富山大学トリチウム科学センター研究報告 5 : 73—78, 1985.

ノート

二重標識 ^{3}H 及び ^{14}C 化合物の放射能測定

朝野武美・桐谷玲子・川西徹朗*

大阪府立放射線中央研究所
大阪府堺市新家町
*近畿大学農学部
太阪府東大阪市小若江

Measurement of Radioactivity of Doubly Labelled $^{3}H-$ and $^{14}C-$Compound

Takeyoshi ASANO, Reiko KIRITANI and Tetsuro KAWANISHI*

Radiation Center of Osaka Prefecture,
Shinke-cho, Sakai, Osaka, Japan.
*Kinki University, Department of Agriculture,
Kowakae, Higashiosaka, Osaka, Japan.
(Received December 25, 1985)

Abstract

The radioactivity of spot of doubly labelled $^{3}H-$ and $^{14}C-$ compound on a thin layer chromatograph plate was measured with a radiochromatogram spark chamber. The results were compared with those obtained from a radio thin layer chromatograph and an autoradiograph. For the radiochromatogram spark chamber, the lower limits on detectable intensity of $^{3}H-$ and $^{14}C-$radioactivities were 1000 dpm and 100 dpm, respectively. Accordingly, it was found that more than 10^{5} dpm of radioactivity was required for the detection of the doubly labelled compound, which has a $^{14}C/^{3}H$ radiactivity ratio of 1/490 on this instrument. Further, it was revealed that the amount of radioactivity of tritium and carbon-14 could be estimated from the brightness of

spark images.

1 Introduction

In a study by use of a doubly labelled $^{3}H-$ and $^{14}C-$ compound, it is sometimes necessary to measure the radioactivities of ^{3}H and ^{14}C, separately. The radioactivity of the doubly labelled compeund is generally measured by a liquid scintillation counter[1]. Petersen investigated the possibility of using a multistep avalanche chamber as an image detector of radio thin layer chromatogram[2]. We have studied a technique to detect the sum of radioactivities of $^{3}H-$ and ^{14}C and the radioactivity of ^{14}C alone in doubly labelled compound on a thin layer chromatograph (TLC) plate by use of a radiochromatogram spark chamber and compared the results with those obtained from a radio thin layer chromatograph and an autoradiograph.

2 Experimental

2-1. Preparation of TLC plate spotted radioactive materials

As specimens, we used commercially available [5−^{3}H] uridine and [2−^{14}C] cytosine, and [2−^{14}C,5−^{3}H] cytosine prepared in our laboratory[3]. The aqueous solutions of the above compounds were so prepared that the radioactivity in 50 μl of each solution was 10^{5}, 10^{4}, 10^{3} and 10^{2} dpm. They were spotted with 4 cm distance from each other on a TLC plate (silicagel plate, 20×20 cm) as shown in Fig. 1. The diameter of spot was about 5 mm. In the doubly labelled compound, $^{14}C-$radioactivity was 1/490[2] of $^{3}H-$radioactivity : 204 dpm of ^{14}C against 10^{5} dpm of ^{3}H, 20 dpm of ^{14}C against 10^{4} dpm of ^{3}H and so on. The radioactivities of the solutions were measured with a liquid scintillation counter (Packard, Tri-cab 460CD).

2-2. Instrument

(1) *Radiochromatogram Spark Chamber* : Model 450A (Birchover Instruments Ltd, England) was used. The principle of the instrument is as follows. The radiochromatogram spark chamber consists of a set of cathode and anode. The cathode is made of a number of helical coils ; anode wires are placed at the center of herical coils. When a high voltage (1900 V) is applied across the electrodes in a PR gas (90 % argon and 10 % methane), ions produced by $\beta-$rays cause a spark discharge just over the radioactive spot. The visible light emitted from the spark is photographed with a Poraloid camera.

