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Note

Synchronized accumulating radioisotope detector for radio gas chromatography

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We have developed a synchronized accumulating radioisotope detector to detect radioactive substances flowing through or alongside the radioisotope detector. The application of this method to the measurement of β-activity on thin-layer chromatoplates was successful and the method offers a great improvement in detection efficiency without sacrificing the resolution[1].

In this paper we describe the application of this method to radio gas chromatography (GC) in which a radioactive substance flows through the radioisotope detector.

EXPERIMENTAL

Radio gas chromatography system

A schematic diagram of the radio-GC system is given in Fig. 1. The GC column eluates are separated so as to flow by two routes in appropriate proportions. One route (route 1) leads to a conventional flame-ionization detector (FID) to measure the amounts of solutes present in the column eluates. The other route (route 2) leads

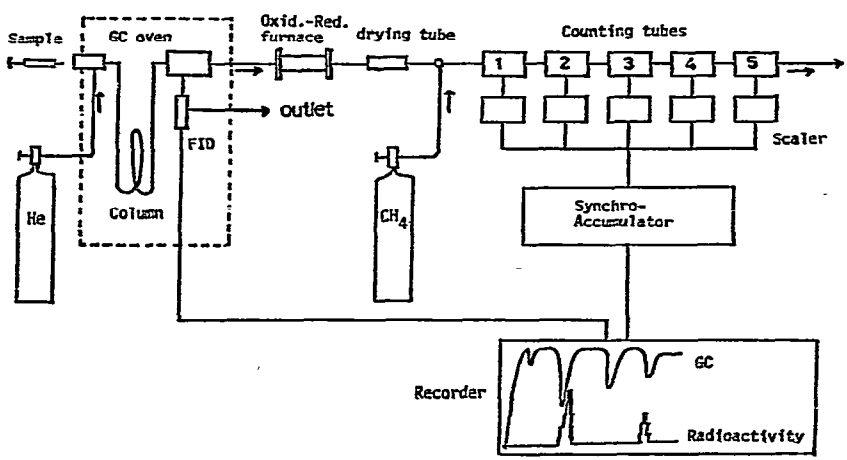

Fig. 1. Radio gas chromatographic system equipped with a synchronised accumulating radiodetector.

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the column eluates to an oxidation–reduction furnace in which ^{14}C- or ^{3}H-labelled compounds in the gas stream are decomposed to produce $^{14}CO_2$ or $^{3}H_2$, respectively. The radioactive gas thus produced then passes into five open detector tubes arranged longitudinally in series, after introduction of methane as a counting gas. The radioactivity signals detected by each individual tube (an effective volume of 30 ml) are accumulated in the respective counting accumulator in a synchronized manner with the flow-rate of the radioactive materials and then sent to the recorder. The operating principle of the synchronized accumulating detector has been described in detail previously[1]. In the comparison of the counting efficiencies of the present and the conventional method, the first detector tube was used not only as part of the synchronized accumulating detector but also as an independent conventional detector having a single counting tube.

Oxidation–reduction furnace

The oxidation–reduction tube placed in the electric furnace is a 180 × 5 mm I.D. quartz tube packed with a volume of about 1.7 ml of copper oxide (dimensions 5 × 1 mm) and about 1.7 ml of reducing iron powder (8–10 mesh). The temperature of the inside of the furnace was about 730°C during operation.

Operating conditions

Unless otherwise stated, the operating conditions were as follows. The carrier gas was helium or nitrogen at a flow-rate of 90 ml/min. One-third of the GC eluate was sent via route 1 and the remainder by route 2. The rate of introduction of methane was 240 ml/min. The amount of radioactive (^{14}C- or ^{3}H-labelled) sample injected was 1–5 nCi. Measurements were performed with a full scale of 1000 cpm at a time constant of 10 sec for the conventional method, and with a full scale of 300 counts per sampling time of 6 sec for the present method.

Radioactive samples

[^{3}H]Hexadecane (0.81 μCi/ml) and [^{14}C]hexadecane (1.31 μCi/ml) were purchased from Daiichi Kagaku Yakuhin (Tokyo, Japan). [^{14}C]Butyryl-4-cinnamylpiperazine (3.73 μCi/mg) and [^{14}C]paeonol (3.11 μCi/mg) were synthesized in our laboratory.

