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SYNCHRONIZED ACCUMULATING RADIOISOTOPE DETECTOR FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A synchronized accumulating radioisotope detector using five counting cells was applied for high-performance liquid chromatography. With the homogeneous counting method, the detection limits were about 45 ɔCi for carbon-14 and about 270 pCi for tritium. This detector system gives better quantitative accuracy than the conventional method using a single counting cell.

INTRODUCTION

In radioisotope tracer experiments, thin-layer chromatography (TLC) and paper chromatography have generally been used for the separation of radioactive compounds. However, there have been few applications of gas chromatography (GC) and high-performance liquid chromatography (HPLC) to such experiments, although these two techniques often exhibit higher efficiencies of separation. One reason may be that with a conventional radioisotope detector using a single counting cell the counting efficiency may not be improved without sacrificing the efficiency of separation.

To overcome this drawback, we have already devised a synchronized accumulating radioisotope detector (SARD), which was successfully used for TLC[1] and GC[2]. The present paper describes the application of this detector to HPLC.

EXPERIMENTAL

Instrument

A Shimadzu LC-2 high-performance liquid chromatograph equipped with a Shimadzu UVD-2 UV detector was used. A block diagram of the radioisotope HPLC system is shown in Fig. 1. SARD was composed of the following units: five counting cells; five pairs of photoelectron multiplier tubes; synchroaccumulator; scaler and two-pen recorder.

Labelled compounds

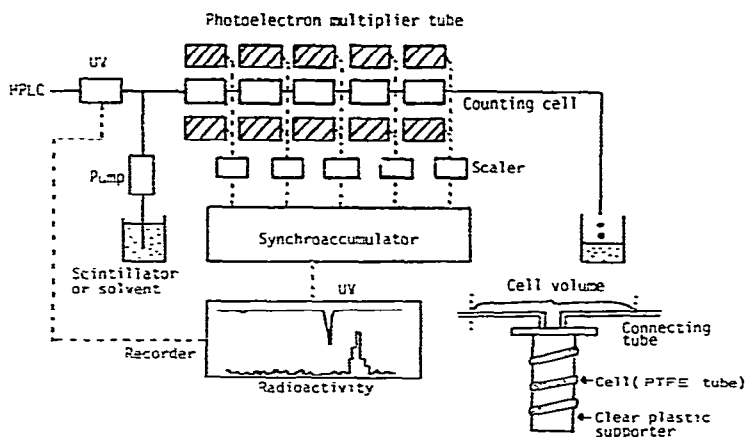[8-^{14}C]Adenine (394 μCi/mg) and [8-^{3}H]adenine (193 mCi/mg) were purchased

Fig. 1. A block diagram of the synchronized accumulating radioisotope detector and of the homogeneous counting cell.

from Radiochemical Centre (Amersham, Great Britain). These compounds were diluted in non-labelled adenine to give aqueous solutions of 450 pCi/100 ng · μl and 45 pCi/10 ng · μl for the ^{14}C-labelled compound, and 720 pCi/10 ng · μl and 72 pCi/ng · μl for the ^{3}H-labelled one.

Operating conditions

The column was Nucleosil 5 C_{18} (0.46 × 15 cm), with a mobile phase of 0.01 M HClO$_4$–methanol (10:1); flow-rate 0.5 ml/min. In the homogeneous counting method, the eluate from HPLC was mixed with a liquid scintillator (7.5 ml/min) and the resulting solution (8.0 ml/min) was passed through the five counting cells having effective cell volume of 1.1 ml per cell. The sampling time was 8 sec under these conditions. In the heterogeneous counting method using a lithium-glass scintillator, the eluate was mixed with twice its volume of the solvent having an identical composition to that of the mobile phase and the resulting solution (1.5 ml/min) was led to the five counting cells having effective cell volume of 0.14 ml (lithium-glass volume: 0.08 ml). The sampling time was 12 sec for this method.

Counting efficiency

Known amounts of [8-^{14}C]adenine or [8-^{3}H]adenine were injected into the chromatograph and the total counts under the peak were calculated manually. The counting efficiency of this detector was estimated by comparing the total counts with the injected radioactivity.

Reproducibility

Sample solutions containing 450 pCi of [8-^{14}C]adenine for the homogeneous counting method or 4500 pCi of [8-^{14}C]adenine for the heterogeneous counting method were injected ten times into the chromatograph.

Linearity

A fixed radioactivity from 90 pCi to 450 pCi of [8-^{14}C]adenine was injected into the chromatograph. The peak intensities were measured by weighing the area on the

chromatogram in the conventional method or by totalling the counts per sampling time of the peak in the SARD method.

