Journal of Chromatography, 108 (1975) 37–41

CHROM. 8118

RADIO GAS CHROMATOGRAPHY OF CARBON-14-LABELLED COMPOUNDS USING CONTINUOUS ACTIVITY ASSAY BY A SEMICONDUCTOR DETECTOR

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(Received December 2nd, 1974)

SUMMARY

A method and apparatus are described for the direct measurement of ^{14}C activity in fractions leaving a gas chromatograph by means of a silicon-diffused detector; the simple device can readily be attached to a conventional chromatograph. The method was tested on substances boiling between 31.5° and 205.8°. With use of a preparative gas chromatograph, with thermal-conductivity mass detection, the limit of detection of the device described was 0.15 μCi.

INTRODUCTION

Semiconductor detectors have been successfully used in non-destructive activity assays of substances labelled with beta-emitters (^{3}H, ^{14}C, ^{35}S and ^{32}P) after their separation by gel electrophoresis[1], paper chromatography[2] or thin-layer chromatography[3]. Particular advantages of such detectors include long-term stability of working conditions, low background, low working voltage and high energy resolution, which makes possible the simultaneous determination of several radionuclides (*e.g.*, ^{14}C and ^{35}S; for details see refs. 3 and 4). Detectors prepared by a special technological process can be operated in an ordinary laboratory atmosphere and make possible the detection of ^{3}H at room temperature[3,5].

Methods for radioactivity assay in gas–liquid chromatography (GLC) have been recently reviewed by Simon[6]; semiconductor detectors have hitherto not been used for this purpose. In view of the simple and rapid technique, continuous assay of the activity of fractions leaving the column is generally preferred to the discontinuous collection of fractions and subsequent determination of their activity[7–10], although discontinuous methods are particularly suitable for measuring lower activities. In the continuous process (regardless of the assay method used), the column effluent may be transferred to the detection volume either directly[11,12] or after conversion of the fractions[11,13] into a gas mixture by oxidation or cracking. The direct measurement of fractions is simpler and the substances to be separated remain intact, but there are often various complications[6,13] due to heating of the detection volume.

In the present work, we have established optimum working conditions for the direct assay of ^{14}C activity by means of a semiconductor detector at the exit of the GLC column. Because, with surface-barrier silicon detectors[3-5], an increase in temperature above 40° results in considerably increased noise and consequently decreased efficiency of detection[14], we used a diffused silicon detector; such a detector can be operated at a higher temperature without affecting the efficiency of detection or other experimental conditions.

EXPERIMENTAL

Materials

The following analytical-grade chemicals (Lachema, Brno, Czechoslovakia) were used: sodium formate, sodium acetate, propionic acid, *n*-butyric acid, pentanoic acid, hexanoic acid, heptanoic acid, and octanoic acid. Dimethyl sulphate was distilled under reduced pressure, technical thionyl chloride was purified according to Rigby[15], and absolute methanol and absolute ethanol were used. Sodium [^{14}C]formate (44 mCi per mmole) and sodium [1-^{14}C]acetate (44 mCi per mmole) were supplied by the Institute for Research, Production and Utilisation of Radioisotopes, Prague Czechoslovakia. The following salts of homologous aliphatic [1-^{14}C]acids were supplied by the Radiochemical Centre (Amersham, Great Britain): sodium propionate (35 mCi per mmole), sodium *n*-butyrate (32 mCi per mmole), sodium pentanoate (16 mCi per mmole), sodium hexanoate (22 mCi per mmole), sodium heptanoate (18 mCi per mmole) and sodium octanoate (17.5 mCi per mmole).

The methyl esters of the first four homologous [1-^{14}C]carboxylic acids were prepared by esterification of the corresponding sodium salts with dimethyl sulphate[16]; the labelled methyl esters of the remaining four acids, and ethyl [1-^{14}C]octanoate were prepared by treating the corresponding sodium salts with sulphuric acid and subsequently esterifying with the appropriate alcohol in the presence of thionyl chloride according to Brenner and Huber[17]; the esters were distilled off in a closed apparatus at low temperature and under reduced pressure. The specific activity of the esters was determined, as carbon dioxide, in an internal gas proportional counter by measurement of combusted aliquots[18].

Gas chromatography

The automatic preparative gas chromatograph used was the APPC 3002 model of the Research Workshops of the Czechoslovak Academy of Sciences and was equipped with a thermal-conductivity mass detector.

The first four methyl esters of the homologous series were separated in a column (3 m × 6 mm) packed with Chromaton N-AW (particle size 0.125–0.160 mm) coated with 10% of Carbowax 400 (Lachema); the column was temperature-programmed from 30° to 50° at 4° per min, and helium was used as carrier gas (50 ml per min). The sample mixture (40 μl, consisting of 10 μl of each component) was applied as a single injection.

The remaining four methyl esters of the homologous series, and ethyl [1-^{14}C]-caprylate were separated in a column having the same dimensions and packed with the same support (particle size 0.200–0.250 mm), but with 15% of Apiezon L (Lachema) as stationary phase; this column was temperature-programmed from 120° to

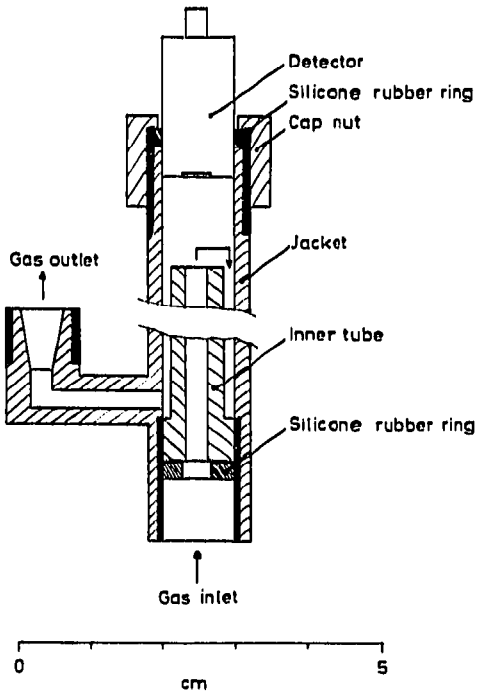

Fig. 1. Chamber for radioactivity measurement.

