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B. E. Gordon

August 1979

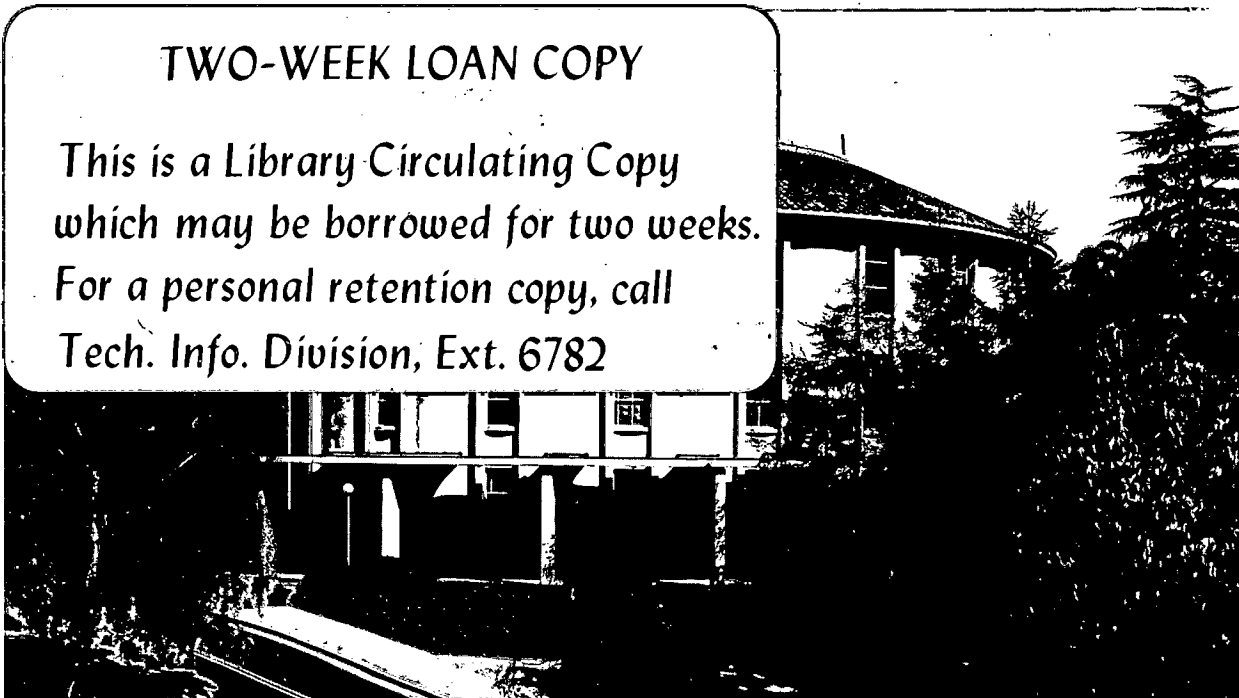
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A CRITICAL EXAMINATION OF SOME
NEWLY AVAILABLE STANDARDS
FOR LIQUID SCINTILLATION COUNTING

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ABSTRACT

Some newly available encapsulated sources of carbon-14 and tritium have been evaluated for their usefulness as accurate, convenient internal standards in liquid scintillation counting. They have been found to be as accurate and reproducible as claimed and provide the practitioner with a clean, rapid method for using the widely applicable internal standard method. The water-soluble tritium standard (sucrose) requires a minimum water concentration (1.3% for Aquasol-2) in a detergent cocktail before it will completely dissolve. Efficiency determination of colored and gelled samples is considerably simplified as is the preparation of quenched standard sets in any cocktail system.

INTRODUCTION

Liquid scintillation counting is one of the central analytical methods in biochemical, biological and medical research. Because results are best expressed in disintegrations per minute (dpm), counters employ several methods to measure the counting efficiency. Among these are two automatic methods, the external standard channel ratio (ESCR), and Compton edge shift (H #)¹, and one manual method, the internal standard method (ISM). The first two depend upon a set of quenched standards to permit one to generate a quench curve wherein the counting efficiency is plotted against one of the above parameters. The ISM, while more widely applicable is also more tedious, requiring the manual addition of a spike and two counts.

