

CHAPTER 2

Scintillation Counting of Harvested Biological Samples with Low-Energy Beta Emitters, Using Solid Scintillant Filters

C.G. Potter and G.T. Warner

INTRODUCTION

Liquid scintillation counting (LSC) of low-energy beta-emitting isotopes has an important role in the quantitation of biological samples. Although the level of radioactivity is low, the use of liquid scintillant gives rise to disposal problems because of the volume of organic solvents involved.

A significant proportion of LSC work employs samples deposited on filter discs to measure the uptake of labeled compounds into cells and the binding of ligands to receptors. Placing many samples into vials for counting by LSC is a tedious procedure open to identification errors.

Recently methods have been developed whereby a complete filter, bearing many samples, is placed in a thin plastic bag with only a small volume of scintillant (typically 10 mL/96-sample filter) and counted using a flat-bed scintillation counter.^{1,2} This counter has a low background count rate and good counting efficiency, and the commercial version has a high sample capacity and rate of throughput. This makes it particularly useful for lymphocyte assays using ³H-thymidine uptake to monitor proliferation, or to measure ⁵¹Cr release in cytotoxic assays.³ The very small volume of scintillant required makes sample disposal easy as well as economical.⁴

Despite these advantageous features, there are other areas of potential application where the flat-bed counter is at some disadvantage. In particular, where samples are soluble in scintillant, diffusion may result in the movement of activity away from the sample area. Movement may also occur if the sample is not firmly bound to the filter and material is released by the addition and spreading of the scintillant while preparing the filter for counting. Another disadvantage not seen in a vial-counting liquid scintillation counter, is that of inter-sample interference, whereby activity in one sample is detected in an adjacent sample area. The degree of this "cross talk" can be reduced to less

than 0.01% for low energy isotopes by printing black lines on the filters. These absorb light emitted laterally as well as help identify and index during harvesting and placement of the filter on the plate.^{1,2} A further reduction in cross talk, however, is desired when using thick filters, higher energy isotopes, and the most stringent requirements.

The possibility that filters could be composed of scintillant has been previously described.⁵ This paper presents preliminary data on a technique using special filters made of fibers coated with a thin layer of solid scintillant. Radioactive samples are deposited on the filter, dried in the usual way and counted without addition of a liquid scintillant. The beta emission excites the solid scintillant and the events are counted using the flat-bed scintillation counter. Cross talk and background are reduced using this method and the samples have little tendency to migrate. Disposal of samples is further simplified because there is no liquid component. The properties of this type of filter are characterized for different solid scintillants as well as describe a heating method to increase the rather low tritium counting efficiency to a level useable for many applications.

MATERIALS AND METHODS

Preparation of Filters

Conventional harvesting uses glass fiber depth filters of a mean porosity suitable for the samples required, e.g., Whatman GF/A or GF/C for cells, the thicker GF/B for membrane preparations, and GF/F for microorganisms. For the flat-bed scintillation counter (Betaplate, Pharmacia-Wallac OY, Finland), special reinforced versions of these filters were available, printed to facilitate orientation of the filter and reduce cross talk. As well as coating these filters with solid scintillant, the counter uses polypropylene depth filters in a variety of thicknesses and porosities. The solid scintillant was prepared from PPO (2,5-diphenyloxazole) dissolved in toluene (Analar grade) at concentrations up to 2.75%, together with a spectral shifter (Bis-MSB, [1,4-di(2-methylsteryl)-benzine]), added at 10% w/w of the PPO. Tetramethylbenzine (durene, from Aldrich, U.K.), which has a low melting point (55°C), was also added at levels of up to 10% w/v of toluene. The filter sheets were immersed in the scintillant solution, drained and hung up to dry in a fume hood having a fast flow of air. After an hour the filters were completely dry and virtually odorless. This method ensured that a thin coating of scintillant covered each fiber of the filter. When durene was used, it formed a solid solvent as a vehicle for the scintillant proper. Even for the finest porosity filters, there was little reduction in filtration capability with the addition of solid scintillant so that suitably cut sheets could be used in a cell harvester or filtration manifold. Similar filter sheets were prepared for tests using polyvinyl toluene/PPO, butyl-PBD, or anthracene in place of PPO as the scintillant.

