

LIQUID SCINTILLATION COUNTING OF PAPER CHROMATOGRAMS

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Several reports have recently appeared in the literature describing techniques for the counting of radioisotope labeled compounds on paper chromatogram by means of scintillation counting methods. Roucayrol et al. (1957) mounted wet paper strips, previously dipped in a solution of phenylbiphenyloxadiazole in toluene, onto a photomultiplier window and reported satisfactory results for the radioactivity assay of C^{14} , I^{131} or P^{32} labeled compounds. Techniques for the solid scintillation counting of H^3 and C^{14} labeled compounds in paper chromatograms were developed by Selinger and Agranoff (1959), in which dried paper strips, previously impregnated in a solution of anthracene in benzene, were placed on the surface of a photomultiplier tube for counting.

Encouraged by these studies, a simple technique has been developed in this laboratory which permits one to count a great number of sectional paper chromatograms (either 1 cm. x 4 cm. or 2.5 cm. x 4 cm.) in a liquid scintillation counter equipped with an automatic sample changer.

It was found in a series of preliminary studies that insertion of a couple of filter paper strips in a standard counting vial (5 dram) containing liquid scintillation mixture and benzoic acid- C^{14} does not appreciably reduce the counting efficiency. This fact led to the devisal of a reliable technique for the counting of paper strips charged with C^{14} or H^3 labeled compound and immersed in liquid scintillation mixtures. In a typical assay of this type, paper strip chromatograms (Whatman No. 1), 4 cm. in width,

are cut into 2.5 cm. sections, each of them is inserted individually into the holder of specially made vials (Figure 1). The vials described herein

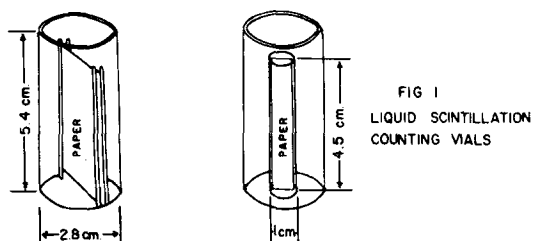


FIG 1
LIQUID SCINTILLATION
COUNTING VIALS

are made through the kind cooperation of Mr. Wade Meeker, Chemical Testing and Service, Department of Chemistry, Oregon State College. To each vial is then delivered 18 ml. of a conventional phosphor solution such as that consists of 4 g. PPO and 50 mg. POPOP or 3 g. of terphenenyl and 30 mg. POPOP in a liter of toluene. The vials are then closed with polyethylene caps and are counted in a Tri-Carb liquid scintillation spectrometer with the proper voltage settings and window widths for the radioisotopes in question. In the event that better resolution of paper chromatogram is desirable, 1 cm. section of a 4 cm. paper strip can be counted by the use of counting vial equipped with a concentric well (Figure 1). The well is approximately 1 cm. in diameter and 4.5 cm. in height and holds about 3 ml. of the phosphor solution. The counting efficiencies for paper chromatograms charged with various C^{14} or H^3 labeled compounds are given in Table I.

It can be seen from these data that the counting efficiency for C^{14} assay is far superior than what can be expected with a conventional Geiger-Müller counter and is comparable with the previously reported techniques, although the counting efficiency for tritium assay is rather low. In view of the simplicity and excellent reproducibility of the present technique and the fact that it is suitable for counting a great number of samples in a counter equipped with automatic sample changers, the technique should prove to be useful in biochemical research involving the use of radiotracers.

TABLE I

Counting Efficiencies and Reproducibility for the Liquid
Scintillation Counting of Paper Chromatograms

Labeled Compound	Radioactivity dpm	Net Counting Rate ^a , cpm	Efficiency per cent	Precision ^b per cent
Benzoic acid-7-C ¹⁴ ^c	4795	4076	85	2
Benzoic acid-7-C ¹⁴ ^d	4795	4059	85	1
Na-benzoate-7-C ¹⁴	4795	2610	54	2
Glucose-U-C ¹⁴	5472	3124	57	2
K-gluconate-U-C ¹⁴	9969	5334	54	2
Glutamic acid-2-C ¹⁴	24640	13347	54	2
Na-benzoate-H ³	2542	39	1.5	2
Amino acids-C ¹⁴ ^e	8881	4884	55	2

- a. Net counting rate refers to the observed counting rate less the background counting rate. Countings were carried out to a standard deviation of no more than 1 per cent.
- b. Precision is expressed as percentage deviation from the mean of four replicate samples.
- c. Labeled compound dissolved in the scintillation solution without the insertion of filter paper.
- d. Labeled compound dissolved in the scintillation solution with the insertion of a piece of blank filter paper.
- e. Paper chromatogram of a mixture of C¹⁴ labeled amino acids in a bacteria hydrolyzate was counted prior and after development in sec-butanol NH₃ solvent. Total recovery from fifteen 2.5 cm. x 4 cm. sections was 90 per cent of the applied activity.

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