

CHAPTER 7

Solidifying Scintillator for Solid Support Samples

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ABSTRACT

Although solid support counting suffers from some disadvantages such as self-absorption and relatively poor reproducibility, it is widely employed for screening assays because it makes sample preparation easy. However, as the number of assays increases, the amount of scintillator consumed makes waste disposal costs problematical in the light of environmental restrictions.

A solidifying scintillator formulation will be described which allows for dispensing of the scintillator in liquid form with subsequent solidification at room temperature. Issues of sample handling, counting performance, and configuration of the counting equipment, will be discussed in comparison to liquid cocktails.

INTRODUCTION

Solid support counting of weak beta emitters is an established radioassay technique. It is widely employed because the advantages obtained by easy sample preparation outweigh the disadvantages resulting from poor reproducibility due to self-absorption. In practice, radioactive material is spotted onto chromatography paper or isolated on a filter which is then dried and immersed in a cocktail for counting.¹⁻³ A common element of many of these assays is that no solubilizer is used and the sample remains on the solid support rather than dispersing into the bulk of the cocktail. This adds the effect of nonreproducible counting geometry to the self-absorption problem. Another drawback of this technique is that excessive amounts of cocktail are used. This leads to a vexing disposal problem when large numbers of samples are processed.

These problems can be overcome by using a paraffin based solidifying scintillator. The solidifying scintillator was formulated to allow the experimenter to reverse the phase of the sample from solid to liquid scintillator. The melting point is chosen to allow for good counting efficiency in the solid phase at room temperature and for easily dispensing the material at moderately elevated temperatures. Since the sample is solid it can be handled easily to obtain good

counting geometry. Also, the smaller volume solid samples are more easily disposed of.

Paraffin Scintillator Formulation and Measurement Geometry

Common to all conventional scintillators, whether solid or liquid, is the fact that the phase does not change after sample preparation. To obtain a cocktail which can be dispensed in liquid form and counted as a solid, a conventional organic scintillator is modified by replacing a part of the solvent (p-xylene, diisopropylnaphthalene) with pure paraffin having an appropriate melting point. The melting point can be adjusted within limits by changing the molecular weight distribution of the linear hydrocarbons which make up the paraffin.

The paraffin scintillator consists of PPO, bis-MSB, paraffin, and solvent. The paraffin can be homogeneously mixed with solvent and liquid scintillation fluors without giving rise to quenching. PPO and bis-MSB were used because they are the most popular liquid scintillation fluors.⁴ The solvent dissolves the fluors and works effectively as an energy transfer medium to the primary fluor, which is essential for obtaining a high counting efficiency.^{5,6}

In use, the scintillator is heated to about 40°C to melt it, and 0.3 mL of the liquid is dropped on a solid support sample. After 10 sec, it solidifies, and a rigid and translucent solid support sample can be obtained. The appearance of the prepared samples is somewhat cloudy, but it is not necessary for them to be fully transparent. Once the solid sample is formed, it is stable and will remain rigid at room temperature.

The solid support samples thus impregnated with the paraffin scintillator can be counted in a liquid scintillation counter with each sample suspended in a polyethylene bag which is vertically supported in a plastic holder as shown in Figure 1. It is well known that the counting efficiency of the solid support sample depends on its orientation in a counter.⁷⁻⁹ Best results are obtained when the plane of the solid support sample is parallel to the photomultiplier faces, due to improved light dispersion from the sample.⁹

EXPERIMENTAL

Solid Support Samples

Aqueous solutions of ³H leucine and ¹⁴C uridine with known activity were employed; 0.1 mL of these solutions were individually deposited on solid supports, and then dried overnight at room temperature. The solid supports used here are glass fiber filters, membrane filter chromatography paper, and thin layer chromatography plates. The samples were counted on a liquid scintillation counter, Packard TriCarb Model 4000 Series, coupled with a multi-channel pulse height analyzer.