Detection limit

A known amount of [8-^{14}C]adenine (from 22.5 to 360 pCi) or [8-^{3}H]adenine (from 36 to 576 pCi) was injected.

Resolution

[8-^{14}C]Adenine (450 pCi/μl) was reinjected at 2.0. 1.5, 1.0 or 0.5 min after the first injection of the same sample solution.

RESULTS AND DISCUSSION

In GC analysis, some compounds lacking in volatility cannot be detected because these compounds are adsorbed on the column. On the other hand, essentially all compounds can analyzed by HPLC, if these compounds dissolve in an available solvent. This technique has been applied in many fields.

The measurement of the radioactivity in HPLC eluates has generally been performed by counting the radioactivity in each fraction of the eluate with a liquid scintillation counter, namely the off-line batch counting method. However, a continuous counting method has been available for 10 years[3,4] and has also been reviewed[5]. This method is classified into two types. One is homogeneous counting in which the eluate is mixed with a liquid scintillator and the resulting solution is continuously passed through the counting cell set between a pair of photoelectron multiplier tubes. The other is heterogeneous counting in which the eluate is passed through a cell containing a solid scintillator. However, in both conventional methods using a single counting cell, it is impossible to improve the counting efficiency without sacrificing the resolution.
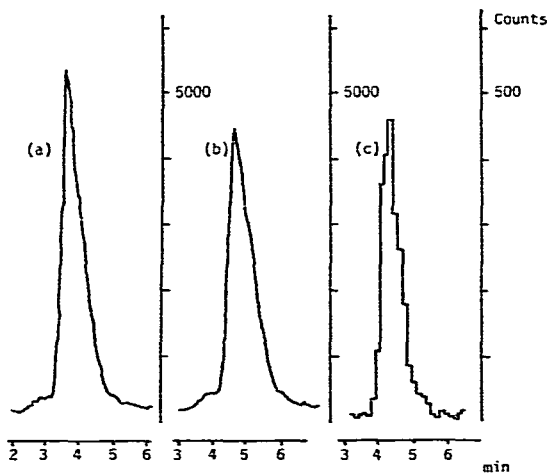

Fig. 2. Comparison of radioisotope HPLC obtained (a) from the first counting cell (time constant, 30 sec), (b) from the fifth counting cell (time constant, 30 sec) and (c) by synchronizing signals from the first to the fifth counting cells (sampling time, 8 sec).
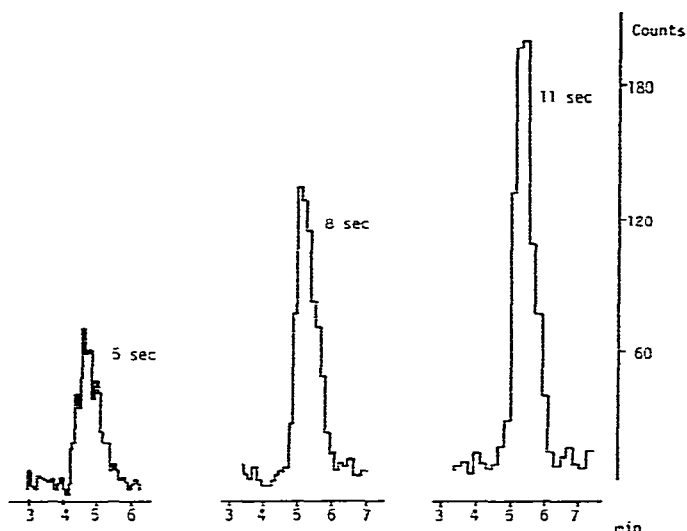
Fig. 3. Comparison of SARD chromatograms obtained with several sampling times. Sample: [8-
^{14}C]adenine 450 pCi.

When SARD was used as a detector for GC, no peak broadening was observed in comparison with the conventional method[2]. Since a liquid shows a greater back diffusion than a gas, it was feared that HPLC analysis might result in greater peak broadening than GC analysis, when the mobile phase was passed through several counting cells. Thus, this was examined by comparing the chromatograms obtained by using the first and the fifth counting cells as a detector (Fig. 2). A large difference in shape between the two chromatograms was not observed. When the signals from the first to the fifth counting cells were synchronized, no peak broadening was observed. From these results, it is apparent that the SARD system can be used for the HPLC analysis.
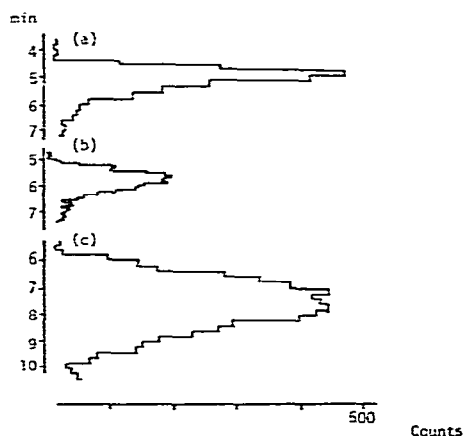