Cell Harvesting

The cells used for harvesting tests were K562 cells grown in RPMI and 12.5% fetal calf serum. They were labeled overnight in the flask with $5\mu\text{Ci/mL}$ ^3H -thymidine or $0.1\mu\text{Ci/mL}$ ^{35}S -methionine. Microtitration plates were plated with 0.2 mL aliquots at cell densities of $1-5 \times 10^5$ cells/mL and the aliquots filtered using a cell harvester (Skaatron, Norway) with a 10-sec water wash, followed by a flow of air for 5-10 sec. The filters were generally dried for 2 or 3 hours in an incubator at 37° , or on a hot plate, or in a microwave oven. A methanol wash could not be used as it washed away some of the solid scintillant.

Heat Treatment and Counting Preparation

After drying, filters were placed in thin plastic bags having a low-melting-point inner layer, laminated to an outer heat resistant plastic. Control filters had liquid scintillant added (Betaplatescint, Pharmacia Wallac OY, Finland) and the bag heat-sealed in the usual way. Samples with solid scintillant were counted directly or after heating to melt the scintillant, which could thus permeate the radioactive samples and be better coupled to the electron emission.⁶ Uniformity of heating was achieved in two ways, one by use of a microwave oven where the dielectric losses in the filter and solid scintillant produced heat enough to melt the scintillant after a few minutes exposure. The second method was to pass the dried filter in its bag through the heated rollers of an office laminator (Coated Specialties Ltd., Basildon, U.K.). Thus the filter became compacted and the bag sealed, producing a thin, rugged set of samples ready for counting. The temperature required should not be high enough to produce shrinkage of the composite during lamination, otherwise registration of the samples with the counter support plate will be lost. Too low a temperature will not melt the scintillant. For polypropylene filters the temperature may be made high enough to melt the plastic also producing a transparent set of samples. These may have a pleasing, solid appearance, but the melted plastic can move while under the rollers giving unreliable results.

RESULTS

Glass Fiber Filters

Cells were harvested onto glass fiber filters (Pharmacia-Wallac) with and without solid scintillant. In the first experiment, the amount of PPO in toluene for the coating solution varied between 5.5 and 27.5 g/L also with or without 5% durene. Table 1 shows that the highest amount of PPO provided the highest efficiency, which was enhanced by heating and passing the filter and bag through heated rollers. Addition of durene generally increased efficiency a

Table 1. Relative Counting Efficiencies of Solid Scintillant, with and without Durene, for Different Amounts of PPO + BisMSB (9:1 w/w), Using Samples (n = 12) of ³H-Labeled Cells Filtered onto Scintillant Coated Glass Fiber Sheets

%PPO + BisMSB ^a	Heating ^b	± Durene	Rel. Eff ^c (% ± SD)
0.55	-	-	5.1 ± 0.4
	-	+	4.3 ± 0.5
	+	-	9.4 ± 1.5
	+	+	12.3 ± 1.3
1.10	-	-	5.6 ± 0.7
	-	+	7.2 ± 0.5
	+	-	12.7 ± 2.4
	+	+	17.9 ± 1.2
1.65	-	-	5.9 ± 0.9
	-	+	6.7 ± 0.6
	+	-	18.9 ± 1.5
	+	+	23.1 ± 1.9
2.20	-	-	7.0 ± 0.9
	-	+	8.5 ± 0.6
	+	-	20.5 ± 2.0
	+	+	26.6 ± 2.0
2.75	-	-	10.3 ± 1.7
	-	+	10.7 ± 0.7
	+	-	26.6 ± 3.5
	+	+	26.1 ± 1.8

^aPPO + BisMSB dissolved in toluene, with or without 5% durene and used to soak filters dried and used in a filtration assay of ³H-thymidine-labeled K562 cells.

^bHeating by the heated rollers of office laminator.

^cRelative efficiency, compared with parallel samples counted in the flat-bed scintillation counter using BetaplateScint cocktail.

little and could partly replace PPO. At the higher quantities of PPO or with durene present, the filters became stiffer and filtration became a little impaired. In another experiment (Table 2), butyl-PBD showed a similar efficiency to PPO before heating, but it was reduced after passing the filters through the heated rollers. Addition of durene did not improve efficiency either before or after heating. Table 3 shows the results of an experiment comparing 2% PVT/toluene with 2.75% of either butyl-PBD or PPO, both with and without durene, (increased to 10%) in toluene. Again there was no difference or reduction in counting efficiency for butyl-PBD and PVT, even when using microwave heating, which would not produce the compaction made by heated rollers. Anthracene with or without durene was also tried and heating efficiency improved slightly. One noteworthy aspect was that the energy spectrum for anthracene was shifted to higher levels compared with the others scintillants tested.