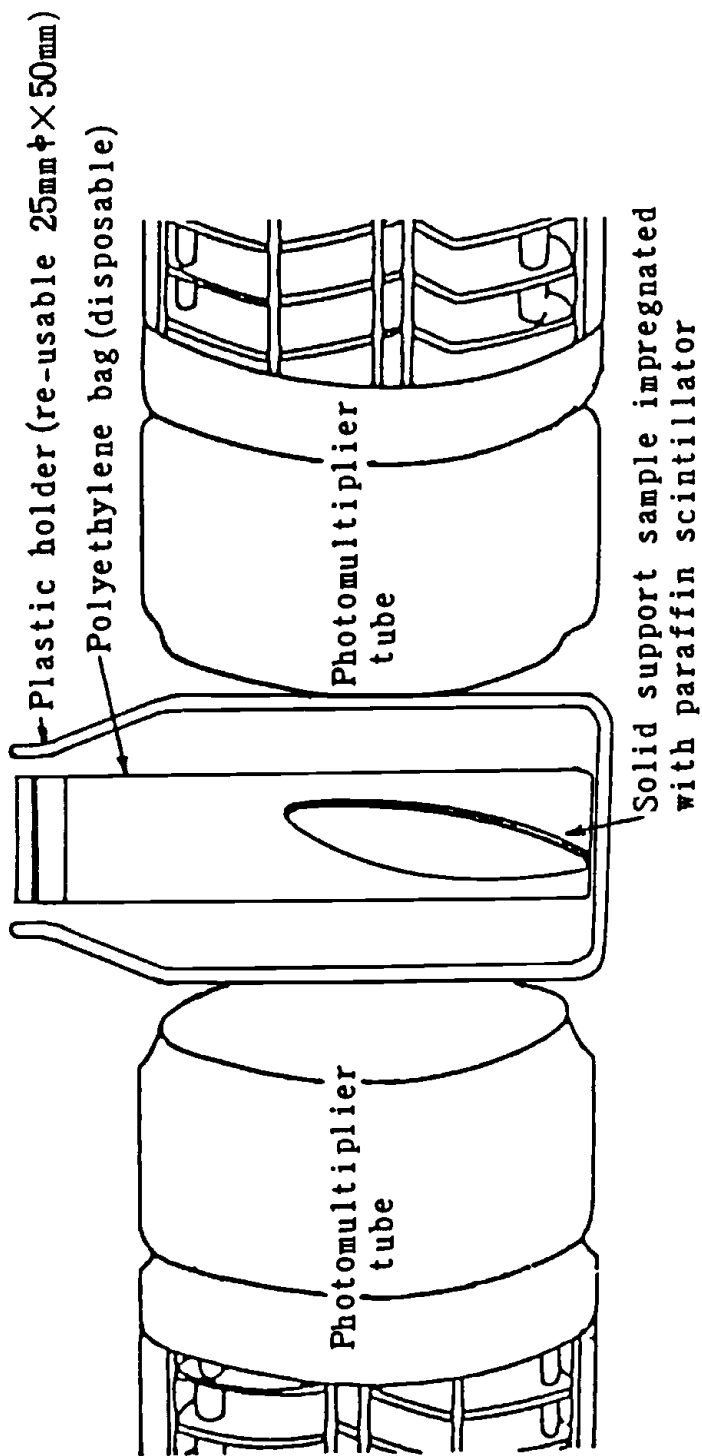


Figure 1. Measurement geometry for solid support sample in LSC.