Because liquid scintillation practitioners employ a multitude of cocktails, it is often of concern whether a quench curve from a sealed, quenched standard set, prepared in one cocktail is suitable for samples in a different cocktail. There are a number of detergent cocktails commercially available, and some of these are of novel (and undisclosed) composition. Samples in these cocktails are counted in the sol or gel phases. In addition, highly colored samples may not be accurately determined by comparison with a chemically quenched standard set. These and gelled samples require the use of the ISM for accurate results.

The foregoing point to two clear needs in modern liquid scintillation practice. The first is a simple, rapid, and accurate method of preparing a quenched set of standards in any desired cocktail. The second is a more rapid and convenient method of using the internal standard method. The latter is particularly desirable

when counting efficiency is determined automatically via reference to a standard quench curve. The ISM should be used with one or more samples from every large set to make sure that there has been no significant shift in the standard quench curve.

Our approach to the above problems has been to dispense internal standard spikes via a Gilman Manostat microburette, 1 ml volume, in increments of 10-20 μ l. Standard carbon-14 toluene, tritiated toluene, and tritiated water are used depending on whether the sample is hydrophilic or oleophilic. This method of dispensing standards has also been used in preparing sealed, quenched standard sets in particular cocktails. In this case, the vial screw cap is permanently sealed by applying epoxy glue to the glass vial threads. Both mini- (7 ml volume) and maxi- glass vials (22 ml volume) have been so prepared. Plastic vials should not be used to prepare sealed standard sets.

The above technique has been both tedious and subject to a somewhat larger error than desirable due to the imprecision of the burette, particularly when the displacing mercury gets very dirty. Disassembly and cleaning as well as the need to calibrate the standard solutions against NBS standards all have mitigated against the wider use of internal standards.

Recently, there appeared on the market capsules of standard carbon-14 tristearin, tritiated tripalmitin, and tritiated sucrose on small glass capsules, OXI-Test(RIC)^a. Micrograms of each of the standards

(a) Radiomatic Instruments and Chemical Co. (RIC), 80 Fairbanks St., Addison, Ill, 60101.

are deposited on the inner surface of the capsule which is then sealed into a tough plastic strip. The capsules are pressed from the strip into the vial where the labeled compound dissolves. The presence of the glass capsule has no effect on the counting efficiency. High precision and accuracy for the amount of radioactivity in each capsule is claimed in an accompanying data sheet.

Experimental

The accuracy and precision of the putative dpm of each isotope type was tested by dissolving five replicates of each in 15 ml of a cocktail. The oleophilic standards of carbon-14 (CO) and tritium (HO) were dissolved in toluene scintillator, the hydrophilic tritium standard (HW) was dissolved in 15 ml of Aquasol-2 detergent cocktail to which 500 ul of water had been added. Counting was to a 2σ value of <0.5%.

Following this first count, spikes of standard carbon-14 toluene, and standard tritiated toluene were added to the toluene scintillators and standard tritiated water to the detergent cocktail. The specific activities of all three had been previously determined by comparison with NBS standards. This experiment determined the accuracy and precision of the capsules.

Table 1. Accuracy and Precision

<u>RIC OXI-Test</u>	<u>cpm</u>	<u>dpm determined by Internal Standard</u>	<u>Stated DPM</u>
¹⁴ CO	40179 <u>±</u> 0.1%	53,026	53,600
³ HO	50626 <u>±</u> 0.8%	99,026	98,600
³ HW	35921 <u>±</u> 1.4%	91,354	89,200

Both the precision and the accuracy are acceptable. The slightly poorer precision of the HW standards is in agreement with the stated precision of $\pm 1.2\%$.

