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SYNCHRONIZED ACCUMULATING RADIOISOTOPE DETECTOR FOR THIN-LAYER RADIOCHROMATOGRAPHIC SCANNING

SHIGEO BABA and YASUJI KASUYA
Tokyo College of Pharmacy, Tokyo (Japan)
and
MASAAKI TAKEDA and NOBORU TOKUNAGA
Aloka Co., Ltd., Tokyo (Japan)
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SUMMARY

A novel method of scanning and measuring the β-activity in thin-layer and paper chromatograms has been developed. The principle involved is to accumulate the signals from multiple detector tubes connected to each other in series in a synchronized way, and a marked improvement in counting efficiency and the resolution has been achieved as compared to the presently available method.

INTRODUCTION

Thin-layer radiochromatogram (RI-TLC) scanners and paper radiochromatogram (RI-PC) scanners have been used extensively in tracer experiments involving soft β-ray-emitting isotopes. In many cases, the amount of the labelled substances recovered and chromatographically separated is very small. Thus, extremely sensitive methods with a high resolution for radiotracer detection are required. The presently available method of radio gas chromatography (RI-GC) does not, however, offer a satisfactory resolution for the tracer quantitation and therefore has limited use. The established procedures of radioisotope detection have been described in books and reviews[1–4]. A review by Prydz[5] discusses the newer types of detectors and some of the possible principles of detectors as yet not fully tested.

When a single counting tube is used in detecting radioactive components after their chromatographic separation, there is the possibility that the signals from the radioactive components lying close to each other become smeared during the activity recording even when they have been satisfactorily separated in the chromatogram. In the conventional method where a single counting tube is used in scanning chromatograms it is therefore theoretically impossible to obtain a high counting efficiency without sacrificing the resolution.

Nowadays, nearly all radiochromatogram scanners use windowless gas-flow GM tubes. For detection on paper chromatograms it is possible to use two detectors,

one at each side of the chromatogram, and to add their signals to get an increased sensitivity (4π-detection). This type of paper-strip scanner is commercially available. For thin-layer preparations a scanner equipped with a single windowless gas-flow GM tube, covering a maximum solid angle of 2π, is often used. However, the possibility of a simultaneous detection with many detectors placed in an array or a matrix to cover the chromatogram area has been suggested[5] and such devices are available commercially. On the other hand, the use of a series of detectors, each of which is equipped with a complete set of electronics for recording the signal, has the drawback that the minimum total width of a windowless gas-flow counter is 10 mm and therefore the windows would either be too wide, thus sacrificing the resolution, or only narrow, thus leaving broad chromatogram zones between the slits uncounted.

An alternative technique is to use a series of counting tubes connected to each other and to add their signals to get an increased sensitivity with an improved resolution. The present paper describes such a synchronized accumulating detector[6] for scanning and measuring the β-activity in thin-layer and paper chromatograms. The detector comprises a particular combination of detector tubes, accumulating counters and synchronizing switches.

WORKING PRINCIPLE OF A SYNCHRONIZED ACCUMULATING DETECTOR

In detecting radioactivity of a radioactive substance flowing through or alongside a radioisotope detector, the detector assembly which has hitherto been in common use has comprised a single detector tube. This type of detector has its inherent drawbacks and it does not give an efficient counting result. This is mainly because the use of a single detector tube does not permit a simultaneous increase in both the detecting efficiency for the radioactivity and the resolving power of, for example, two components separated by gas chromatography. This is illustrated in Fig. 1.

