example, the effects of background reduction at a gate width of 100 μs are shown in Fig. 3. In general,



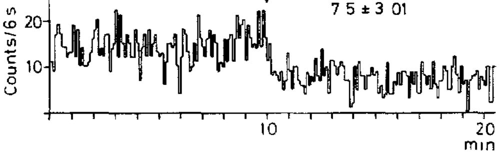


Fig. 2. Arrangement of the five counting tubes. Arrows indicate external radioactive rays.

Fig. 3. Effects of mutual anti-coincidence guard on background. * Counts per fraction (mean ± S.D., n=100). The anti-coincidence circuit was switched on at the point of the arrow.

anti-coincidence counting is likely to cause counting losses. An examination was therefore made to see if counting losses occur on fixing the gate width at 100 μs.

[^{14}C]Hexadecane in an amount from 0.5 to 4.5 nCi was injected into the radio-GC—SARD system to examine the linearity between injection radioactivity and radioactive peak intensity. An excellent linear relationship was found, with a correlation coefficient of 0.9999. Radioactivity less than a few nCi is normally the amount injected in biochemical studies. The counting loss was thus proved to be negligible in practical samples. The above experiment results show that the mutual anti-coincidence method is effective for background reduction in the SARD. The detection limits of the low-background SARD were examined.

A typical example of a detection limit is shown in Fig. 4. Detection limits have been usually defined on the basis of signal-to-noise ratios (S/N) and S/N = 2—3 used as detection limits. If S/N = 2.6 (where N is the mean background) is used as a detection limit, those of a conventional detector and the low-background SARD are 52 and 26 pCi, respectively. It is therefore evident that a detection limit twice that of the conventional method can be achieved as a result of the effects of both the synchronized accumulation and mutual anti-coincidence methods. However, the above method disregards the chromatographic peak width and statistical errors in radioactive disintegration.

In the SARD, fractions are obtained every 6 s (see Experimental) and the counts of each fraction by digital display. The detection limit could be determined more accurately on the basis of total counts of multiple successive fractions rather than counts of one fraction, as the radioactivity in a peak appears over several fractions. The following parameters are presented to define the detection limits: TCSF_n = total counts of n successive fractions; $\mathrm{TCSF}_{n,\mathrm{back}}$ = TCSF_n in the background region; $\mathrm{TCSF}_{n,\mathrm{max}}$ = the TCSF_n that gives the highest value in the region of the peak under consideration (corrected for background), where n is defined as the number of fractions making $\mathrm{TCSF}_{n,\mathrm{max}}$ approximately 90% the total counts under the peak in question. In Fig. 5, the distribution of $\mathrm{TCSF}_{3,\mathrm{back}}$ is approximately the same as the Gaussian distribution. The distribution of $\mathrm{TCSF}_{n,\mathrm{back}}$ has thus been discussed as such in the present work. The detection limit of the SARD is defined as follows [8]:

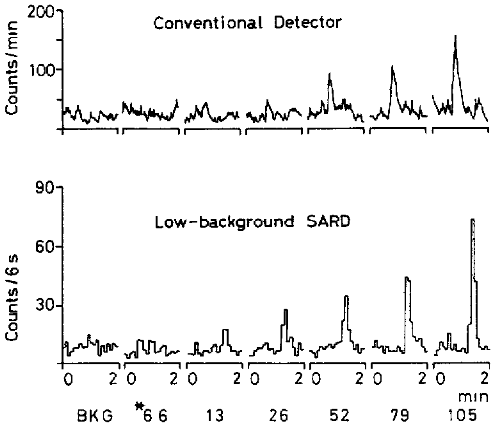
$$\mathrm{TCSF}_{n,\mathrm{max}} = 3(\mathrm{S.D.\ of\ TCSF}_{n,\mathrm{back}})$$

The injection radioactivity, x pCi in the detection limit, is calculated as follows [9]:

$$\begin{aligned}\mathrm{TCSF}_{n,\mathrm{max}} &= x \times 2.22 \times \text{GC peak yield} \times \text{combustion efficiency} \times \\ &\times \text{counting efficiency} \times \text{transit time in the SARD} \times \\ &\times \mathrm{TCSF}_{n,\mathrm{max}}/\text{total counts of peak} = 3\ (\mathrm{S.D.\ of\ TCSF}_{n,\mathrm{back}})\end{aligned}$$

where S.D. is the standard deviation.