³⁵S-labeled Samples

For cells labeled with ³⁵S-methionine and harvested onto plain or printed glass fiber filters with PPO/BisMSB/durene solid scintillant, the efficiency

Table 2. Relative Counting Efficiencies of Solid Scintillant, with and without Durene and for Different Amounts of Butyl-PBD, Using Samples (n = 12) of ³H-Labeled Cells Filtered onto Scintillant-Coated Glass Fiber Sheets, with and without Heating

Relative Counting Efficiency of BetaplateScint (% ± SD)			
% of Butyl-PBD ^a	Heating ^b	- Durene	+ Durene
0.5	-	3.8 ± 1.3	5.4 ± 1.8
	+	3.4 ± 1.4	4.0 ± 1.2
1.0	-	8.5 ± 2.6	7.8 ± 2.9
	+	6.9 ± 2.1	7.0 ± 2.3
1.5	-	8.1 ± 2.4	9.0 ± 2.5
	+	6.7 ± 2.2	8.1 ± 2.2
2.0	-	13.0 ± 3.5	7.2 ± 1.8
	+	10.7 ± 3.0	7.0 ± 2.0
2.5	-	10.8 ± 2.6	8.3 ± 2.0
	+	8.0 ± 1.8	7.3 ± 1.9

^aButyl-PBD dissolved in toluene, with or without 5% durene, and used to soak glass fiber filter, which was dried and used in a filtration assay of ³H-thymidine-labeled cells.

^bHeating by the heated rollers of an office laminator.

Table 3. Relative Counting Efficiencies of Solid Scintillants with Different Compositions, Coated on Glass Fiber Filters and Used to Harvest ³H-Labeled Cells

Fluor	Heating ^a	± Durene ^b	Rel. Eff. ^c (% ± SD)	N
Liquid scintillant control	None	-	100.0 ± 8.8	20
Polyvinyltoluene plastic scintillant 2% in toluene	None	-	8.4 ± 0.5	20
	μ	-	8.5 ± 0.6	
	μ + roll	-	4.3 ± 0.5	
Butyl-PBC 2.5% in toluene	None	-	8.9 ± 0.7	10
	μ	-	8.9 ± 0.4	
	μ + roll	-	7.6 ± 0.4	
	None	+	9.0 ± 0.4	
	μ	+	9.1 ± 0.7	
PPO + 10% w/w BisMSB; 2.75% in toluene	None	-	7.7 ± 0.7	20
	μ	-	10.8 ± 1.4	
	μ + roll	-	19.2 ± 1.7	
	None	+	7.5 ± 1.3	
	μ	+	10.3 ± 0.8	
	μ + roll	+	22.7 ± 2.5	

^aμ = microwave oven heating (400 W for 120 sec); roll = microwaved samples passed through office laminator (>90°C).

^bDurene as 10% of the solution used to make the solid scintillant filters.

^cRelative efficiency compared with parallel samples counted in the flat-bed scintillation counter using BetaplateScint cocktail.

Table 4. Relative Counting Efficiencies, Cross Talk and Background Count Rates for Glass Fiber Filters Coated with Solid Scintillant (PPO/BisMSB/durene) and Used to Harvest ³⁵S-methionine-Labeled Cells, with and without Subsequent Heating

Treatment	Plain Glass Fiber				Printed Glass Fiber			
	bg	cpm	CV%	xt%	bg	cpm	CV%	xt% ^a
Solid scintillant set 1 (n = 12)	4.3	42181	9.2	0.024 (10.0 cpm)	3.0	50065	6.8	<0.001 (0.3 cpm)
Set 1 + liquid scintillant	11.1	128590	5.0	0.020 (25.7 cpm)	14.8	126234	4.5	0.011 (14.3 cpm)
Relative efficiency			32.8				39.7	
Solid scintillant set 2 (n = 12)	3.9	43746	8.8	0.007 (3.0 cpm)	2.2	53463	4.8	<0.001 (0.4 cpm)
Set 2 + heating by laminator	5.1	53160	8.0	0.002 (1.1 cpm)	2.6	68628	4.9	0.002 (1.6 cpm)
Relative efficiency			40.0				50.1	
Increase in relative efficiency with heat			22				28	

^aCross talk—mean of 12 adjacent blanks less backgrounds.

was only slightly increased by heating. Table 4 shows that the efficiency reached up to 50% of glass fiber using liquid scintillant. Background count rates were also low, but in addition, these filters showed good inter-sample cross-talk characteristics even without printing, although with the addition of printed lines the cross talk fell to very low levels which are not easily measured, i.e., <0.001%. Similar experiments with ³H-labeled cells gave no measurable cross talk, with or without printing.