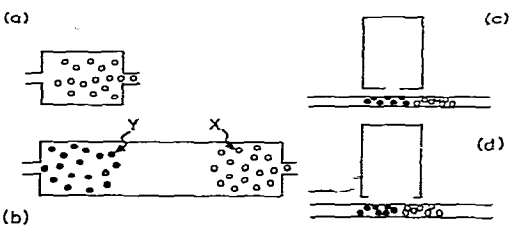


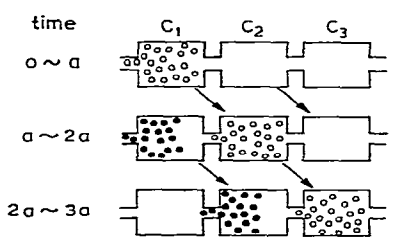
Fig. 1. Inherent drawback of the single counting-tube method. Left, radio gas chromatography; right, radio paper or thin-layer chromatography.

Fig. 1a indicates that, although a detector tube having a relatively small inner volume provides a high resolving power, when a radioactive component X travels through the tube, the detection efficiency must be less than when a tube of larger volume is used since the time of the component X within the detector tube is correspondingly shorter. However, an increase in the volume causes a problem in detecting the radioactivity of another component Y, when both X and Y are in the detector tube at the same time, as is shown in Fig. 1b. Fig. 1c indicates that a narrow detector tube

provides a high resolving power but the detection efficiency must be less than when a wider detector tube (Fig. 1d) is used. However, an increase in the window width results in insufficient resolving power for each component. These facts indicate the fundamental difficulty in increasing both the detecting efficiency and the resolving power simultaneously when only one detector tube is used.

It is possible to overcome or reduce the above drawbacks when a series of detector tubes are arranged longitudinally in series, either adjacent to each other or at uniform fixed distances apart. When open tubes are used, to examine gaseous materials, the tubes are connected end to end so that the gas may flow from one to the other. As the radioactive substances travel through or alongside the detector tubes, the radioactivity present in a certain portion is detected individually by each tube in turn and is accumulated in one of the counters by commutating switches which direct pulses from the tubes to the respective accumulating counters. The switches can be synchronized with the flow-rate of the radioactive materials.

Figs. 2 and 3 show a synchronized-accumulating radiodetector using three open detector tubes. Fig. 2 illustrates the passage of two gaseous radioactive components through the detector tubes C_1–C_3 in times $t = 0$–a, a–$2a$ and $2a$–$3a$, where a is equal to the volume of the counting tube divided by the flow-rate of the carrier gas. The radioactivity signals detected by tubes C_1, C_2 and C_3 during the above time intervals are accumulated in the counting accumulator A_1 shown in Fig. 3. The accumulated count is then passed to an appropriate auxiliary scaler. The same procedure is followed for the next radioactive component entering the detector tubes, the counts being accumulated in the accumulator A_2, and so on with subsequent radioactive components. C_1, C_2 and C_3 have equal inner volumes and are connected in series at certain distances. B_1, B_2 and B_3 are commutating switches, each having one output terminal and three input terminals 1–3. The connection between the output terminal and one of the input terminals is controlled electronically according to a time interval based on the flow-rate of the radioactive substance. Thus, when switching devices B_1, B_2 and B_3 are connected to C_1, C_2 and C_3 during a time interval 0–a, as shown in Fig. 3, each switching device is continually switched to the next input terminal as the time intervals 0–a, a–$2a$ and $2a$–$3a$ elapse.



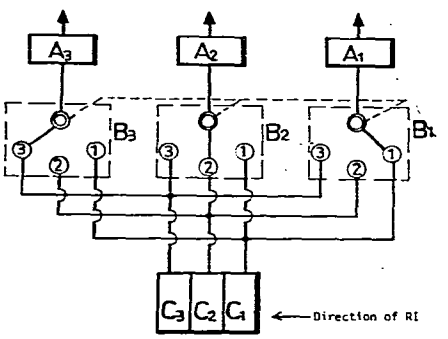


Fig. 2. Principle of a synchronized accumulating radioisotope detector. C = Detector tube.

Fig. 3. Block diagram of a synchronized accumulating radioisotope detector. C = Detector tube; B = switching unit; A = accumulating counter.