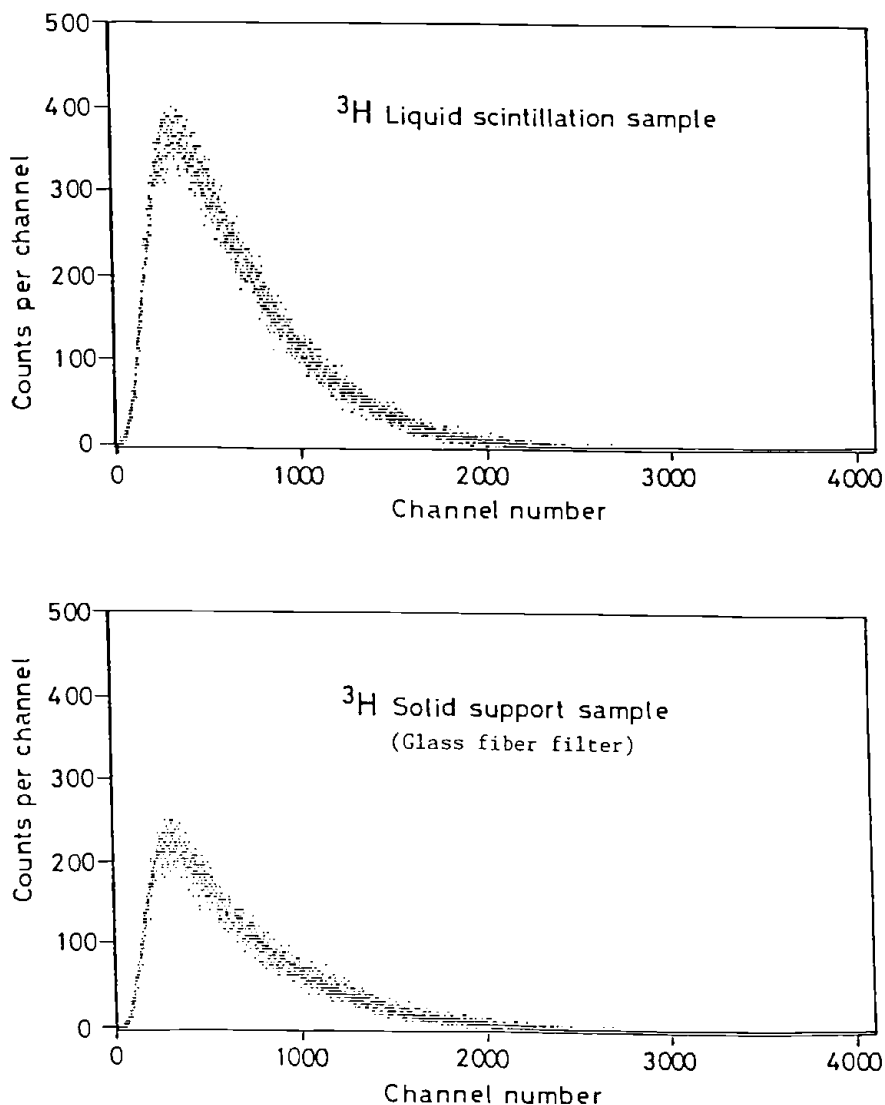


Figure 2. Pulse height spectrum of a ^3H solid support sample: in a conventional cocktail (top), treated with the solidifying scintillator.

Homogeneous Samples

Several formulations were also prepared as homogeneous mixtures to test the relative counting performance. In these formulations, 5 g PPO and 0.8 g bis-MSB were dissolved in 250 mL of solvent and mixed with 500 mg of paraffin. After thorough mixing at an elevated temperature, 15 mL aliquots were dispensed into 20 mL scintillation vials. The solvents tested were xylene

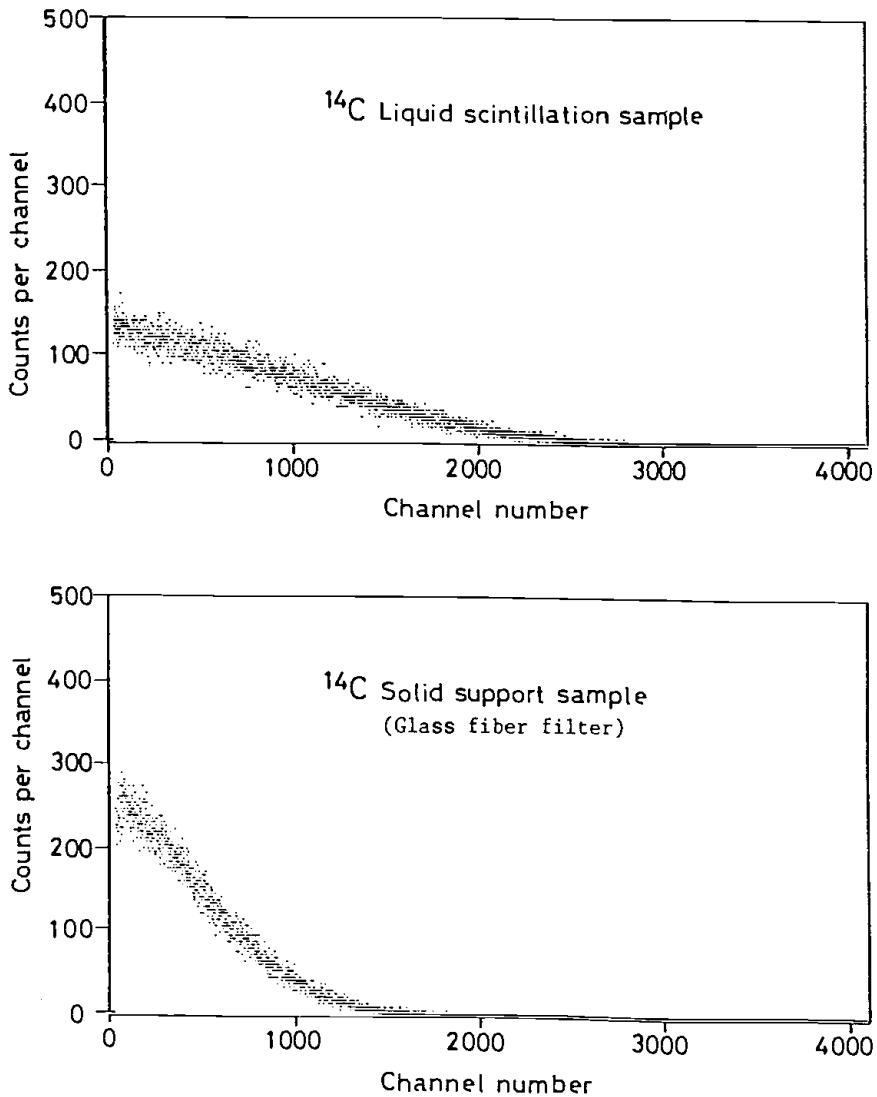


Figure 3. Pulse height spectrum of a ^{14}C solid support sample: in a conventional cocktail (top), tested with the solidifying scintillator.

(X), pseudo-cumene (P), and diisopropylnaphthalene (K0, K5). Different batches of paraffin were used for formulations K0 and K5.

