

EVALUATION OF LIQUID SCINTILLATION COUNTING ACCURACY:
EFFECT OF VARIOUS CHEMICAL QUENCHING AGENTS AND
EFFECT OF MILKY SAMPLE PREPARATIONS

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ABSTRACT

In liquid scintillation, the quality of sample preparation is as important to accurate results as the quality of instrumentation. When a researcher evaluates a sample preparation, the most important factor is the accuracy of his results. A particularly useful method of evaluation is by dpm error. By starting with a sample with accurately known dpm, one counts the sample under experimentally relevant conditions, such as sucrose gradients, deposition on filter support or preparation in a saline solution. The dpm of the sample is calculated from a quench curve and compared to the known dpm value. An error of less than 5% in the calculated value would indicate a valid preparation. While this is not a new concept, it is extremely sensitive and simple. The sensitivity of the technique is demonstrated in the two evaluations reported here.

Commercial preparation of sealed quenched standards is not uniform. Various quenching agents are used by different manufacturers. Carbon tetrachloride and nitromethane are the most common quench agents used by commercial manufacturers. Examination of several different quenching agents, chloroform, carbon tetrachloride, 1-bromopropane, p-bromotoluene, nitromethane, nitrobenzene, 1,2,4-trichlorobenzene, α , α , α -trichlorotoluene, by the dpm error method demonstrated only small quench correction errors associated with different quench agents. One notable exception is 1,2,4-trichlorobenzene which seems to involve some undefined annihilation process. In the case of tritium this led to a 10%-15% dpm error at high quench (5-10% efficiency) levels. The results were independent of method of quench monitor (H# or SCR).

Recently some manufacturers have touted counting in milky/chalky preparations. The dpm error of milky sample preparations in standard

20 ml glass vials ranges from 10-60% error depending on cocktail and sample preparation but independent of quench monitor method. The calculated CPM value is always low. Comparison of a clear preparation with a milky preparation of the same tritium activity and quench value results in lower cpms of the milky preparation while the relative distribution of the sample pulses remains the same. This implies some type of annihilation process. Apparently, the opaqueness of the sample is acting as a light trap for those photons generated in the interior of the vial. This was confirmed by an experiment where the dpm error is greatly reduced by reducing the diameter of the vial containing the milky preparation.

INTRODUCTION

Problems with sample preparation can lead to large errors in sample activity in liquid scintillation counting. Sometimes these errors can be subtle and investigators unknowingly fall into them. This paper will discuss two problems that can lead to large counting errors. The first problem is the effect of the quenching agent used when making the quench curve. The second problem discussed will be errors introduced by the turbidity of the sample.

The method for determining errors is a simple yet quantitative technique. Using samples with known dpm, one determines the experimental dpm and compares the two values. The unit of measure is dpm error. It is calculated using formula 1.

$$\text{Formula 1: } \frac{(\text{dpm known} - \text{dpm calc})}{\text{dpm known}} \times 100\% = \text{dpm error}$$

Repeatable and accurate pipetting of known quantities of radioisotope is the crux of this method. One monitors the precision of pipetting of the radioactive spike by counting the samples before addition of quench agent or other manipulation. If prepared well, the cpm of each sample should be the same. The dpm of the samples are known if prepared with calibrated pipets from a solution of accurately known dpm.

EXPERIMENTAL

Samples and standards were prepared using ^3H and ^{14}C solutions from NEN with dpm reported to $\pm 3\%$. The preparations were counted on a Beckman LS 9800 or LS 1800 system. The standards were counted three times each to a 0.5% two sigma error (160,000 counts). When monitoring with H number, the average of 10 H numbers was taken for each sample. When monitoring by SCR, the sample was counted until the channel with the least activity achieved 0.5% two sigma error. The same standards were used to establish both the H# and SCR quench curves. Samples were counted two times each to a 1% two sigma error (40,000 counts). All samples were prepared in duplicate. Preparations used for standards had the same cpm $\pm 0.8\%$ before quenching with nitromethane. Preparations used for samples had the same cpm $\pm 1.2\%$ before further manipulation.