This switching mechanism works in such a manner that the radioactivity present in the same portion is detected by each detector tube and the counting information from each detector tube is accumulated in the same accumulating counter. The counter is reset after a full cycle of the terminals 1–3.

The accumulating counter A_3 is connected to C_3 through terminal 3 of the switch B_3 during the interval 0–a and the resulting count is immediately transferred to the auxiliary scaler at $t = a$, followed by resetting of B_3. In the subsequent time intervals a–$2a$ and $2a$–$3a$, A_3 is connected to C_1 and C_2 through terminals 1 and 2 of B_3, respectively, and A_3 accumulates the radioactivity counts in the second portion of the radioactive flow.

The intermittent movement of the contact arms in each switching mechanism is synchronized with the switching signal, so that throughout each counting period (duration a) the arms are stationary, each connecting different tubes to different counters as described above. Therefore, there should be no difference in radioactivity detection efficiency or in the resolving power of a radioactive component through each switching system.

One of the advantages of the present method is that it is not necessary to ensure uniformity in the performance, background count and counting efficiency of all the detector tubes, since all portions of the radioactive gas are treated equally by all the detector tubes.

The synchronized accumulating detector has now been applied to RI-TLC scanning. The technique involves the introduction of a new term, the sampling time S (expressed in sec), which is automatically determined from the equation

$$S = \frac{w/n}{b/60} \tag{1}$$

where w is the width of the detector tube expressed in mm, b is the scanning rate in mm/min and n is an integer. The purpose of the term n is to divide the width of each tube into subfractions to obtain a better resolving performance in counting.

Fig. 4 shows a synchronized accumulating radioisotope detector consisting of five detector tubes each of 10 mm width and having a thin mica slit (0.95 mg/cm^2) of 2×20 mm. n is set at 8. The fifth detector tube is used not only as a part of the synchronized accumulating radiodetector but also as an independent conventional detector having a single counting tube.

EXPERIMENTAL

Operating conditions

Unless otherwise stated, the scanning rate was 12.5 mm/min. Measurements were performed in a full scale of 1000 cpm at a time constant of 10 sec for the conventional method, and in a full scale of 300 counts per sampling time for the present method. The peak intensity of the radioactivity on the scannogram obtained by both methods was quantitated by area measurements.