160° at 8° per min, and the helium flow-rate was 65 ml per min. The esters were injected in 10-μl portions.

Radioactivity assay

The chamber (Fig. 1) for the activity measurement is made from brass; its inner surface is lined with PTFE. The detection volume is placed between the column exit and the cell of an independently thermostatted katharometer and is heated at 85°. The entire column effluent passes through these two chambers. The upper part of the chamber, together with the pre-amplifier is attached to the thermally insulated cover of the chromatograph. The detector can thus easily be adjusted or exchanged without affecting the thermal state of the katharometer. Detection was carried out with use of commercially available equipment (General Electric, Philadelphia, Pa., U.S.A.), *viz.*, a diffused detector based on silicon (model NE-203) with a recording device (Nucle Eye Monitor). Count rates were recorded by means of an integrating device on a chartrecorder moving at the same speed as the chart-recorder of the thermal-conductivity detector.

RESULTS AND DISCUSSION

Fig. 2 shows the traces from the thermal-conductivity cell and from the activity chamber during the separation of a mixture of methyl esters of a homologous series of saturated aliphatic [^{14}C]acids. It can be seen that the widths of the corresponding peaks on the mass and activity traces are almost identical.

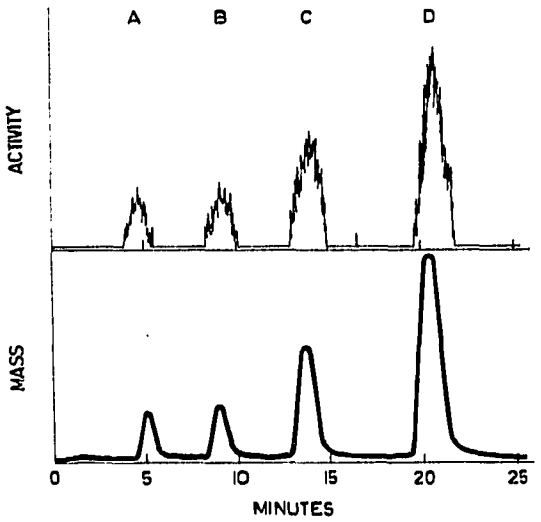

Fig. 2. Mass and radioactivity record of the GLC of a mixture of esters of homologous [^{14}C]acids: A, methyl formate; B, methyl acetate; C, methyl propionate; D, methyl butyrate.

The accuracy of the method is shown by the results in Table I. The activity of each fraction was calculated from the amount of each ester in the starting mixture and its specific activity (designated A_1 for methyl formate and A_n for the other esters). The calculated ratios of A_n to A_1 do not differ from the experimental values by more than 1 %.

The limit of detection was determined from relationships previously applied in GLC with a gas counter[13]. As, with the present device, the detector background is lower than 0.1 cpm and the measured fraction passes through the detection volume in less than 2 min, activity in the fraction is assumed to be detected when at least 3 counts are recorded; it can be seen from Table I that the activity required for this value is somewhat less than 0.15 μCi. For more precise measurement, the radioactivity must be correspondingly higher[11].

The limitations of the present method with respect to the b.p. of substances to be separated were determined by means of esters of higher [^{14}C]acids. For esters up

TABLE I

SEPARATION OF METHYL ESTERS OF HOMOLOGOUS [^{14}C]ACIDS

Fraction	*Activity, A (μCi)*	*Ratio of specific activities, A_n/A_1*		*Counts per peak**
		Calculated	*Found*	
Methyl formate	2.3	—	—	53
Methyl acetate	2.7	1.18	1.17	62
Methyl propionate	6.5	2.82	2.80	149
Methyl butyrate	15.4	6.57	6.62	351

* Parallel measurements of separate fractions from the display of the Nucle Eye Monitor.

to ethyl octanoate (b.p. 205.8°), there was no tailing of the activity peak when compared with the corresponding mass peak.

None of the substances having b.p. in the range tested [31.5° (methyl formate) to 205.8°] caused any contamination of the device leading to memory effects.

In view of the proximity of emitted beta-spectra, the above conditions can also be regarded as valid for the GLC of ^{35}S-labelled substances. However, the present detector is not suitable for use with tritiated substances. When compared with surface-barrier detectors[3–5], the detector used exhibits very low energy resolution; thus, it cannot be used for the simultaneous detection of several radionuclides, but other advantageous features of semiconductor detection are retained.

CONCLUSIONS

The method described makes possible direct assay of ^{14}C activity after GLC without the necessity for prior oxidation or cracking. The simple device for the activity assay can readily be attached to conventional gas chromatographs, and the semiconductor detector and counting equipment are commercially available. The method is particularly useful for substances of low b.p. and high radioactivity.

ACKNOWLEDGEMENTS

This work was performed under contract No. 1019/R2/RB and with financial support from the International Atomic Energy Agency in Vienna. Another grant was obtained from the Czechoslovak Atomic Anergy Committee under contract P 09-159-003-04-06. The technical assistance of Mr. V. Pánek is gratefully acknowledged.

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