Counting efficiency

A 3-μl volume of [^{14}C]hexadecane or [^{3}H]hexadecane was injected into the gas chromatograph and the GC eluates were led to route 2 by closing the two-way separator outlet leading to route 1. Helium and methane were introduced at flow-rates of 60 and 240 ml/min, respectively. The peak intensity of the radioactivity was quantitated by totalling the counts of each sampling time (6 sec) under the peak (total counts under the peak). Measurements were performed in triplicate under the same conditions.

Linearity

[^{3}H]Hexadecane (0.33 μCi/ml) was injected into the gas chromatograph using volumes of 1, 2, 3 and 4 μl at about 3-min intervals. The radioactivity was measured by the present and the conventional methods. The peak intensity of the radioactivity

on the chromatogram obtained by the conventional method was quantitated by both peak-area and peak-height measurements.

Reproducibility

An ethanolic solution of 1 μl of [^{14}C]butyryl-4-cinnamylpiperazine (8.4 nCi) was injected into the gas chromatograph five times. The radioactivity measurements were performed with a full scale of 10,000 cpm for the conventional method, and with a full scale of 3000 counts per sampling time of 6 sec for the present method.

Determination of metabolites

A rat was given orally 30 μCi/kg of [^{14}C]paeonol (20 mg/kg with carrier paeonol) and the 24-h urine sample was collected. Non-radioactive paeonol, 2,5-dihydroxy-4-methoxyacetophenone and resacetophenone (2 mg each) dissolved in methanol were added as carriers to a 25-ml portion of the 24-h urine. The urine sample was then acidified with sulphuric acid to pH 1.0, heated at 90°C for 1 h and extracted with diethyl ether. The ether extract was washed with water, dried over anhydrous sodium sulphate and evaporated to dryness under reduced pressure. After derivative formation with 0.5 ml of anhydrous acetic acid–pyridine (1:1), the residue was dissolved in 100 μl of acetone and five 1-μl portions of the acetone solution were injected into the gas chromatograph at 20-min intervals. The radioactivity was measured by both the present and the conventional methods and the signals were recorded on the same chart paper with a two-pen recorder. The amounts of solutes in the GC eluates were also recorded on another recorder paper. The peak intensity of the radioactivity on the chromatogram was measured for quantitation.

RESULTS AND DISCUSSION

GC is one of the most frequently used methods for the separation of chemical compounds, and there have been reports[2,3] and reviews[4] on radio-GC. In a previous paper we concluded that the presently available method of radio-GC in which a single counting tube is used does not offer satisfactory resolution with high detection efficiency, and is therefore of only limited use. Our success with the application of a synchronized accumulating detector to thin-layer radiochromatograms[1] prompted us to extend the use of this new radiodetector to radio-GC. Our primary objective was to apply the new radiodetector to radio-GC in order to obtain a higher detection efficiency without sacrificing the resolution.

The usefulness of the application of the radiodetector to radio-GC is largely dependent on how much peak broadening occurs due to the prolonged residence time of the radioactive substance in the multiple detector tubes in series. We have already shown that the use of multiple detector tubes does not result in appreciable peak broadening[1]. This raised the possibility of the application of the synchronized accumulating detector to a continuous flow of a radioactive gas stream from the gas chromatograph.

In this study we first carried out linearity experiments and compared the resolutions obtained by the present and the conventional methods. The results are shown in Fig. 2. The volume of a counting tube (30 ml) is much larger than the volume of the FID, which resulted in a lower resolving power for radio-GC than for ordinary