Reagents

All reagents used in the experiments were purchased from Wako (Tokyo,

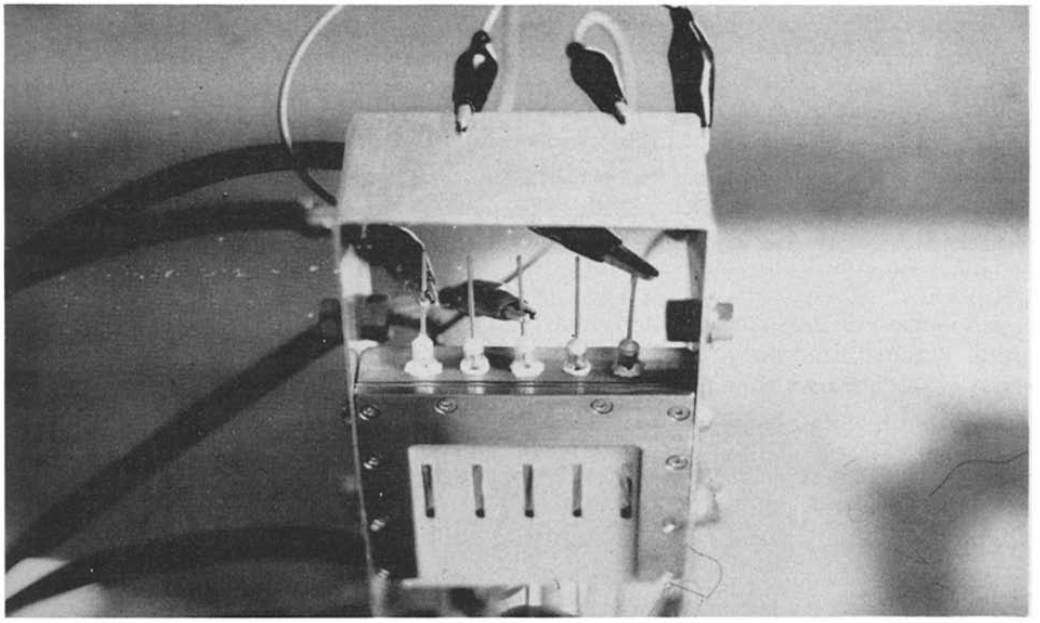


Fig. 4. Counting unit of the synchronized accumulating radioisotope detector for a radiochromatographic scanner.

Japan) and were of reagent grade. [Methyl-^{14}C]caffeine and 1-butyryl-4-cinnamyl[γ^{14}C] piperazine were synthesized in this laboratory. Phenyl[α-^{14}C]alanine was purchased from Daiichi Kagaku Yakuhin (Tokyo, Japan).

Comparison of scannograms obtained by the conventional method and the present method

Volumes of 2, 4, 6 and 8 μl of a chloroform solution of [methyl-^{14}C]caffeine (*ca.* 3000 dpm/μl) were spotted on a TLC plate at equal intervals. On the same plate, 6 and 8 μl of the above solution were spotted close to each other. After drying, the plate was scanned under the conditions described above.

Linearity and reproducibility

For the linearity experiment, an ethanol solution of phenyl[α-^{14}C]alanine (560 dpm/μl) was spotted on a TLC plate using volumes of 5, 10, 20 and 27 μl at *ca.* 3-cm intervals. Ten microlitres of the ethanol solution was spotted on a TLC plate for the reproducibility experiment. The plate was dried and scanned five times for the linearity, and eight times for the reproducibility, determination. The intensity of each radioactive peak was then calculated.

Measurements of low activity

Rats were intravenously injected with 1 μCi of phenyl[α-^{14}C]alanine. The blood was drawn 5 min later and deproteinized. The deproteinized blood was spotted on a cellulose TLC plate. The plate was then scanned at rates of 12.5 mm/min (sampling time S = 6 sec), 5.0 mm/min (S = 15 sec) and 2.5 mm/min (S = 30 sec).

RI-TLC in the determination of metabolites

A guinea pig was subcutaneously given 20 mg/kg of 1-butyryl-4-cinnamyl-[γ-^{14}C]piperazine (1.86 μCi/mg), and an aliquot of urine obtained from the animal 24 h later was treated with Amberlite XAD-2. The effluent (*ca.* 1.5×10^5 dpm) was spotted on a TLC plate and the plate was developed with benzene–acetone–methanol–28% ammonium hydroxide (100:24:12:2). Measurements were performed five times under the same conditions.

RESULTS AND DISCUSSION

A series of experiments demonstrated that the present method permits the accurate synchronized accumulation of the signals from five detector tubes connected in series. Fig. 5 shows that the radioactivity peaks measured by the present method were not broadened as in the conventional method. Moreover, two closely lying radioactive peaks were separated more clearly by the present method, *i.e.*, the resolution has been improved. There was a strict linearity between the signal intensity and the radioactivity in the measured spot as shown in Fig. 6. The results presented in Table I demonstrate the very good reproducibility in counting.

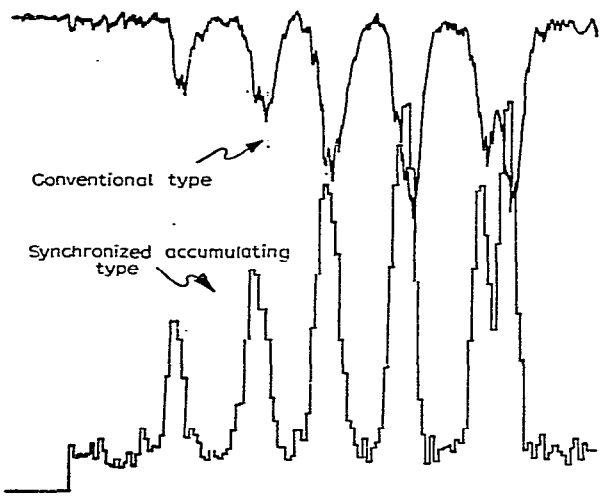


Fig. 5. Comparison of the scannograms obtained by the synchronized accumulating method (lower) and the conventional method (upper).