The formulations were evaluated by spiking them with ^3H or ^{14}C labeled toluene. After thorough mixing, each sample was allowed to solidify. The samples were assayed in a Packard TriCarb Model 2000CA as well as an experimental single tube time-resolved counting apparatus.¹⁰

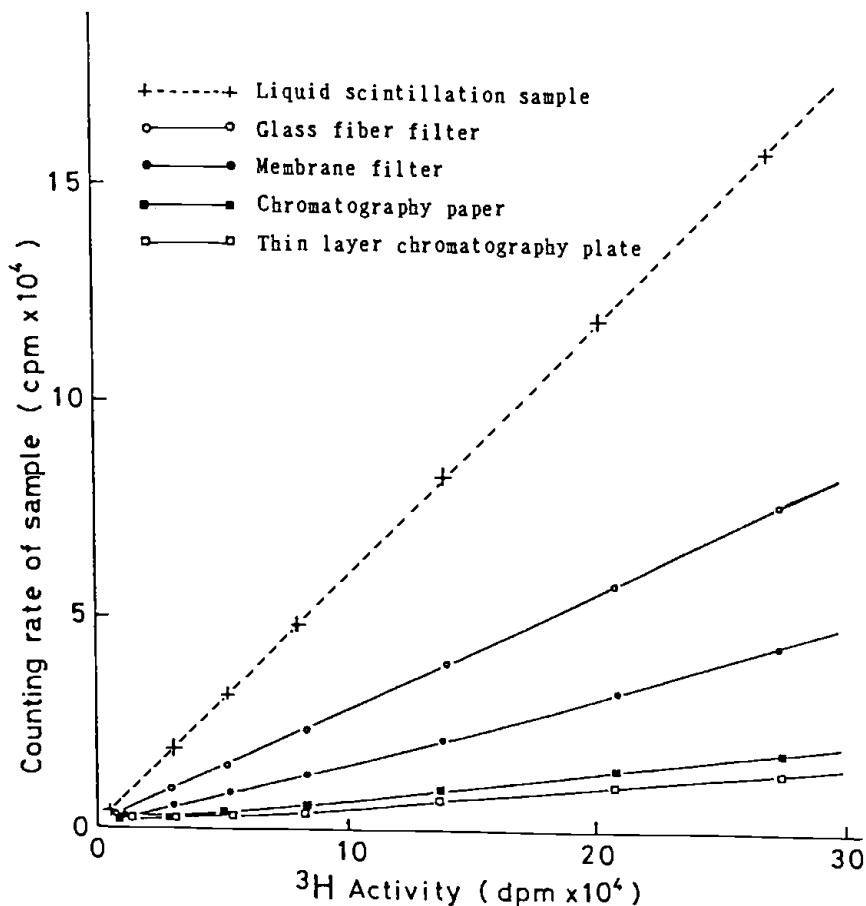


Figure 4. Count rate as a function of ^3H activity for various solid support samples.

Results

The scintillation pulse height spectrum was investigated in order to better interpret the counting data. The pulse height distribution of the ^3H solid support sample, prepared in the technique described in Figure 1, is very similar to that of the conventional ^3H sample, although the counting rate for each channel may be reduced (Figure 2). As for a ^{14}C sample, however, the volume and dispersion area of the paraffin scintillator are not large enough to absorb the β -ray energy of the ^{14}C completely, which seems to cause a shifted pulse height distribution (Figure 3).

The optimum fluor concentration and volume ratio of paraffin to p-xylene were also investigated. The formulation for best counting efficiency was found to be; PPO: 10 g; bis-MSB: 1.0 g; paraffin: 670 mL; and p-xylene: 330 mL.