RESULTS AND DISCUSSION

Samples with known dpm were prepared with a variety of chemical quenching agents and counted against a standard curve. The results of the dpm error are summarized in Table 1 (H number quench correction) and Table 2 (SCR quench correction). Errors of greater than 6% are indicated in italics. For the great majority of cases, there is little or no error introduced by using different chemical quenching agents. When the tritium efficiency is greater than 20%, very little effect is noted among any of the quenching agents. (Aqueous samples seldom quench below 20% tritium efficiency, even at a 50% aqueous sample load). No effect was observed using different chemical quench agents when ^{14}C was the isotope.

There are anomalies, however. Samples quenched with 1,2,4-trichlorobenzene count at a lower efficiency than predicted by either H number or SCR. The errors become very significant at tritium efficiency below 12%. In a separate experiment, 5 samples of 440,000 dpm HTO quenched with trichlorobenzene to an H number of about 300 were compared to 5 samples of 440,000 dpm of HTO quenched with nitromethane to the same H number and SCR value. The cpm of the trichlorobenzene quenched sample were 20% lower than the "equivalent" nitromethane quenched sample (see Table 3).

Table 1. % DPM Error for Chemical Quenches
 Quench Monitor H# Label H3, H₂O, 400,000 dpm
 Quench Curve Based on Nitromethane Quench
 Cocktail Ready-Solv HP/b, 10 mL

Tritium Efficiency	CCl ₄	CH ₃ Cl	1-Br Propane	CH ₃ COCH ₃	CH ₃ NO ₂	NO ₂	CCl ₃	Cl	Cl	CH ₃ Br
50-40%	1.0%	1.0%	1.0%	1.0%	1.0%	1.0%	1.0%	1.0%	1.0%	1.0%
39.9-30%	-4.0%	-	-2.7%	1.0%	-3.5%	-3.5%	-3.3%	1.0%	1.0%	-4.1%
29.9-20%	-4.3%	-	-3.3%	1.0%	-2.8%	-4.4%	-5.4%	-7.2	-6.2%	-2.8%
19.9-12%	-2.6%	-2.6%	-2.3%	-1.1%	+1.5%	-1.4%	-4.4%	-6.2%	-10.0%	-2.6%
11.9-8%	-3.4%	-3.8%	-3.7%	-2.9%	+1.5%	-1.4%	-5.3%	-10.0%	-11.2%	-3.8%
7.9-3%	-4.0%	-6.1%	-4.9%	-2.7%	+2.8%	1.0%	-6.0%	-11.2%	-11.2%	-5.9%

Table 2. % DPM Error for Chemical Quenchers
 Quench Monitor SCR Label H3, H O, 400,000 DPM
 Quench Curve Based on Nitromethane Quench
 Cocktail Ready-Solv HP/b, 10 mL

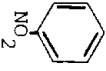
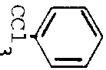
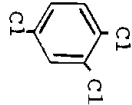

Tritium Efficiency	CCl ₄	CHCl ₃	1-Br Propane	CH ₃ COCH ₃	CH ₃ NO ₂				
50-40%	1.0%	1.0%	1.0%	1.0%	1.0%	1.0%	1.0%	1.0%	1.0%
39.9-30%	-2.2%	-	1.0%	-3.8%	1.0%	1.0%	1.0%	1.0%	1.0%
29.9-20%	-2.9%	-	1.6%	-9.6%	-1.0%	-1.0%	1.0%	-3.9%	1.0%
19.9-12%	-2.3%	-2.5%	1.0%	-12.9%	+3.3%	-1.8%	1.0%	-5.0%	+1.9%
11.9-8%	-1.7%	-1.4%	1.0%	-17.9%	+2.8%	1.0%	1.0%	-8.6%	+2.0%
7.9-3%	-2.0%	-1.8%	-2.7%	-20.0%	+1.3%	1.0%	-1.0%	-10.9%	1.0%

Table 3. Quench Effects of Trichlorobenzene Sample 10 mL Ready Solv MP, 0.8 mL H₂O, 0.2 mL HTO