A great advantage of the present method is its usefulness for detecting the low radioactivity distribution on thin-layer or paper chromatograms. The conventional method, in which a rate meter is used to regulate the scanning speed, does not allow any improvement in counting efficiency upon decreasing the scanning speed. In the present method, however, reduction of the scanning speed b results in an increase in the sampling time S according to eqn. (1). This increase in the sampling time results in an increase in the total counts, leading to an improvement in the accuracy in counting. The effect of the scanning rate on the rate of movement of the various peaks in the RI–TLC sample was examined and the results are shown in Fig. 7. When the radio-

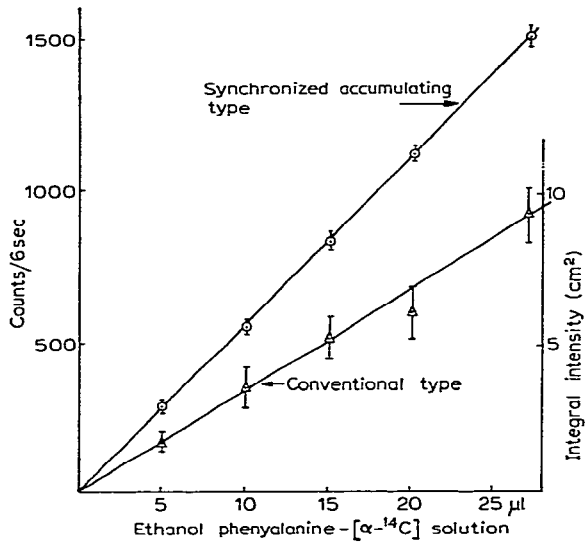


Fig. 6. Comparison of linearity between the signal intensity and the radioactivity.

TABLE I

COMPARISON OF REPRODUCIBILITY IN COUNTING

Conventional type (cm^2)	*Synchronized accumulating type (counts per 6 sec)*
3.51	545
2.89	556
3.59	546
2.69	574
4.24	549
3.58	546
3.24	570
5.03	540
Mean 3.47	553.3
S.D. ±0.49	± 11.6

activity in the TLC plate was scanned and measured at three different scanning rates of 12.5, 5.0 and 2.5 mm/min (corresponding sampling times of $S = 6$, 15 and 30 sec, respectively), there was observed an obvious improvement in the counting efficiency by the present method. Fig. 7 also demonstrates the improved detection of a very weak radioactive peak and that the ratios of peak-to-background counts in a relatively slowly moving peak were much higher in the present method.

After the subcutaneous injection of 1-butyryl-4-cinnamyl[γ-^{14}C]piperazine to a guinea pig, the radioactivity distribution profile in the RI-TLC sample of urine revealed three distinct peaks (Fig. 8). After repeated measurements of the radioactivity (five times under the same conditions), the intensity of the three radioactive peaks on the scannogram was calculated and expressed as a percentage of the total radioactivity. The results are presented in Table II. There is close agreement in the mean