The relationship between the measured activity and the count rate is shown

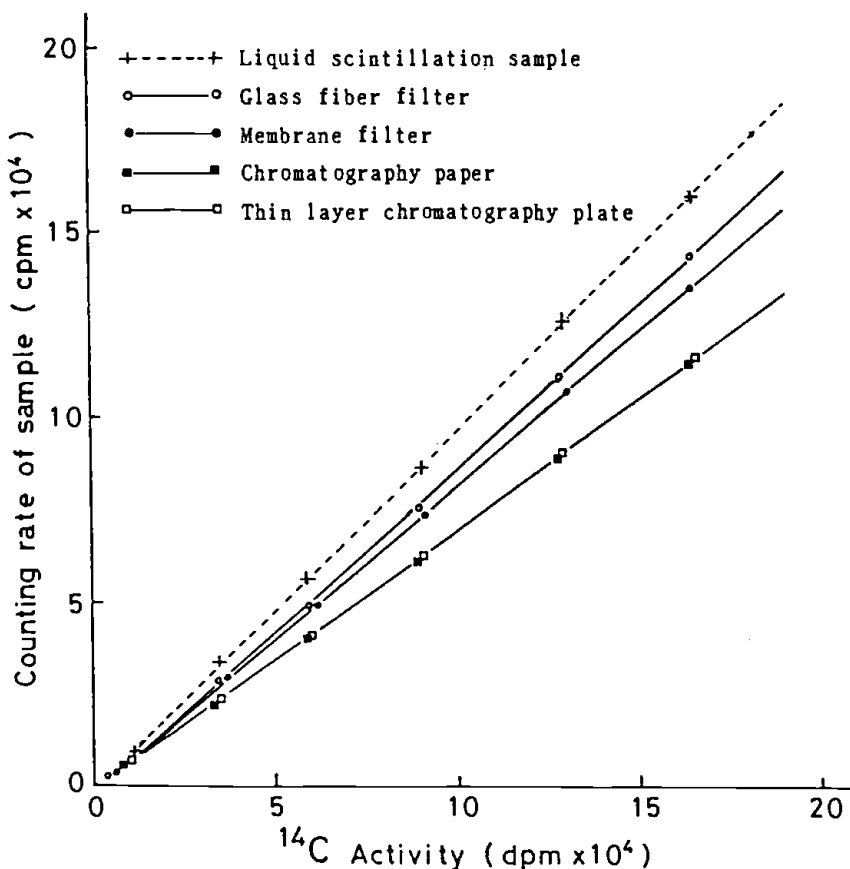


Figure 5. Count rate as a function of ^{14}C activity for various solid support samples.

in Figures 4 and 5 for various kinds of solid support samples. The relationship is linear over a wide range of activity for each nuclide.

Freshly prepared samples were monitored for several days (Figure 6). The count rate of the sample does not decrease with the time elapsed for more than two weeks. This indicates the absence of any significant vaporization of the paraffin scintillator from the prepared sample.

The counting efficiencies for each support material determined are displayed in Table 1; ^3H and ^{14}C can be measured with counting efficiencies of 6–30% and 70–87% respectively. The difference in the counting efficiency for each support material can be attributed to that of the β -ray self-absorption and photon reduction inside the solid support.

Figure 7 displays the ^3H and ^{14}C efficiency values of homogeneous paraffin solutions measured on a two tube coincidence counter. The formulations tested used the solvents xylene (X), pseudo-cumene (P), and diisopropylnaph-

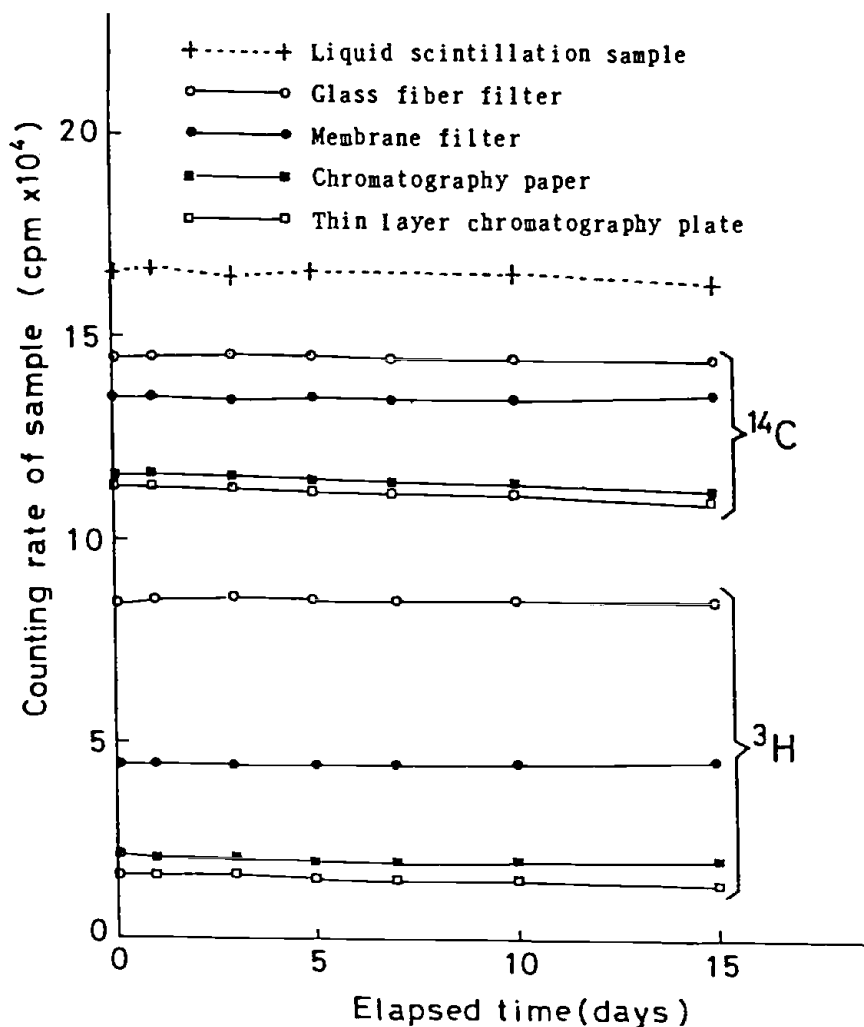


Figure 6. Count rate as a function of elapsed time for various solid support samples.

thalene (K5, K0). We see that excellent efficiencies are obtained for the solid samples.