Quench Agent	Quantity (Microliters)	H#	SCR	dpm	dpm
CH ₃ NO ₂	64	296.1	0.4251	440,000	24,400
C ₆ H ₃ Cl ₃	490	299.5	0.4243	440,000	19,600

Table 4. Effect of Aqueous Halide Ions on DPM Error
 Sample: 1 mL Aqueous Solution
 Solution: 10 mL Ready Solv-MP

Aqueous Solution	CPM	H#	DPM Error
D.I. Water	81,670	95.1	- 2.6
0.1M NaCl	81,076	96.4	- 2.7
0.2M NaCl	81,325	97.0	- 2.0
0.6M NaCl	81,007	98.2	- 1.8
1.0M NaCl	79,706	99.2	- 2.9
1.4M NaCl	81,173	99.3	- 1.0
2.0M NaCl	81,345	99.2	- 0.9
0.2M NaBr	80,382	101.1	- 1.0
0.6M NaBr	77,431	104.8	- 2.8
1.0M NaBr	77,807	106.5	- 1.4
1.6M NaBr	77,126	108.3	- 1.2
2.0M NaBr	76,515	109.8	- 1.2

The low count rate appears to be due to an annihilation process since sample pulse height spectra have the same distribution as similarly quenched samples with other quench agents. Only those samples quenched with 1,2,4-trichlorobenzene had lower efficiency. The mechanism is unclear. It is not due to high chlorine content. Results in Table 4 indicate that increasing halide content has little

effect on the cpm and no effect on the dpm error¹. Even if the chlorine is organic soluble such as chloroform, no similar error is detected (See Table 5). Counting in small vials does not decrease the error. These results indicate a Beta annihilation process of unknown origin.

Table 5. Effect of Organic Chlorine on DPM Error

Quench Agent	Volume (Microliters)	mMoles	H Number	DPM Error
CHCl_3	500	6.25	258	- 3.8%
$\text{C}_6\text{H}_3\text{Cl}_3$	500	4.01	289	- 11.2%

There is a second anomaly. Samples quenched with acetone dpm correct well with H number but very poorly with SCR. Even at rather high tritium efficiency, 20-29%, the error is almost 10% by SCR. An additional experiment compared three samples with the same tritium efficiency, one quenched with acetone, one with nitromethane and one with carbon tetrachloride. Each of the samples had the same H number and ESCR value. However, the SCR value of the acetone sample was significantly different from the other two (See Table 7).

Not only does the acetone sample have a different SCR value but the pulse height spectrum has been shifted towards higher pulses. This is most unusual since there is no increase in count rate! This shift is true whether the tritium label is on toluene or water. This unusual shift to higher pulses for acetone quenched samples can also be seen in the spectra of Cadmium-109 (22.1 KeV Silver X-rays) and Iodine-125 (27.5 KeV X-rays). Speculation about this unusual behavior has not produced a viable explanation.

For the experimenter, these results indicate that use of commercial quench sets quenched with either carbon tetrachloride or nitromethane pose no serious source of error for most chemical quench applications. Caution should be exercised with tritium when efficiencies are below 15% because the species causing quench might

Table 6. Comparison of Quench Monitor Values for Samples with Different Quench Agents at same Counting Efficiency

	CCl ₄	Quench Agents CH ₃ COCH ₃	CH ₃ NO ₂
CPM	43469	43875	44874
DPM	400,000	400,000	400,000
EFF	10.9	11.0	11.2
H #	243.3	242.6	243.0
ESCR	.2146	.2151	.2060
SCR	.4265	.4594	.4242

not correct to the commercial quench set. Obviously acetone and 1,2,4-trichlorobenzene should be avoided.

The second section deals with errors introduced by counting milky or turbid samples. While these milky emulsions may provide stable counting conditions^{2,3} and be single phase, they are quench correctible only under certain conditions. Three different preparations were examined in vials of three different inside diameters. Single phase was assured by the Beckman two phase monitor and dual ratio plot techniques. The three solutions were: a clear aqueous preparation; an aqueous gel preparation and an aqueous milky preparation. The results are summarized in Figure 1. For the clear aqueous preparation, the size of the vial had little effect on the dpm error. As the sample increased in turbidity from the example of the gel to the milky preparation, the amount of error greatly increased with the increase of the diameter of the vial. This was true whether monitoring quench with H number (Figure 1) or SCR (Table 7).