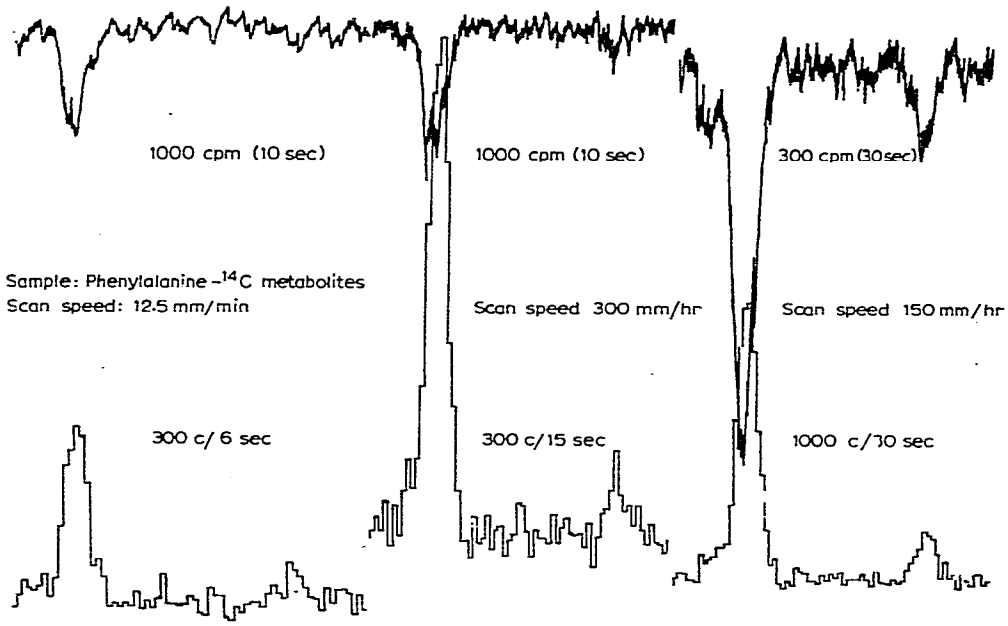


Fig. 7. Effect of the scanning rate on the counting efficiency of the synchronized accumulating method (lower) and the conventional method (upper).

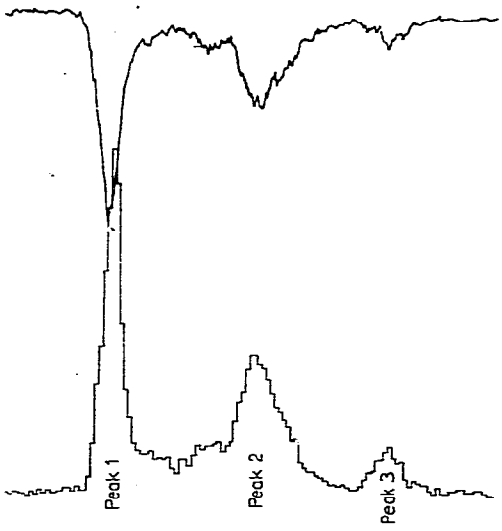


Fig. 8. Example of the application to a metabolic study of the synchronized accumulating method (lower) and the conventional method (upper). Full scale: 1000 cpm for the conventional method and 300 counts per 6 sec for the accumulating method. Sample: 1-butyryl-4-cinnamyl[γ-^{14}C]piperazine; scanning speed: 12.5 mm/min.

values between the present method and the conventional methods. However, there is a relatively large difference in the S.D. values. This indicates that the present method offers a more precise and accurate determination of the radioactive substance on the TLC plate.

TABLE II

EXAMPLE OF THE DETERMINATION OF METABOLITES AND THE ACCURACY IN MEASUREMENTS

Expt. no.	*Synchronized accumulating type*			*Conventional type*		
	Peak 1	*Peak 2*	*Peak 3*	*Peak 1*	*Peak 2*	*Peak 3*
1	40.54	35.27	8.42	38.89	36.25	9.993
2	40.82	35.47	10.23	41.44	34.59	8.84
3	41.27	36.26	8.60	38.69	35.47	11.82
4	40.90	36.52	8.93	41.36	34.49	8.85
5	40.88	36.35	8.97	43.75	37.06	7.08
Mean	40.88	35.97	9.03	40.83	35.57	9.30
S.D.	±0.23	±0.50	±0.63	±1.87	±0.98	±1.55