For the detection of $^{14}C-$activity, a given metalized film (Melinex) was placed

between the cathode and the TLC plate, and for the detection of both $^{3}H-$ and $^{14}C-$activity, the film was removed. In the former, the flow rate of PR gas was 0.8 l/min and exposure time was 3 h at aperture of f 11. In the latter, the flow rate was 2.5 l/min and exposure time was 2 h at aperture of f 11. The film of type 667 was used for the Poraloid camera.

(2) *Radio thin layer chromatograph* : JTC 201 (Aloka Co. Ltd, Japan) was used. A windowless flow type counter (counting box : $8\times30\times20$ mm, window : 2×10 mm) was used as a detector. A Q gas (90 % herium and 10 % isobutane) was used as a counting gas. Operating voltage was 1200 V. A distance between the detector and the TLC plate was 1 mm. Scanning speed of the detector was 2.5 cm/min.

(3) *Autoradiograph* : A standard X-ray film, Fuji RX, was used. After being exposed to the radioactive TLC plate for 30 days in the dark, the film was developed.

3 Results and Discussion

(1) *Radiochromatogram spark chamber* : The lower limit on detectable intensity of radioactivity is dependent on exposure time and distance between the helical cathode and the TLC plate, and is varied by covering high radioactive spots with $\beta-$ray absorver. In the case that a number of spots having various intensities of radioactivity were placed on the TLC plate, sparks over high radioactive spots suppressed those over low radioactive spots, because there were a limited number of sparks available per second. This effect could be overcome by increasing the exposure time and covering the high radioactive spots with aluminum foils.

Following results were given by the experiment with no metalized film (Melinex). At the exposure time of 1 h and the distance of 2 mm, and without covering of high radioactive spots, merely the images of $^{14}C-$spots which have radioactivity more than 10^{3} dpm were detected and those of the $^{3}H-$spots were not. At the exposure time of 2 h and the distance of 0.5mm, and without covering, the image of $^{3}H-$spot which have radioactivity of 10^{5} dpm was detected, whereas the image of $^{14}C-$spot of 10^{2} dpm was hardly detected. At the exposure time of 2 h and the distance of 0.5mm, and with covering over the $^{14}C-$spot, (3)-10^{5} dpm with aluminum foil (44 mg/cm^{2}), the images of $^{14}C-$spots which have radioactivity more than 10^{2} dpm and those of $^{3}H-$spots which have radioactivity more than 10^{3} dpm were detected as shown in Fig. 1-A-(1) and -(3), respectively. It can, therefore, be pressumed that the image of spot of (2)-10^{5} dpm, which consist of 10^{5} dpm of ^{3}H and about 200 dpm of ^{14}C, in Fig.1-A is due to the sparks related to $\beta-$ray of ^{3}H and ^{14}C. On the

other hand, the images of spots of (2)-10^4 dpm and (2)-10^3 dpm in Fig. 1-A are pressumed to be due to the sparks related to β-ray of ^3H, because these spots have ^{14}C−radioactivity of less than 20 dpm whose radioactivity was not detected in another experiment under the present conditions.