The results appear to point to an annihilation mechanism. However, in this case, it is not annihilation of the Beta particle but rather of light pulses since dpm correction can be made in the smaller diameter vials. If annihilation, then one should expect that the sample pulse height spectrum would have the same boundaries and same SCR value. Indeed, they do. Figure 2 shows the pulse height spectrum for a clear solution with H# 63.6 and 100,000 dpm of tritium superimposed on a pulse height spectrum for a milky sample with an H#

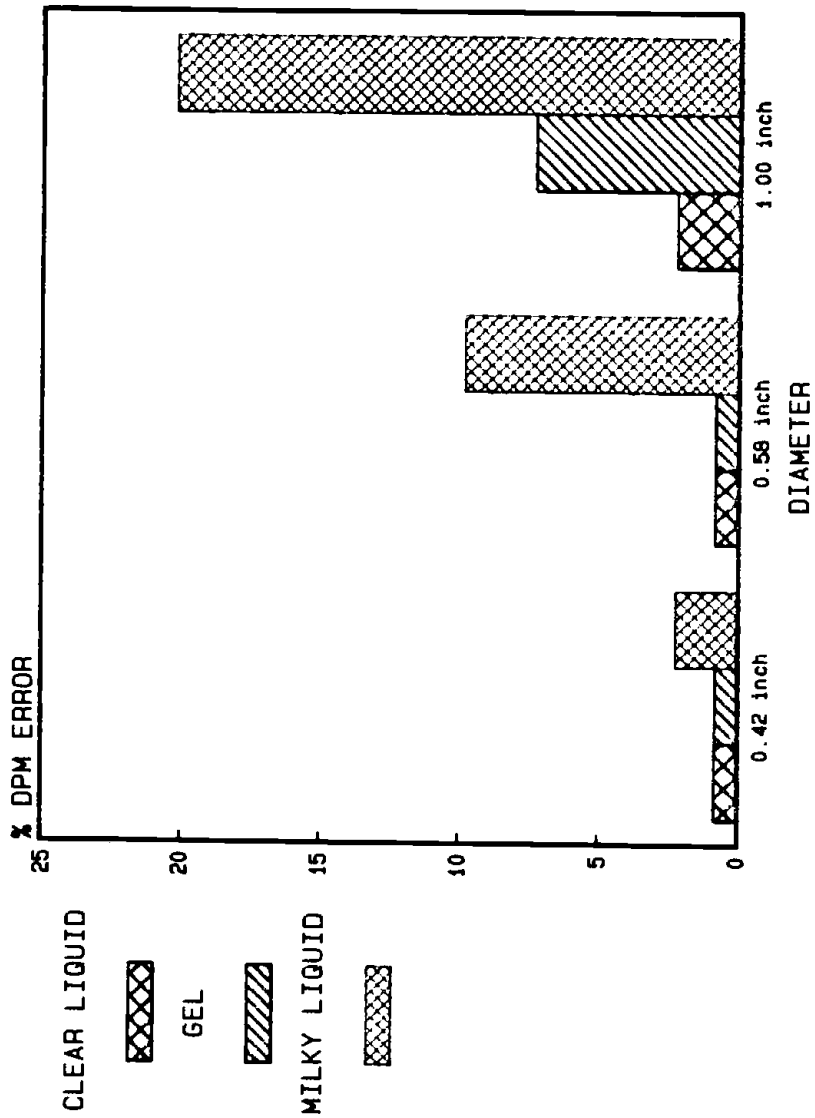


Figure 1. Effect of vial diameter on error.

