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AN IMPROVED METHOD FOR COLLECTION AND MEASUREMENT OF RADIOACTIVITY IN COMPOUNDS SEPARATED BY GAS–LIQUID CHROMATOGRAPHY

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SUMMARY

^{14}C-Labeled compounds in the effluent of a gas–liquid chromatograph were collected intact by condensation and adsorption to small glass beads coated with silicone fluid in short glass cartridges and then eluted into scintillation vials for counting.

The overall recovery of radioactive fatty acid methyl esters and trimethylsilyl derivatives of glycerol and glucose was greater than 90%.

INTRODUCTION

The combination of gas–liquid chromatography (GLC) and subsequent radioassay of labeled compounds by liquid scintillation counting (LSC) provides a rapid and convenient method of determining the specific activities of the wide variety of materials which can now be separated on a GLC apparatus.

Radioactivity in GLC effluents can be measured in a number of ways: of these, fraction collecting offers several advantages over flow-through or combustion methods[1]. A major difficulty in fraction collecting, however, has been the choice of suitable material and containers for trapping the GLC effluent. KARMEN *et al.* have published several papers on this and related aspects of radioassay by gas chromatography[2–5] and Packard Instrument Co.* has produced a commercial fraction collector. Their method is to convey the chromatographic effluent in a heated steel tube to short glass tubes or cartridges filled with anthracene or *p*-terphenyl crystals. The cartridges are placed directly in a scintillation counter for assay or their contents emptied into standard counting vials with a suitable fluor. Complete details of the methods have been published[2–6].

This paper presents a modification of the above method in which small glass beads coated with silicone oil are substituted for the more commonly employed trapping agents, increasing the efficiency of trapping to almost 100%. The trapped

* Packard Instrument Co., Inc., 2200 Warrenville Rd., Downers Grove, Ill. 60515, U.S.A.

material can be readily eluted with standard toluene-based fluor, reducing quenching and increasing efficiency in the LSC assay.

EXPERIMENTAL

Materials

Radioactive ^{14}C-labeled fatty acid methyl esters were obtained by hydrolyzing radioactive mono- and digalactolipids from *Vicia faba* leaves with 5% H_2SO_4 in methanol[7]. The methyl esters were extracted with hexane and concentrated for injection. The weight percent composition of the fatty acid methyl ester mixture was (14:0)* trace, (16:0) 7.06%, (18:0) 2.54%, (18:1) 2.47%, (18:2) 7.98% and (18:3) 79.96%.

Four different standards of known amounts of [^{14}C]glycerol and [^{14}C]D-glucose, both uniformly labeled, were prepared with the addition of unlabeled glycerol and glucose as carrier. Tri-Sil** was used to form the trimethylsilyl ethers of glycerol, α- and β-D-glucose. The reaction mixtures were injected directly into the chromatograph.

Gas–liquid chromatography and fraction collection

GLC was carried out on a dual column, Model 7401, (Packard Instrument Co.) equipped with flame ionization detectors, glass columns, and on-column injection. The GLC effluent was passed through a stream splitter installed in the detector oven and connected directly to the column outlet septum. The splitter permitted a flow ratio of approximately 1 part to the detector and 5 parts to a Packard fraction collector, Model 852.

For initial tests of recovery of injected radioactive compounds, the column and splitter were bypassed with a length of 16 gauge Teflon tubing attached directly to the fraction collector. Later, the stream splitter was inserted immediately prior to the fraction collector inlet and joined to the collector and detector with short (2-in.) pieces of Teflon tubing.

When the column was replaced by the Teflon tube, the column oven was maintained at 180° and the carrier gas (N_2) flow rate at 60 cm^3/min.

The column employed for fatty acid methyl ester separation measured 6 ft. × 4 mm I.D., was packed with 15% EGS on 80–100 mesh Chromosorb W, AW, and was run isothermally at 180°. For separating glucose and glycerol trimethylsilyl ethers a 1.5 ft. × 4 mm I.D. column of 5% SE-30 on 60–80 mesh Chromosorb W, AW, DCMS treated, was temperature-programmed for an initial hold of 10 min at 105°, an increase of 10°/min to 170°, then held at 170° until the last component was eluted.

Inlet- and outlet-detector temperatures were 190°, and N_2 carrier gas flow rate was 80 cm^3/min. The temperature of the fraction collector arm was maintained at 200° and the carrier gas effluent split ratio was determined by a bubble flow meter attached to the detector outlet of the splitter and to the outlet end of a typical collection cartridge in the fraction collector.

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* Number of carbon atoms in the fatty acid: number of double bonds.