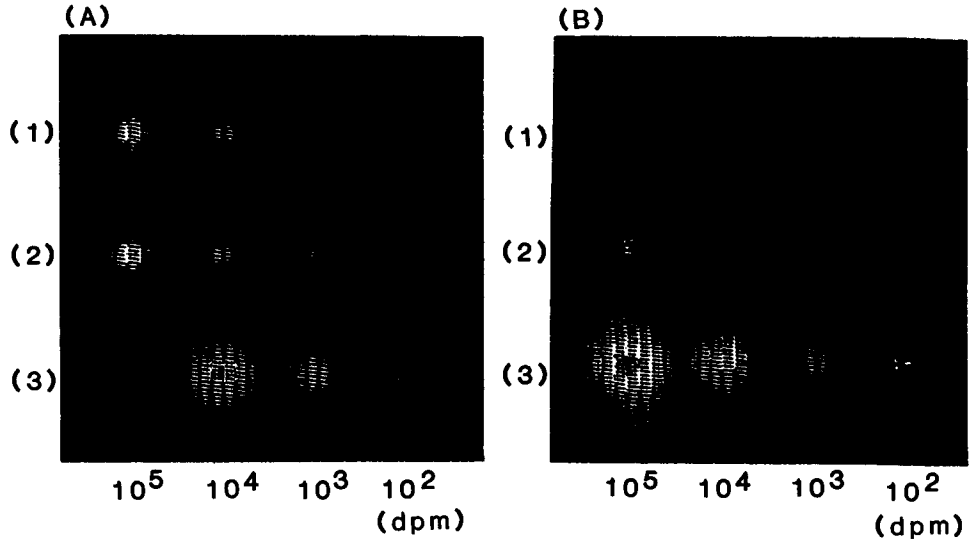

Fig. 1. Spark chamber images photographed with metalized film removed and exposure of 2 h (figure A), and with metalized film placed and exposure of 3 h (figure B).
(1) ^3H−labelled compound (upper row)
(2) doubly labelled ^3H− and ^{14}C−compound (middle row)
(3) ^{14}C−labelled compound (lower row)

With use of the metalized film, and at the exposure time of 3 h and the distance of 2 mm, only images of ^{14}C−spots which have radioactivity more than 10^2 dpm were detected with expectation as shown in Fig. 1-B.

In Figs. 1-A and -B, the brightness of image increased with increase in intensity of radioactivity.

Accordingly, it was revealed that 1) more than 10^5 dpm of radioactivity was required for the detection of the doubly labelled compound and 2) the amount of radioactivity of the doubly labelled compound could be estimated from the brightness of image of sparks.

(2) *Radio thin layer chromatograph*: Fig. 2 shows the scanning patterns of the spots on the TLC plate. From the result of the measurement of the ^3H−labelled compound Fig. 2-(1) and the ^{14}C−labelled compound Fig. 2-(3), the counting efficiency of ^3H and ^{14}C were calculated to be 0.1 % and 10 %, respectively. In Fig. 2-(2), the spot