Polypropylene Filters

Samples of several types of polypropylene depth filters were obtained from three suppliers. Each was tested by harvesting cells labeled with ³H-thymidine onto the polypropylene filters with solid scintillant and comparing them with controls harvested on glass fiber or polypropylene filters with liquid scintillant. Any reduction in the effectiveness of filtration, together with any self-absorption or quenching, should produce a reduced count rate. As before, filters bearing solid scintillant were counted directly or after heating by microwave or passing through heated rollers. Some filters were found to have too coarse a mesh to filter out the majority of cells and others were too flimsy to handle satisfactorily. The properties of some suitable filters are illustrated by data on three examples, designated A, B, and C. Table 5 shows that the count rate for the polypropylene filters with liquid scintillant was slightly reduced compared to glass fiber. Counting solid scintillant filters directly gave relative efficiencies between 7.4 and 17.9%, which was increased by microwave radiation. A further increase was produced by hot rolling, to a maximum of 33.5% for sample B. At temperatures high enough to melt the polypropylene, counting efficiency was reduced and variation increased. Background count rates

Table 5. Relative Efficiencies (% \pm CV) for ^3H -Labeled Cells Filtered onto Solid Scintillant Polypropylene Filters

Treatment	Type of Filter (Porosity)		
	A (2.5 μ)	B (10 μ)	C (?)
Liquid scintillant ^a	91.8 \pm 5.5	78.5 \pm 6.8	89.7 \pm 7.5
Solid scintillant ^b	13.3 \pm 8.1	17.9 \pm 4.9	7.4 \pm 10.6
+ microwave	16.6 \pm 8.4	24.8 \pm 15.7	9.0 \pm 12.8
+ microwave and heated rollers	23.6 \pm 7.8	33.5 \pm 8.4	21.3 \pm 11.5

^aBetaplateScint, CV for glass fiber was 5.9%.

^b2.75% PPO/BisMSB, 10% durene/toluene.

were very low unless the samples were melted, which probably increased optical cross talk between the pairs of photomultipliers.

Linearity with Cell Density

Using glass fiber filters and liquid scintillant, linearity of count rate with cell density is possible over a wide range.³ In order to test whether this was also true for solid scintillant filters, microtitation plates were set up with 6 replicate wells at cell densities ranging from approximately 10^3 to 4×10^5 cells/well and harvested on glass fiber filters with or without solid scintillant. Table 6 shows that linearity was maintained over this range, before and after passing the samples through heated rollers.

Uniformity of Samples

A complete microtitre plate was set up with approximately 2.5×10^5 cells/well and harvested using a glass fiber filter, coated with solid scintillant, giving a matrix of 6×16 samples. Twelve more samples in another plate were also harvested using a standard glass fiber filter with liquid scintillant and gave a mean of 107,800 cpm with a 2.2% coefficient of variation (CV). The solid scintillant filter was counted directly (mean of 10,995 cpm) and after passing through heated rollers (mean of 18,203 cpm). Table 7 shows that the normalized means for each row exhibit a significant positive regression, either when

Table 6. Linearity of Relative Counting Efficiency for Different Cell Densities Harvested onto Glass Fiber Filters Coated with Solid Scintillant.^a Expressed as Solid Scintillant Filter Count Rate/Liquid Scintillant Control Count Rate (% \pm SD)

Cell Density $\times 10^3$ /Well	Before Heating	After Heating
380	6.49 \pm 0.70	11.19 \pm 1.15
114	6.14 \pm 0.83	10.97 \pm 2.03
38	6.73 \pm 1.46	11.82 \pm 2.62
11.4	6.18 \pm 0.66	10.31 \pm 1.71
3.8	5.79 \pm 1.15	9.47 \pm 2.16
1.1	6.98 \pm 1.65	12.25 \pm 3.00

^a2.75% PPO/BisMSB/5% durene in toluene.

Table 7. Regression for Normalized Data ($y = a + bx$) of Means of Rows ($n = 16$, 6 in each row) for Cells Filtered onto Glass Fiber, and for Glass Fiber or Polypropylene Filters Coated with Solid Scintillant Spiked with ^3H -hexadecane, Before and After Heating.