Fig. 4. Comparison in peak shapes in the heterogeneous counting method. (a) Total flow-rate, 1.5 ml/min; sampling time, 12 sec. (b) Total flow-rate, 1.5 ml/min; sampling time, 6 sec. (c) Total flow-rate, 0.5 ml/min; sampling time, 12 sec. Sample: [8-^{14}C]adenine, 4500 pCi.

In the homogeneous counting method, the sampling time was calculated from the equation:

$$\text{Sampling time (sec)} = \frac{\text{effective cell volume (1.1 ml)}}{\text{flow-rate through the cells (8.0 ml/min)}} \approx 8 \text{ sec}$$

When a shorter sampling time (*e.g.*, 5 sec) was employed, the signals from each cell did not synchronize and the chromatographic peak became lower and wider. The employment of a longer sampling time (*e.g.*, 11 sec), however, resulted in a slightly wider peak (Fig. 3). In the heterogeneous counting method, an unexpected peak broadening occurred when the total flow-rate was 0.5 ml/min (Fig. 4c). An increase of the total flow-rate to 1.5 ml/min made the peak more sharp. The signals from each cell, however, did not synchronize when the sampling time (6 sec) employed was calculated from an equation analogous to that mentioned above (Fig. 4b). These phenomena may result from the large extent of back diffusion of the eluate due to the solid scintillator. In this case, an appropriate sampling time was selected by changing flow-rates and sampling times until a good shape peak was obtained. The appropriate sampling time and the total flow-rate were 12 sec and 1.5 ml/min, respectively (Fig. 4a).

The counting efficiency of this detector was estimated as follows. An accurate disintegration count in an [8-^{14}C]adenine or [8-^{3}H]adenine sample solution was made by a liquid scintillation counting method. The average total counts under the peak were 6182 counts per 8 sec when 4390 pCi of [8-^{14}C]adenine were injected. The total flow-rate of eluate and scintillator was 8 ml/min and the effective volume of a counting cell was 1.1 ml. The residence time of the HPLC eluate in the detector components was thus calculated to be 0.667 min. From these data, the counting efficiency for ^{14}C was determined to be 95.1%. In a similar manner, the counting efficiency for ^{3}H was calculated to be 34.6%. In the heterogeneous counting method, the counting efficiencies were 32.3% for ^{14}C and less than 0.1% for ^{3}H, respectively.

The reproducibility of this counting method and the conventional one are shown in Table I. It is apparent that the present detector system has a better reproducibility than the conventional one, the coefficient of variation of the former (3.7%) being smaller than that of the latter (8.6%).

By comparing the linearity of the calibration curve of injected amount *vs.* count for the two methods, the quantitative accuracy was determined. The coefficients of correlation were 1.00 for the SARD method and 0.98 for the conventional one (Fig. 5), demonstrating that the present method gives better quantitative accuracy.

There are several factors which affect the detection limit of radioisotope HPLC. The cell volume and the total flow-rate passing through the cell are most important. Since a higher detection limit is attained by use of a longer counting time, it is possible to use a larger cell volume and a lower flow-rate. Lowering of the resolution power, however, should always be accompanied by use of the method mentioned above. The signal-to-noise ratio may also affect the detection limit. A typical example of the detection limit of this detector is shown in Fig. 6. In the homogeneous counting method, the detection limits were about 45 pCi for ^{14}C and about 270 pCi for ^{3}H using a cell volume of 1.1 ml and total flow-rate 8 ml/min. For 90 pCi of ^{14}C, there was no large difference in a signal-to-noise ratio between the

TABLE I

REPRODUCIBILITY

S.D. = Standard deviation; C.V. = coefficient of variation.

	Homogeneous*		Heterogeneous**, SARD (counts/12 sec)	UV (nm)
	SARD (counts/8 sec)	Conventional (mg)		
1	673	77.8	1287	132.9
2	621	79.3	1282	133.3
3	650	65.4	1218	134.6
4	660	66.5	1188	137.4
5	685	78.4	1215	137.0
6	662	84.6	1168	137.2
7	661	76.7	1259	132.3
8	692	87.4	1227	136.9
9	668	76.0	1276	128.0
10	719	72.0	1245	134.3
Mean ± S.D.	669 ± 25	76.4 ± 6.6	1237 ± 38	134.4 ± 2.8
C.V. (%)	3.7	8.6	3.1	2.1

  * Sample: [8-^{14}C]adenine, 450 pCi.
 ** Sample: [8-^{14}C]adenine, 4500 pCi.