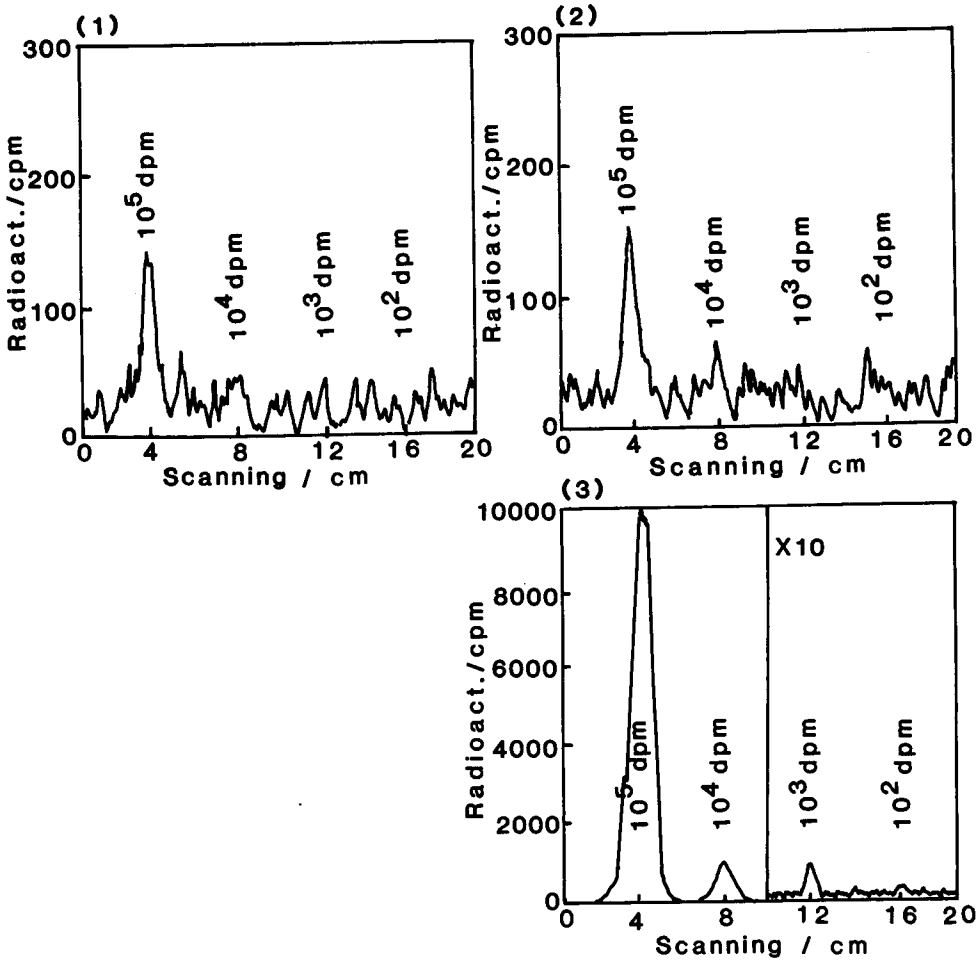

Fig. 2. Radio thin layer chromatograms.
(1) ^{3}H−labelled compound
(2) doubly labelled ^{3}H− and ^{14}C−compound
(3) ^{14}C−labelled compound

of (2)-10^5 dpm (duobly labelled ^{3}H− and ^{14}C−compound) gave a significant counting rate of about 150 cpm. However, it is difficult to recognize the presence of ^{14}C−radioactivity, which was estimated to be about 20 cpm, for this spot in consideration of the statistical errors of the total couting rate and a back ground counting rate (30 cpm).

(3) *Autoradiograph*: Fig. 3 shows an autoradiogram of the spots on the TLC plate. The positional resolution of image of autoradiograph was better than that of radio-chromatogram spark chamber. The sensitivity of autoradiograph was similar to that of

radiochromatogram spark chamber. This method, however, required long exposure time.

Thus, in present study, a technique of radioactivity measurement of doubly labelled $^{3}H-$ and $^{14}C-$compound was established by use of a radiochromatogram spark chamber. It was proved that radiochromatogram spark chamber was an useful tool for detection of radioactivity of the doubly labelled compound on a TCL plate, in comparison with the radio thin layer chromatograph and the autoradiograph.

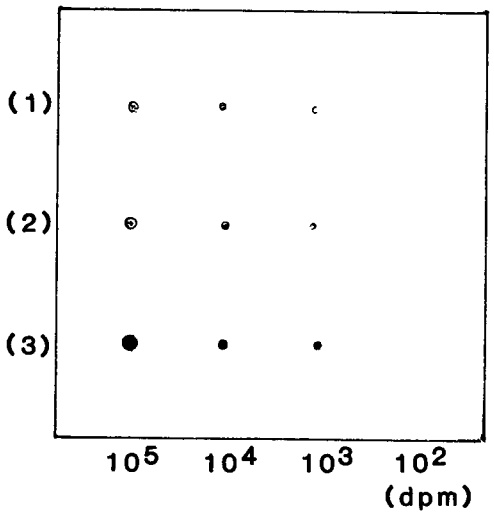

Fig. 3. Images of autoradiogram.
(1) $^{3}H-$labelled compound (upper row)
(2) doubly labelled $^{3}H-$ and $^{14}C-$compound (middle row)
(3) $^{14}C-$labelled compound (lower row)

References

1) Y. Kobayashi and D. V. Maudsley, "Biological Applications of Liquid Scintillation Counting" (Academic Press, 1974), p.22.
2) G. Petersen, G. Charpak, G. Melchart and F. Sauli, Nuc. Instr. and Meth., **176** (1980) 239.
3) T. Asano, R. Kiritani and S. Fujita, This journal, **4** (1984) 23.