In Figure 8, ³H efficiency values for the formulations are displayed as obtained from the experimental time-resolved single tube counter (Valenta). The apparatus is able to operate as a two pulse coincidence counter—requiring that a second pulse be registered in the tube after the first—or as a three pulse triple coincidence counter. While cocktail formulation had little effect on efficiency in the two-tube counter, we see a dramatic improvement for diisopropyl naphthalene in the single tube counter. The results for ¹⁴C (see Figure 9) are similar.

Table 1. Comparison of Counting Efficiencies Obtained from this Study for some Kinds of Solid Support Samples

Nuclide	Sample No.	Glass Fiber Filter (14 mg/cm ²) ^a	Membrane Filter (5.5 mg/cm ²)	Chromatography Paper (8.5 mg/cm ²)	Thin Layer Chromatography Plate (0.1 mm) ^b
³ H	1	29.9	15.5	5.77	7.60
	2	30.9	15.7	5.60	7.02
	3	31.0	15.3	5.56	7.43
	4	30.7	15.3	5.60	7.38
	5	30.0	16.2	5.54	7.58
	Mean ± S.D. ^c	30.5 ± 0.2	15.6 ± 0.2	5.61 ± 0.04	7.40 ± 0.10
¹⁴ C	1	85.6	80.1	71.1	71.1
	2	86.5	82.2	71.4	72.8
	3	86.8	82.5	70.2	71.1
	4	86.5	81.9	70.4	69.5
	5	87.1	81.5	70.6	69.6
	Mean ± S.D.	86.5 ± 0.3	81.6 ± 0.4	70.7 ± 0.2	70.8 ± 0.6

^aSurface density.^bThickness of thin layer.^cStandard deviation.