For example, the detection limit of the low-background SARD for [^{14}C]-hexadecane was estimated as follows. The ratio of $\mathrm{TCSF}_{3,\mathrm{max}}$/total peak counts (see Fig. 4) was 0.92 ± 0.02. TCSF_3 was then used as the criterion for the detection limit. $\mathrm{TCSF}_{3,\mathrm{back}}$ was 24.3 ± 5.25 (mean ± S.D., n=100). The GC



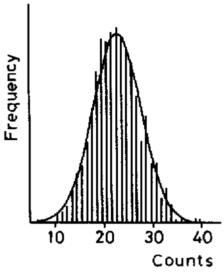
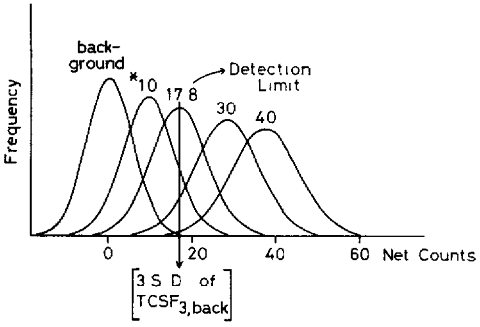


Fig. 4. Detection limit. * Injection radioactivity of [^{14}C]hexadecane (pCi).

Fig. 5. Frequency distribution of TCSF$_{3,\mathrm{back}}$. The histogram shows the experimental data for TCSF$_{3,\mathrm{back}}$. The theoretical curve of the Gaussian distribution is drawn on the basis of the experimental data (TCSF$_{3,\mathrm{back}}$: mean ± S.D. 22.4 ± 4.91, n=1000).



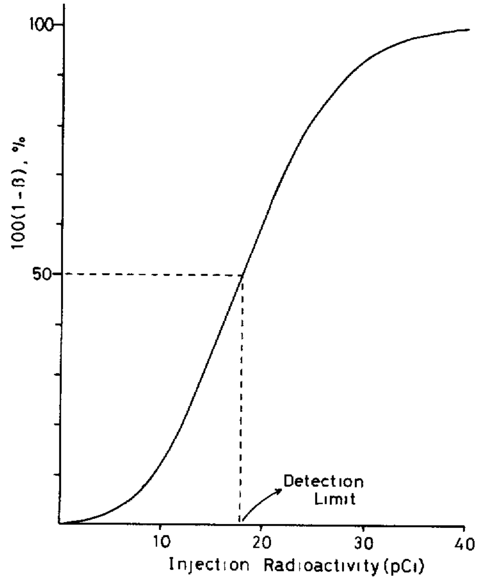


Fig. 6. Gaussian frequency distribution for TCSF$_{3,\mathrm{back}}$ and TCSF$_{3,\max}$ of [^{14}C]hexadecane. * Injection radioactivity (pCi). GC peak yield, 100%; combustion efficiency, 100%; counting efficiency, 96.5%; transit time, 0.5 min; TCSF$_{3,\max}$/total counts of peak, 0.92; TCSF$_{3,\mathrm{back}}$, 25.1 ± 5.85 (mean ± S.D., n=100).

Fig. 7. Relationship between injection radioactivity and detection level for [^{14}C]hexadecane. All conditions as in Fig. 6.

peak yield and the combustion efficiency of [^{14}C]hexadecane were 100% and the counting efficiency and transit time were 96.5% and 0.5 min, respectively. The detection limit for [^{14}C]hexadecane was thus calculated as follows:

$$\mathrm{TCSF}_{3,\mathrm{max}} = x \times 2.22 \times 1.00 \times 1.00 \times 0.965 \times 0.5 \times 0.92 = 3 \times 5.25$$

$$x = 16.0 \text{ pCi}$$

As radioactive disintegration is accompanied by statistical fluctuations, two probabilities should be considered in discussing the detection limit [10, 11]: the probability α, that $\mathrm{TCSF}_{n,\mathrm{back}}$ exceeds the detection limit, giving a false peak, and the probability β, that $\mathrm{TCSF}_{n,\mathrm{max}}$ of a sample containing a certain amount of radioactivity is below the detection limit, giving no peak. The Gaussian distributions for $\mathrm{TCSF}_{3,\mathrm{back}}$ and $\mathrm{TCSF}_{3,\mathrm{max}}$ of [^{14}C]hexadecane are shown in Fig. 6. When 3 (S.D. of $\mathrm{TCSF}_{n,\mathrm{back}}$) is the detection limit, 100α = 0.14%. The detection limit defined above has a detection level $100(1 - \beta)$ of 50%. The relationship between the injection radioactivity and detection level is theoretically predicted in Fig. 7. When [^{14}C]hexadecane of activities 10, 20, 30 and 40 pCi is injected, the detection levels are 12.3, 61.6, 93.4 and 99.5%, respectively. In fact, when [^{14}C]hexadecane of activity 11 and 22 pCi was injected ten times each, characteristic peaks were noted to occur one and five times, respectively.

It has been demonstrated that the precision of measurement with a radio-gas chromatograph was improved by a factor of $\sqrt{5}$ using the SARD equipped with five counters without a reduction in chromatographic resolution. The background of the SARD was reduced by approximately half by the mutual anti-coincidence method.

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