SARD (1.52) and the conventional method (1.59). Schutte[+] reported that the detection limits were 2 nCi for ^{14}C and 5 nCi for ^{3}H using a homogeneous counting cell volume of 1.4 ml. In the heterogeneous counting method, several solid scintillators such as cerium-activated lithium glass, europium-activated calcium fluoride and organic scintillator were used. There were large differences in the detection limits among the reported data. For example, Schutte[+] reported that the detection limits were 10 nCi for ^{14}C and 1 μCi for ^{3}H by using a U-shaped heterogeneous detector with
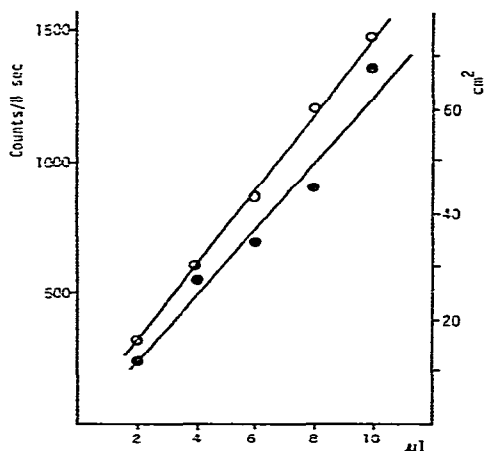

Fig. 5. Linearity of calibration curves. Data obtained by a conventional method (right axis) (●), and with SARD (left axis) (O). Sample: [8-^{14}C]adenine, 45 pCi/μl.
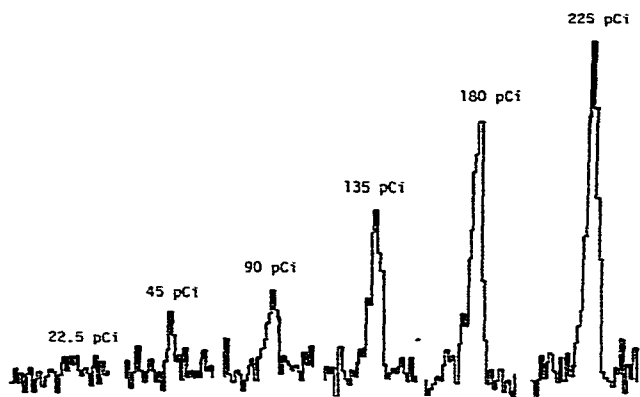
Fig. 6. Example of detection limit. Data obtained by using a counting cell volume of 1.1 ml and total flow-rate of 8.0 ml/min. Sample: [8-^{14}C]adenine.

volumes of 160–500 μl filled with cerium-activated lithium glass beads. On the other hand, using 1,4-bis(5-phenyloxazolyl-2)benzene (POPOP). Sieswerda and Polk[3] achieved detection limits of 0.03 nCi for ^{14}C and 0.07 nCi for ^{3}H at a signal-to-noise ratio of 1:1. In our heterogeneous counting method, the detection limit was about 270 pCi for ^{14}C.

Finally, an investigation on the resolution power was performed. In both the homogeneous counting method and the heterogeneous one, two peaks appeared on the chromatograms, when the radioactive samples were injected at 1-min intervals. However, the resolution of the former method was superior to that of the latter one, because the two peaks were clearly separated in the former method. An example of the application of the homogeneous counting method is shown in Fig. 7. Two peaks appeared on the chromatogram after injection of samples at 1-min intervals, but samples injected at 0.5-min intervals showed a single peak. Under these conditions, the separation of peaks was attained at 0.75-min injection intervals.
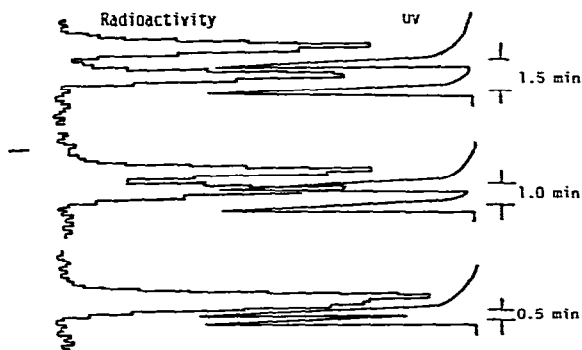

Fig. 7. Resolution. Data obtained by using a cell volume of 1.1 ml and total flow-rate of 8.0 ml/min. Sample: [8-^{14}C]adenine, 900 pCi.

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