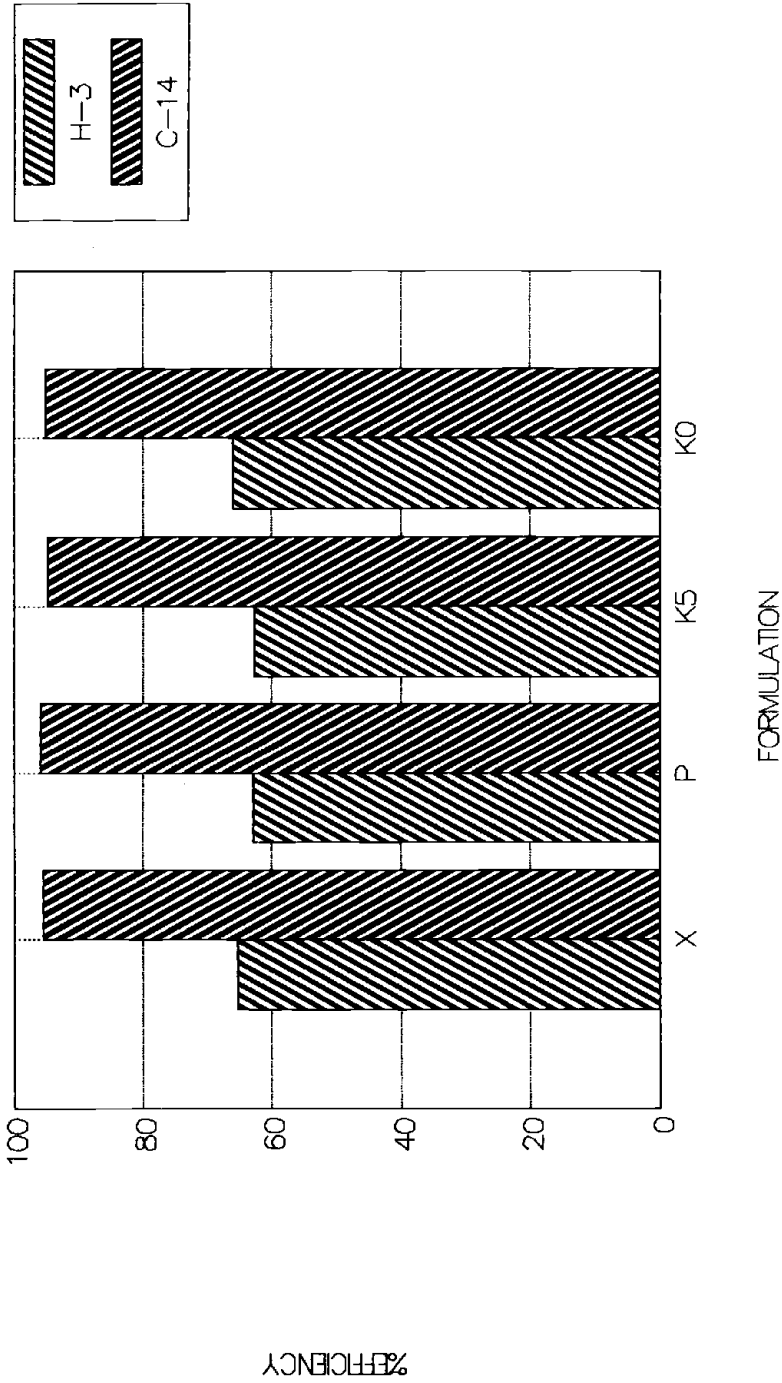


Figure 7. LSC counting efficiencies for paraffin scintillator samples using various solvents: (X) xylene, (P) pseudo-cumene, (K5, K0) diisopropylnaphthalene.

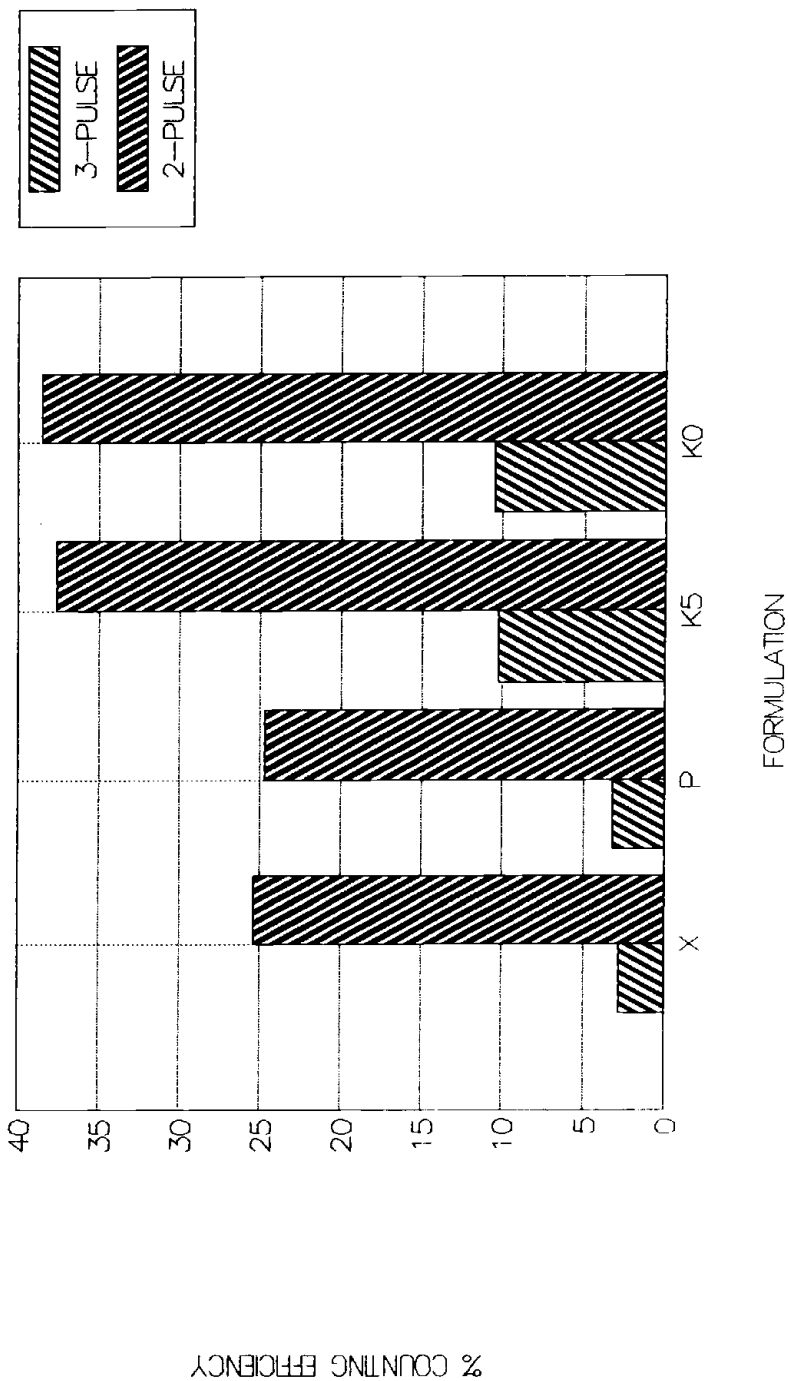


Figure 8. Time resolved single tube counting efficiencies for ³H paraffin scintillator samples using various solvents: (X) xylene, (P) pseudo-cumene, (K5, KO) diisopropylnaphthalene.