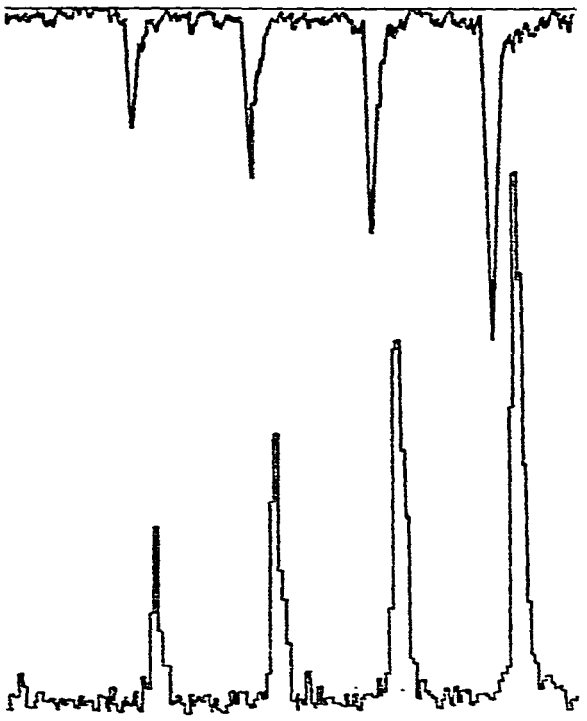

Fig. 2. Comparison of the radio gas chromatograms obtained by the synchronized accumulating method (lower trace) and the conventional method (upper trace). Sample: [^{3}H]hexadecane; from left to right, 1, 2, 3 and 4 μl.

GC. There was no appreciable difference in resolution between the present and the conventional methods. The resolution obtained seems to be reasonable if we take into consideration the sum of the flow-rates of the carrier gas and the counting gas (300 ml/min) and the volume of a counting tube.

The average total counts under the peaks were 2478 counts per 6 sec when 2.43 nCi of [^{14}C]hexadecane were injected and 2436 counts per 6 sec when 3.93 nCi of [^{3}H]hexadecane were injected. The total flow-rate of carrier gas and counting gas was 300 ml/min and the volume of a counting tube was 30 ml. The residence time of the GC eluate in the detector components was thus calculated to be 0.50 min. From these data, the counting efficiency was determined to be 91.9% for [^{14}C]hexadecane and 55.8% for [^{3}H]hexadecane.

The results of the linearity experiment are shown in Fig. 3. It is apparent that the present method gave much better linearity between the signal intensity and the amount of sample injected than the conventional method.

The results of the reproducibility experiment using [^{14}C]butyryl-4-cinnamylpiperazine are presented in Table I. The present method obviously provided very good reproducibility of counting.

As an example of the application of the present method to a metabolic study, the radioactivity distribution profile in the radio-GC chromatogram of a urine sample obtained after oral administration of [^{14}C]paeonol to a rat is given in Fig. 4A.

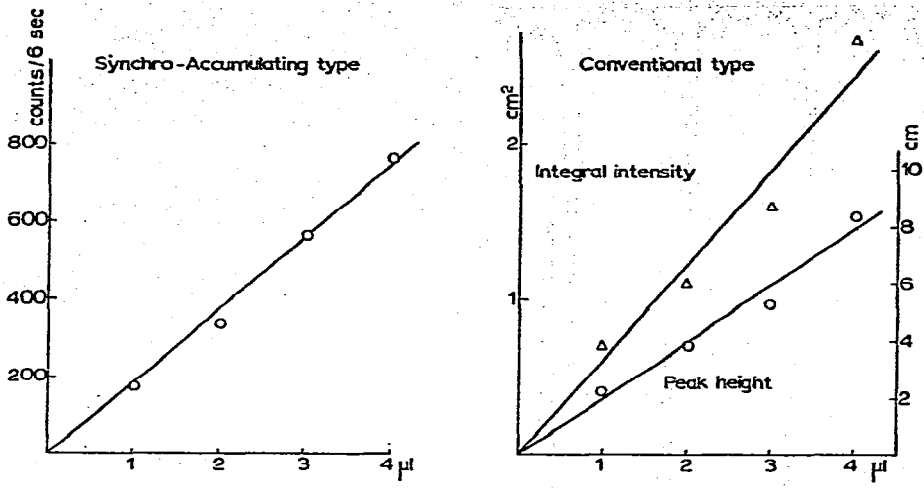

Fig. 3. Comparison of linearity between the signal intensity and the radioactivity (from the data in Fig. 2). Conventional method: quantitation of signal intensity by peak-area (△) and peak-height (○) measurements.