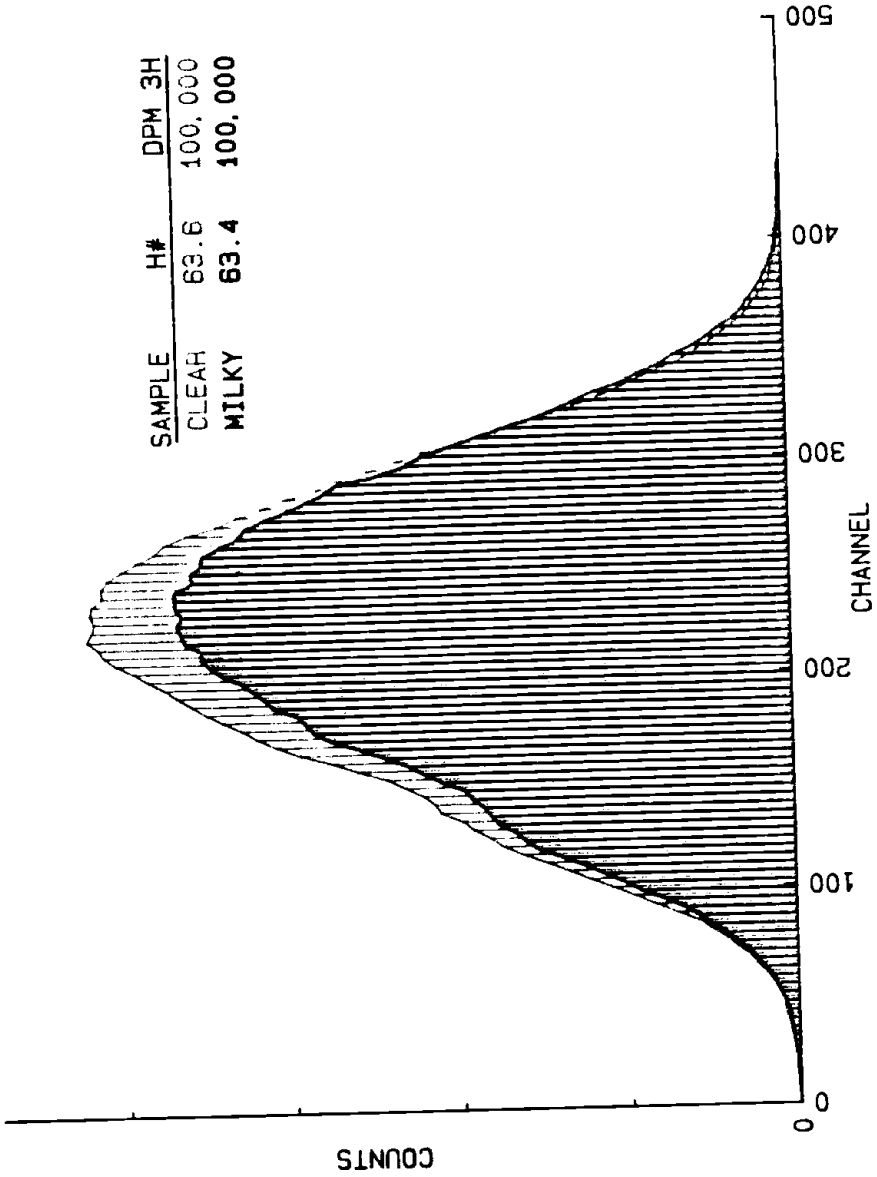


Figure 2. Pulse height spectrum for a clear (light) and milky (dark) sample.

Table 7. DPM Error of Milky Sample by SCR

Vial I.D.	DPM \pm 3%	CPM \pm 0.5%	SCR \pm 0.5%	DPM Error	Theoretical Error
0.99	1 x 10 ⁵	38,300	0.82	21.5	\pm 4.0%
0.58	1 x 10 ⁵	43,700	0.83	13.8	\pm 4.0%
0.42	1 x 10 ⁵	51,090	0.85	5.8	\pm 4.0%

of 63.4 and 100,000 dpm of tritium both samples in one inch diameter vials. The limits of the curves are the same. The SCR for the clear sample is 0.590 while for the milky sample, it is 0.603, essentially the same.

Clearly, these results indicate that "light annihilation" can occur in turbid samples caused by internal reflectance causing either loss of light or loss of coincidence. Errors in counting turbid samples can be substantially reduced if not cured by using smaller diameter vials.

REFERENCES

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