SIMULTANEOUS DETERMINATION OF ^3H AND ^{14}C BY LIQUID SCINTILLATION IN VERY SMALL SAMPLES

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In both quenched and unquenched samples simultaneously labelled with ^3H and ^{14}C, the sensitivity of detection of the two nuclides was determined in a liquid scintillation spectrometer with the use of polyester bags which made possible to perform measurements with only a few tenths of milliliters of the liquid scintillator. The influence of the bag size and the scintillator volume on the differentiation of the two nuclides was measured. With identical channel settings in the scintillation spectrometer, the differentiation in bags is more efficient than in usual glass vials containing 5—20 ml of the scintillator.

Particularly in molecular biology and biochemistry, only a very small amount (micrograms or even less) of the radioactive sample is often accessible. Experimental systems of compounds labelled both by ^3H and ^{14}C have become very frequent. The simultaneous determination of the radioactivity of each of the two nuclides may be performed by various methods[1] from which the liquid scintillation technique is most widely used. In this technique, glass, polyethylene or quarz vials are used containing usually from 5 to 20 ml of the scintillator. Gupta[2,3] developed a new procedure of liquid scintillation counting for the separate estimation of a single β-nuclide in small volumes of solvents. This procedure is based on the use of small plastic bags. The samples are introduced along with a small amount of scintillator into bags which are then placed into usual scintillation vials. In this manner, the contamination of vials is removed and the scintillator uptake is considerably decreased.

In the present paper, we analyse the geometry of a small volume of the liquid scintillator in polyester bags from the standpoint of the simultaneous determination of ^3H and ^{14}C. With the use of both quenched and unquenched samples, the differentiation of nuclides in bags is compared with that in usual glass vials which require from 5 to 20 ml of the scintillator.

EXPERIMENTAL

Measurements were performed in a Tri-Carb Model 3375 liquid scintillation spectrometer at 7°C with the use of glass vials purchased from Packard Instrument Co, La Grange, Ill., U.S.A.; channel settings, $^3H(^{14}C)$ and $^{14}C(^3H)$. The scintillator was prepared according to Bray[4]. Plastic bags were handmade in three sizes by modification of the original procedure of Gupta[2]. The polyester foil (3 M Comp., U.S.A., Scotchpak Type, No 20 A 5) is cut into strips which are folded, placed between two glass plates and the edges are sealed to obtain tubes of the girth of 2 cm, 3 cm and 5 cm (in the following text, the bags are characterised by these data). The sections of the tube, about 14 cm each, are then sealed with heated intestinal forceps and the tube is cut both in cross seams and roughly in the middle between these seams. The bottom is formed by pressing into the bag a heated round-end glass rod. Bags are placed into vials and sealed with heated intestinal forceps just below the neck of the vial; the upper end of the bag is then snipped and the vial is snap-capped. Reference sources, toluene-^{3}H, 2·24 μCi/g, and toluene-^{14}C, 0·66 μCi/g, were used (10 μl each) to determine the counting efficiency. The quenching is effected by the addition of 0·1 vol.% of analytical reading (A.R.) grade nitromethane. The standard deviation of all measurements was less than ±0·5%.

RESULTS AND DISCUSSION

The size of the bag to be used is determined by the amount of the sample to be measured and its solubility in the scintillator at the temperature of the measuring chamber of the spectrometer. Higher temperatures cause a higher solubility but lead to an increased thermal noise of photomultipliers and thus to a higher background. The temperature of 7°C makes possible a sufficient solubility at a relatively low back-

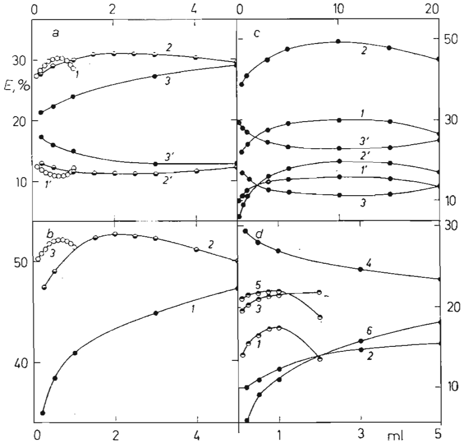

FIG. 1

Dependence of Detection Efficiency on Scintillator Volume

● Glass-vial, bag girths: ○ 2 cm, ◓ 3 cm, ◒ 5 cm. *a* unquenched samples; channel, ^{3}H(^{14}C); 1,2,3 ^{3}H; 1′,2′,3′ ^{14}C; *b* unquenched; channel, ^{14}C(^{3}H); *c* 1,2,3 unquenched; 1′,2′,3′ quenched; 1,1′ ^{3}H in ^{3}H(^{14}C); 2,2′ ^{14}C in ^{14}C(^{3}H); 3,3′ ^{14}C in ^{3}H(^{14}C); *d* quenched; 1,2 ^{3}H in ^{3}H(^{14}C); 3,4 ^{14}C in ^{3}H(^{14}C); 5,6 ^{14}C in ^{14}C(^{3}H).

ground (thus *e.g.*, 1 ml of the scintillator according to Bray[4] contains up to 0·4 ml of water). At such a solubility, plastic bags of the girth of only 2 cm are satisfactory for aqueous solutions of many biological samples. There is a relatively low difference in background for a scintillator in the plastic bag (placed in a glass vial) and for the same volume of a scintillator placed directly in the glass vial. On the other hand, an increasing volume of the scintillator in the glass vial leads to a higher background (Table I).