** Pierce Chemical Co., from Chromatographic Specialties Inc., P.O. Box 758, Brockville, Ontario, Canada.

GC effluent was collected by condensation and adsorption onto 40–60 mesh plain glass beads (Chromatographic Specialties Inc., Brockville, Ontario, Canada) coated with 2% (w/w) Dow Corning-560 silicone fluid. The beads are coated by dissolving the silicone fluid in enough acetone to cover them in a Pyrex baking dish, stirring well and allowing the acetone to evaporate. The beads were packed lightly into borosilicate glass tubes 45 mm long × 9 mm O.D. (7 mm I.D.) (Packard Instrument Co., Cat. No. 6001095) which were plugged first at the lower end with one-half of a cellulose acetate cigarette filter* and filled to within 5 mm of the top. The collector bowl and cartridges were maintained at 7° by cold water circulating through copper cooling coils submerged in the turntable well.

Liquid scintillation counting

The activity of each fraction was determined by LSC in a Packard liquid scintillation spectrometer (Model 3375) after attaching the upper end of each cartridge to the stem of a small plastic funnel and eluting the silicone oil and the radioactive material directly into glass scintillation vials with 15 ml of toluene containing PPO (6 g/l) and POPOP (300 mg/l). Aliquots of the radioactive samples that were injected were counted in the same fluor to determine the total activity injected. Counting efficiency was approximately 90%.

The glass beads and cartridges can be washed with 95% ethanol and acetone, the beads recoated with silicone fluid and reused.

RESULTS

The results in Table I indicate the recoveries obtained using this method of collecting effluents containing trimethylsilyl derivatives of glycerol and glucose and fatty acid methyl esters.

The best average recoveries occurred when the samples were injected and, bypassing the column and stream splitter, applied directly to the fraction collector. The value of 99% found here with a standard deviation of ± 0.6% for the 30-min samples represents almost total recovery of the samples. Losses with this system may be due to incomplete injection of the sample, eddying of the carrier gas and sample in the Teflon tube inlet (particularly after only 3-min collection), and variation in LSC efficiency.

Introducing the stream splitter into the system increases the possibility of error in determining recovery (Expts. 3–6). The low standard deviations of 35 samples used in these four experiments suggest that the apparent losses are most likely due to inaccurate determination of the splitter ratio on that occasion. The results of Expt. 4 are evidence that activity, once collected, is not desorbed even after 1-h exposure to hot effluent gas flow.

In Expts. 9–11 a number of fractions were collected from each injection. When totalled, the counts from these fractions indicate a recovery of 96–99% despite small losses on the GLC columns used. The high standard deviation in Expt. 11 is believed to be due to variable injection volumes, column adsorption losses, impurities in the

* Canadian Filtrona, 5720 Ferrier St., Montreal 9, Quebec, Canada.

TABLE I

RECOVERY OF INJECTED ^{14}C-LABELED COMPOUNDS FROM CARTRIDGES OF SILICONIZED GLASS BEADS

Injected radioactive compounds: TMS–Gly/Gluc = trimethylsilyl ether derivatives of [^{14}C]glycerol, [^{14}C]α- and [^{14}C]β-D-glucose in different proportions in 4 weighed standards A, B, C, and D; FAME = Fatty acid methyl esters of 14:0, 16:0, 18:0, 18:1, 18:2 and 18:3 fatty acids.

Experiment	*Sample type*	*Replicates*	*Collection period (min)*	*Injected activity (d.p.m.)*	*Injected weight (μg)*	*Activity recovered (d.p.m.)*	*Mean recovery (%)*	*S.D. (%)*
Teflon tubing[a]								
1	TMS–Gly/Gluc(A)	6	3	35,025	118	33,984	97.03	± 0.66
2	TMS–Gly/Gluc(A)	6	30	35,025	118	34,667	98.98	± 0.57
Teflon tubing + stream splitter[a]								
3	TMS–Gly/Gluc(B)	10	10	27,760	121	25,460	91.71	± 0.94
4	TMS–Gly/Gluc(B)	5	60	27,760	121	25,814	92.99	± 0.72
5	TMS–Gly/Gluc(B)	10	10	27,760	121	25,078	90.34	± 1.09
6	FAME	10	10	25,800	118	23,555	91.30	± 2.84
7	TMS–Gly/Gluc(B)	10	5	27,760	121	26,100	94.02	± 6.37
8	TMS–Gly/Gluc(C)	10	10	11,390	125	10,972	96.33	± 3.28
GLC column + stream splitter[a]								
9	TMS–Gly/Gluc(A)	10	40[c]	35,025	118	34,342	98.05	± 4.53
10	TMS–Gly/Gluc(D)	20	40[c]	5,100	50	5,024	98.52	± 1.90
11	FAME	[b]	35[d]	3,000–360,000	73–188	2,892–347,000	96.40	±11.04

[a] Apparatus connecting GLC inlet to fraction collector.
[b] Twenty-five different samples run once each.
[c] Four fractions 10 min each.
[d] Seven fractions 5 min each.

samples, and other common errors normally found in GLC; and not to variable recoveries by the collecting system.