A tentative comparison of the counting achievements of the present and the conventional methods is given diagramatically in Fig. 9, where a slit width of 2 mm and a scanning rate of 12.5 mm/min are used. In the conventional method, empirical determination of a time constant depends on the scanning rate and the intensity of the radioactivity in the sample. For the radioactivity levels in our samples, a time constant of *ca.* 10 sec was generally employed for the measurements. Fig. 9A shows how the radioactivity of a particular portion of the TLC plate scanned during the past 10 sec (indicated by +6 to 0) affects the radioactivity counting (expressed as count effect %) of the next interval (0 to −2). The shaded area in Fig. 9A indicates a count effect of 100% but only instantaneous counting is made. In Fig. 9B, the sampling time S is calculated to be 6 sec from eqn. (1) in which $b = 12.5$ mm/min and $n = 8$. The dark area in Fig. 9B represents a portion of the TLC plate where the radioactivity counting is not instantaneous but is made during 6 sec, and the shaded area represents a portion where counting is made partially during the sampling time of 6 sec. The signals detected at each sampling time are recorded independently and not affected by the previous counting. In this way it is possible to perform the measurements with a better resolution. However, it is not practical to apply this technique to the radioactivity measurements of the samples used in the present experiments because of the low counting efficiency and the large statistical variation when only one detector tube is used as in the case of the conventional method. On the other hand, the use of multiple detector

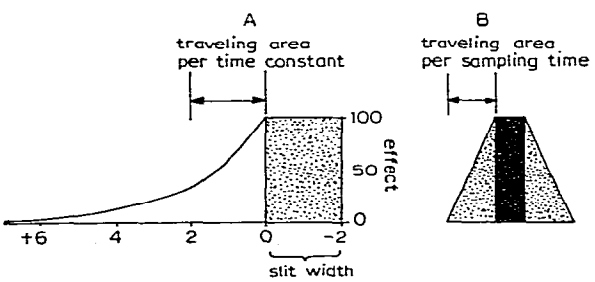


Fig. 9. Comparison of the counting conditions of the synchronized accumulating method (right) and the conventional method (left).

tubes in our method permits the application of the above technique and thus offers a great improvement in the resolution and the counting efficiency with higher precision.

We attempted to apply the new radiodetector to RI–GC. The use of this detector in RI–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 placed in series. Fig. 10 shows the result of simultaneous measurements of radioactivity from the GC effluent by operating the first and fifth detector tubes of a synchronized accumulating radiodetector consisting of five tubes. It can be seen that the use of multiple detector tubes does not result in appreciable peak broadening.

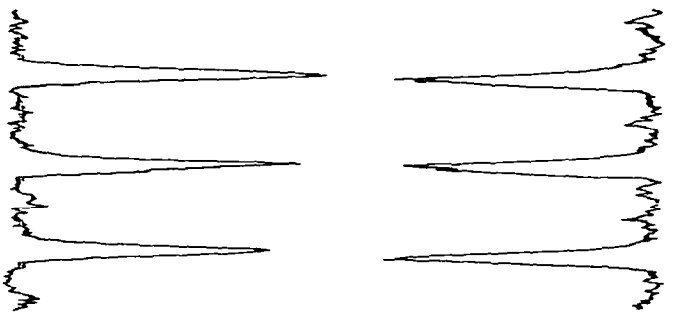


Fig. 10. RI–GC scannograms using the first (right) and fifth (left) detector tubes of a synchronized accumulating radioisotope detector. Sample [^{14}C] hexadecane (1 μl); 1800 dpm. Full scale: 1000 cpm per 10 sec.

Arrays of stationary detectors aligned along a liquid chromatography (LC) column have been suggested for obtaining integrated values for all counts corresponding to the passage of a particular portion of the LC effluent[7]. The RI–GC data mentioned above raise another possibility of the application of the present method for the continuous monitoring of a column effluent in liquid column chromatography.

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