TABLE I

Background with Scintillator without Quenching in Glass-Vials and in 2 cm Girth Bags

The values given are arithmetic means of three samples measured with the standard deviation of ±3·2%.

Scintillator ml	Bag, c.p.m.		Vial, c.p.m.		Scintillator ml	Vial, c.p.m.	
	$^{3}H(^{14}C)$	$^{14}C(^{3}H)$	$^{3}H(^{14}C)$	$^{14}C(^{3}H)$		$^{3}H(^{14}C)$	$^{14}C(^{3}H)$
0·1	10·5	13·5	10·9	14·6	5·0	12·7	16·5
0·5	10·2	13·6	10·5	15·8	10·0	14·4	19·4
1·0	11·7	13·7	10·5	15·5			

The differentiation of the two nuclides requires[5,6] the highest detection efficiency in the $^{3}H(^{14}C)$ channel for ^{3}H and the lowest efficiency for ^{14}C while the requirements of the two nuclides in the $^{14}C(^{3}H)$ channel are just opposite. Figs 1*a* and 1*b* show the dependence of detection efficiency for an unquenched sample in both channels on the scintillator volume (the detection efficiency for ^{3}H in the $^{14}C(^{3}H)$ channel does not exceed 0·02%). The optimum scintillator volume for differentiation of the nuclides under investigation is from 0·6 to 0·8 ml for the plastic bag of the 2 cm size and from 1·5 to 3·0 ml for the size of 5 cm; such volumes are not suitable for the glass-vial. The optimum scintillator volume for a glass-vial is about 10 ml (Fig. 1*c*) but the detection sensitivity is lower because of a higher background and a lower detection efficiency especially for ^{14}C in the $^{14}C(^{3}H)$ channel (Fig. 1*b*). As shown by comparison of the optimum figure of merit values (efficiency^{2}/background) for an unquenched sample in vials and bags, the possibility to differentiate the two nuclides is more favourable with bags than with vials: An identical dependence of the detection efficiency in both channels is also valid for quenched samples when the amplitude spectrum is shifted toward a lower energy region (Figs 1*c* and 1*d*): under the same channel settings, the optimum scintillator volume lies between 0·75 and 1·0 ml for the bag of the size of 3 cm. The volume dependence of the detection efficiency (Figs $1a-d$) may be explained by geometry of the liquid scintillator towards the photomultipliers. The geometry improves with the increasing height of the scintillator column, attains the optimum at a certain range, and then becomes gradually worse.

Nuclide and channel	^{3}H; $^{3}H(^{14}C)$	^{14}C; $^{3}H(^{14}C)$	^{14}C; $(^{14}C^{3}H)$
Bag[a]	88·6	11.1	20·0
Vial[b]	67·6	11·9	14·1

[a] Girth, 2 cm; scintillator volume, 0·6 ml; [b] scintillator volume, 5 ml.

The narrower is the column, the narrower is the optimum range. The higher detection efficiency with bags than with vials may be explained by a greater loss of light quanta in the broader column. Furthermore with ^{14}C, the increase in detection efficiency in a bag in comparison to a vial is somewhat higher than with ^{3}H. This effect which was also observed by Gupta[2] in separate measurements of single nuclides, may be ascribed to greater losses with ^{14}C than with ^{3}H owing to an edge effect on the walls of a broad column in the glass vial (at a greater initial kinetic energy, the emitted electron escapes from the scintillator without forming the necessary number of light quanta).

REFERENCES

1. Tykva R.: Chem. listy *61*, 690 (1967).
2. Gupta G. N.: *Proc. Symp. Isotopes and Radiation in Soil Organic-Matter Studies*, p. 31. International Atomic Energy Agency, Vienna 1968.
3. Gupta G. N.: *Proc. Int. Conf. Org. Scint. and Liquid Scint. Counting* (D. L. Harrocks, Ed.), p. 747. Academic Press, New York 1971.
4. Bray G. A.: Anal. Biochem. *1*, 279 (1960).
5. Herberg R. J.: Anal. Chem. *36*, 1079 (1964).
6. Bush E. T.: Anal. Chem. *36*, 1082 (1964).

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