Counting the eluted radioactivity in solution was found to be more efficient and reproducible than counting the filled anthracene cartridges directly or emptying the cartridges and glass beads into vials. The adsorbed radioactivity was quantitatively eluted from the beads along with the silicone fluid by the first 1–2 ml of fluor, although in practice it was convenient to pour all of the 15-ml portion of scintillation fluid into the funnel and through the beads. Complete elution was confirmed by adding a known amount of GLC sample directly to the beads and eluting with different volumes of fluor. Compounds not readily soluble in toluene could be eluted with a small amount of another solvent followed by the bulk of the fluor. The efficiency of scintillation counting of 90.40% for the standard fluor was reduced to only 89.98% by the silicone washed off the beads in ^{14}C determinations.

DISCUSSION

Anthracene crystals were reported by STEINBERG[8] to be efficient scintillators in the assay of [^{14}C]amino acids from aqueous solution. KARMEN AND TRITCH's finding[2] that short sections of GLC column could be used to trap GLC effluent vapours, and that a liquid phase-coating of silicone fluid on the stationary support was necessary for a high retentivity of fatty acid methyl esters suggested the use of anthracene crystals as both stationary support and solid scintillator. Anthracene in solution proved to be a poor scintillator[6] and *p*-terphenyl was substituted. COOKE[9] found *p*-terphenyl to be a better trapping agent than anthracene but preferred glass wool packing for labeled steroids. Glass wool was also suggested as an inexpensive alternative to anthracene by BENNETT AND COON[10] for adsorbing fatty acid methyl esters. The activity was equally well adsorbed to both glass wool and anthracene and not removed by a 3-min exposure to hot carrier gas flow. No mention was made of the overall recovery of their system.

HAMMARSTRAND *et al.*[11] list eleven references to collection methods which they rejected on criteria of expense, being too cumbersome, non-disposability, and non-reproducibility. Their solution was to impale lengths of cigarette filters coated with silicone fluid (DC-703) on a hypodermic needle attached directly to the carrier gas effluent tubing. Mean recovery was 91.4% (± 3.5% S.D.) for ten determinations.

It was decided that small glass beads would be more reproducibly packed into the cartridges ensuring a constant low resistance to gas flow, that they provide a better surface for condensation and retention of the effluent substituents, and would in general be more convenient to use.

The beads are easily uniformly coated with silicone fluid. Dow Corning-560 fluid was chosen over several other fluids possible, including the DC-550 used by KARMEN[2], because of its higher polarity. It could be readily eluted from the beads by several solvents and it exhibited little or no quenching in LSC. The 2% loading (w/w) of fluid on the 40–60 mesh beads could be increased to 5 or 6% on 80–100 mesh beads without their becoming unduly oily. There was no difference in collection or counting efficiency between the two sizes of beads, but the larger size was deemed easier to manipulate.

Intermittent losses caused by uneven cartridges and hardened, leaky silicone

rubber pads are difficult to detect. Therefore, precautions must be taken to ensure gastight contact between cartridges and the fraction collector nozzle exit pad.

We believe that this method is one of the most convenient, reliable and inexpensive available for collecting samples from GLC effluents.

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REFERENCES

1 A. KARMEN, in J. M. LOWENSTEIN (Editor), *Methods in Enzymology*, Vol. XIV, Academic Press, New York and London, 1969, p. 465.
2 A. KARMEN AND H. R. TRITCH, *Nature*, 186 (1960) 150.
3 A. KARMEN, L. GIUFFRIDA AND R. L. BOWMAN, *J. Lipid Res.*, 3 (1962) 44.
4 A. KARMEN, I. MCCAFFREY, J. W. WINKELMAN AND R. L. BOWMAN, *Anal. Chem.*, 35 (1963) 536.
5 A. KARMEN, *J. Amer. Oil Chem. Soc.*, 44 (1967) 18.
6 A. KARMEN, *Packard Instrument Co., Tech. Bull.*, No. 14, June, 1965.
7 D. E. BOWYER, W. M. F. LEAT, A. N. HOWARD AND G. A. GRESHAM, *Biochim. Biophys. Acta*, 70 (1963) 423.
8 D. STEINBERG, *Nature*, 183 (1959) 1253.
9 B. A. COOKE, *Anal. Biochem.*, 32 (1969) 198.
10 M. BENNETT AND E. COON, *J. Lipid Res.*, 7 (1966) 448.
11 K. HAMMARSTRAND, J. M. JUNTUNEN AND A. R. HENNES, *Anal. Biochem.*, 27 (1969) 172.