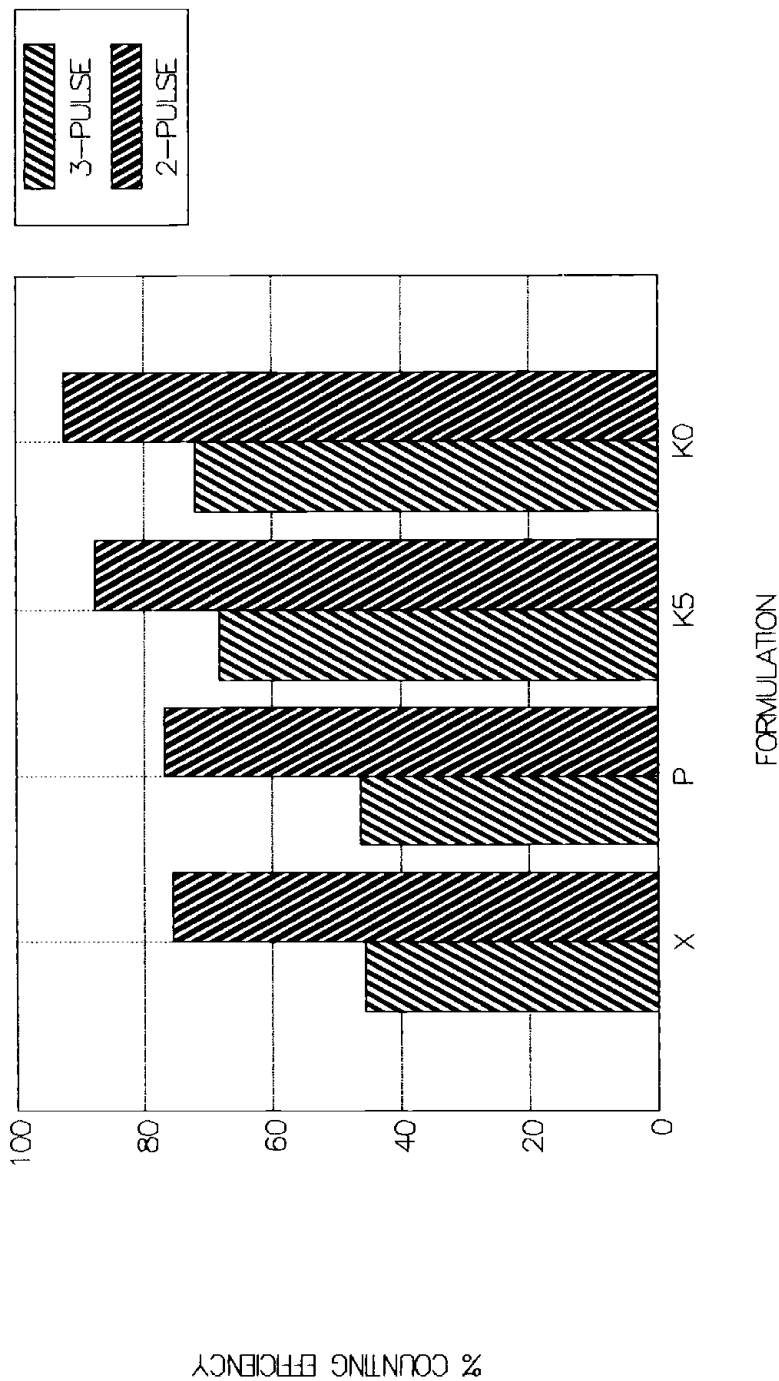


Figure 9. Time resolved single tube counting efficiencies for ^{14}C paraffin scintillator samples using various solvents: (X) xylene, (P) pseudo-cumene, (K5, KO) diisopropyl-naphthalene.

Discussion

The results described above indicate that a solidifying scintillator with good counting performance can be formulated using paraffin as a base material. The scintillator is applied to samples isolated on a solid support while melted. This allows it to impregnate the sample while it is in the liquid state. Because it is solid on cooling, an approximate sample geometry for different counting geometries can be assured.

Chemical quenching is not a concern in solid support counting, since the paraffin scintillator becomes rigid before the quenching substance is eluted into the scintillator. Color quenching, on the other hand, may be present when a colored compound is counted. However, considering the thinness of the sample and the possibility of spreading it over a large area, the color quenching effect can be minimized. These considerations mean that samples prepared using a paraffin scintillator will have uniform counting efficiency, although that efficiency will depend, to a large extent, on the kind of solid support.

In summary, the main advantages of this technique are:

1. Decreased cocktail consumption
2. Reduced disposal costs because each sample generates a small volume of solid radioactive waste
3. Good reproducibility due to improved measurement geometry
4. Easy and rapid sample preparation

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