Type of Filter	\pm Heating	a \pm SD	b \pm SD	t	p	r
Glass fiber	-	90.5 \pm 2.6	1.12 \pm 0.27	4.1	<0.01	0.74
+ cells	+	84.8 \pm 2.2	1.78 \pm 0.23	7.8	<0.001	0.90
Glass fiber +	-	94.4 \pm 1.1	0.72 \pm 0.12	6.1	<0.001	0.85
^3H -hexadecane	+	100.4 \pm 1.1	-0.05 \pm 0.11	0.5	NS	0.13
Polypropylene +	-	86.1 \pm 2.6	1.64 \pm 0.27	6.1	<0.001	0.85
^3H -hexadecane	+	85.5 \pm 5.7	1.70 \pm 0.59	2.9	<0.02	0.61

counting the filters directly, or after heating. The average CV for the rows, which allows for the effects of this regression, was 7.9% for direct counting and 9.5% after heating. These values and the regression when using glass fiber filters, show that the performance falls short of standard methods using liquid scintillant. In contrast, in another test, using polypropylene filters with solid scintillant, a single set of samples from 12 wells was harvested and the sample was dried and passed through the hot rollers. These gave a CV of 4.5%, compared with controls on a standard glass fiber filter with liquid scintillant, which gave a CV of 5.6%. Therefore, these polypropylene filters were capable of as good a consistency in any row as for the standard glass fiber filters using liquid scintillant, although any regression according to the sample row detracts from the technique.

It is possible that these problems of variation could be explained if the deposition of solid scintillant on the filter was not homogeneous. To test this, a standard glass fiber filter was prepared with solid scintillant spiked with ^3H -hexadecane. After preparation, the filter was dried and counted, before and after heating. The results, also in Table 7, showed a clear positive regression according to the sample row when counted direct, but the regression became insignificant after heating. The count rate also decreased, indicating that some quenching or self absorption of the activity had occurred which was associated with compaction of the glass fiber filter while melting the solid scintillant. A similar experiment with a thin grade of polypropylene showed no decrease in count rate after heating, and the regression was also unchanged (Table 7). These results are in contrast to the samples derived from cells which showed an increase in count rate after heating. Presumably the increased counting efficiency due to scintillant permeation of the samples is greater than any reduction due to compaction-produced self-absorption. The variation in count rate for harvested cells therefore appears to be associated with different amounts of deposited solid scintillant and is probably due to the solid-scintillant solution pooling at the lower end of the filters during the drying process. It is therefore evident that any production of these filters should minimize this variation.

DISCUSSION

The experiments described, indicate that a useful efficiency can be obtained for solid scintillant filters especially when the scintillant is melted using the hot rollers of an office laminator. Further improvements occur when polypropylene filters are substituted for glass fiber. In general, the higher the efficiency the smaller the coefficient of variation obtained, and satisfactory results were obtained with a PPO/durene/polypropylene combination. There is a rather complex interaction between the increased efficiency obtained by filter compaction, which may bring particles of the sample closer to the solid scintillant, and any possible losses of light output, due to the increased absorption by the filter/scintillant matrix. Reduced optical efficiency produced by compaction was most apparent where the scintillant did not melt at the temperatures achieved during the hot rolling. This reduction was greatest for the PVT plastic coating and less for crystalline butyl-PBD. For anthracene, however, there was an increase in counting efficiency. Perhaps, because the energy output is higher for this scintillant, fewer events are lost through quenching, while with the proximity of the compacted sample to the solid scintillant, more of the sample may be exposed to the scintillant. Any reduction in efficiency produced by compaction appeared to be less deleterious using polypropylene. When polypropylene filters bearing cells were used with liquid scintillant the polypropylene is a slightly less effective filter. Rather than being due to a poor optical performance, preliminary experiments show that these filters, with or without solid scintillant, trap a smaller proportion of material harvested than do glass fiber filters, even though the porosity of 10μ should retain whole cells entirely. It is likely however, that cells are rapidly lysed when washed from the microtitre plate with water. Reduced retention of this partial lysate is due to differences in the surface properties of the polypropylene or the solid scintillant (which are both hydrophobic), as compared with the (hydrophilic) surface of glass fiber. It is possible that the surfaces of other filters and solid scintillants will be developed for other porosities and may retain nearly 100% of the sample. Indeed, other scintillants and solid solvents that increase the efficiency still further may also be developed, although the level described here would be adequate for many applications. The technique is useful in that it reduces background count rate and cross talk as well as eases disposal. Furthermore, the lack of sample movement during preparation or its diffusion afterwards, makes the counting of many filtered particulate samples possible using the flat-bed scintillation counter.

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