TABLE I

COMPARISON OF REPRODUCIBILITY IN COUNTING

Synchronized accumulating type (counts per 6 sec)	*Conventional type (peak height, cm)*
6141	6.4
6117	6.8
5787	5.8
6183	6.6
5820	6.1
Mean ± S.D. 6009.6 ± 169.9	6.34 ± 0.36

TABLE II

EXAMPLE OF THE DETERMINATION OF METABOLITES AND ACCURACY OF THE MEASUREMENTS

Expt. No.	*Synchronized accumulating type*			*Conventional type*		
	Peak 1 (%)	*Peak 2 (%)*	*Peak 3 (%)*	*Peak 1 (%)*	*Peak 2 (%)*	*Peak 3 (%)*
1	11.93	23.31	64.75	11.11	24.07	64.81
2	12.32	23.50	64.18	4.84	24.19	70.97
3	12.97	24.57	62.46	13.11	21.31	65.57
4	11.79	25.87	62.34	11.29	29.03	59.68
5	11.62	28.04	60.33	17.74	24.19	58.06
Mean ± S.D.	12.13 ± 0.48	25.06 ± 1.75	62.81 ± 1.56	11.62 ± 4.15	24.56 ± 2.49	63.82 ± 4.59

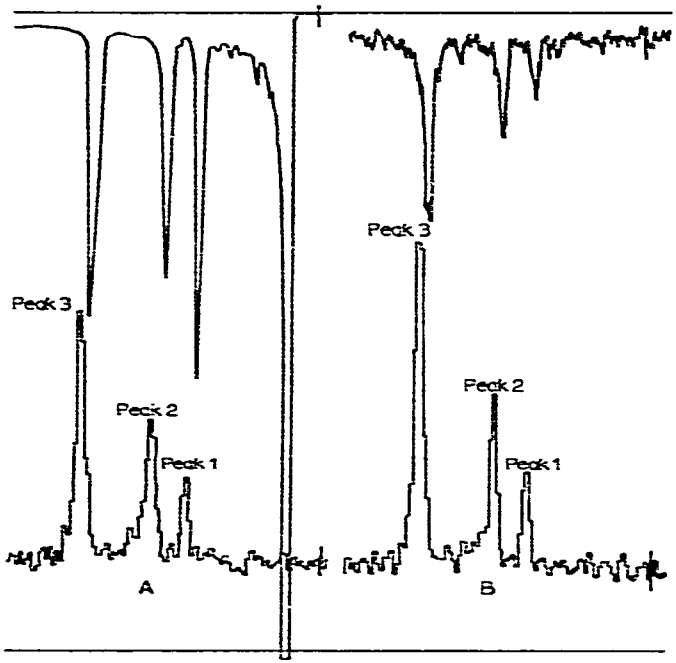

Fig. 4. Example of application to a metabolic study of the synchronized accumulating method. A, Synchronized accumulating radiodetector (lower trace) and flame-ionization detector (upper trace). B, Synchronized accumulating radiodetector (lower trace) and conventional method (upper trace). Sample: [^{14}C]paeonol rat urine metabolites. Peaks: 1 = paeonol acetate; 2 = 2,5-dihydroxy-4-methoxyacetophenone diacetate; 3 = resacetophenone diacetate.

Fig. 4B shows the results of simultaneous measurements of radioactivity in the GC effluent by the present and the conventional methods. These results also confirmed that the present method provided reasonable resolution. The intensities of three distinctive peaks originating from the metabolites were calculated after five repeated measurements of the radioactivity under the same conditions. Table II compares the results of measurements made by the present and the conventional methods. The present method permitted the quantitative analysis of the metabolites with higher precision. Fig. 4A compares the chromatographic patterns of the rat urine sample obtained by the present radio-GC method and using an FID detector. The use of multiple detector tubes in the present method did not result in significant peak broadening.

In conclusion, the applicability of the synchronized accumulating detector to continuous radioactive gas flows from a gas chromatograph has been fully tested and its usefulness confirmed.

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