When these capsules were put into use by some of the research workers in this laboratory, the tritiated sucrose capsules (HW) received considerable criticism due to erratic data. This was traced to their low solubility in the detergent cocktail when no water was added to the cocktail. No such problem arose with the oleophilic tracer (HO). To define the solubility problem, time studies were run of the detergent cocktail, 15 ml, containing increasing amounts of water. The results, shown in Fig. 1, clearly demonstrate that a minimum of 200 μ l of water/15 ml of detergent cocktail (1.3%) are required to completely dissolve the tritiated sucrose. If the aqueous sample is larger than this amount, no solubility problems will be encountered. Even at 200 μ l about one hour is required for the proper dpm to be reached. Note that at 0 and 100 μ l of water constant dpm were reached in 1-4 hours but that these were incorrect values. The criteria that a stable count be reached before accepting the data is a necessary but not sufficient condition. All of the sucrose must dissolve and to achieve this, at least 200 μ l water should be present. It should be stressed that this minimum water concentration may vary among the various commercial cocktails. Those using other brands should determine the minimum concentration of water required.

While the various automatic methods for determining efficiency are substantially in agreement when the detergent system is in the sol phase, quench curves based on solution standards cannot be applied to gelled samples. Many of our samples require counting in the gel

phase-- i.e. >3 ml water per 15 ml detergent cocktail. Gel phase counting is also useful for counting polar molecules present in trace amounts because these may adsorb on the vial walls. These cannot be accurately counted because one is never certain whether all or part are adsorbed. Gelling the cocktail increases the viscosity of the medium to the point where the diffusion rate of the labeled sample to the wall is slow enough to avoid significant loss.

The RIC capsules make it possible to conveniently prepare a quenched set of gelled standards to provide a quench curve or to determine the counting efficiency of samples directly. The former has the problem that gelled cocktails undergo phase change with time so that each time the standard set is used, the set must go through a heating/cooling cycle to restore the original phase. If the number of samples is small, then the ISM is recommended. To see if RIC capsules could be used for efficiency determination in gels, an experiment was run wherein standard toluene- ^{14}C , toluene- ^3H , and HTO were added in known amounts to 15 ml of detergent cocktail followed by 5 ml water. After vigorous shaking to set up the gel all were counted. Following the first count RIC capsules were added to each (CO to the toluene- ^{14}C , HO to the toluene- ^3H , and HW to the HTO). The gels were then gently warmed until liquid, shaken vigorously, allowed to cool, and counted again. Using the stated dpm of the RIC capsules as internal standards, the counting efficiencies were computed. These were compared with the true counting efficiency. The External Standard Channels Ratio (ESCR) method is included only for comparison using a quench curve developed from the usual sealed standards in toluene

scintillator. All samples were run in triplicate and the results are shown in Table 2.

Table 2. Efficiency Determination in Gels by Two Methods

<u>Radioactivity</u>	<u>True</u>	<u>ESCR</u>	<u>RIC OXI-Test</u>
¹⁴ C-toluene	.676 ± .007	.744 ± .002	.680 ± .005
³ H-toluene	.181 ± .002	.208 ± .002	.180 ± .007
³ H-water	.171 ± .004	.208 ± .0	.170 ± .004

Table 2 clearly demonstrates that the ISM gives accurate efficiency measurements. The ESCR does not as is well known. If a quenched standards set in the gel phase were prepared, the ESCR would give satisfactory results.

Color quenching also poses problems for liquid scintillation counter users when the efficiency and dpm are determined automatically by reference to a chemically quenched standard curve. To test this, the Beckman LS-9000 employing the Compton edge shift (H #) was used. A known amount of HTO (34000 dpm) was added to 15 ml of a detergent cocktail containing 500 μ l water. To this was added several drops of a saturated solution of 2,4 dinitrophenylhydrazine in ethanol. Three such were prepared containing different amounts of the color quench. Efficiencies were determined in the counter using the Compton Edge shift (H #) and also by the ISM using the HW capsule. The results, shown in Table 3, demonstrate the advantage of the ISM for color quenched samples.

Table 3. Efficiency Determination of Color Quenched Samples by Two Methods.

<u>Vial</u>	<u>True</u>	<u>RIC OXI-Test</u>	<u>H #</u>
1	.129	.132	.180
2	.086	.087	.116
3	.0075	.0078	.03

Conclusion

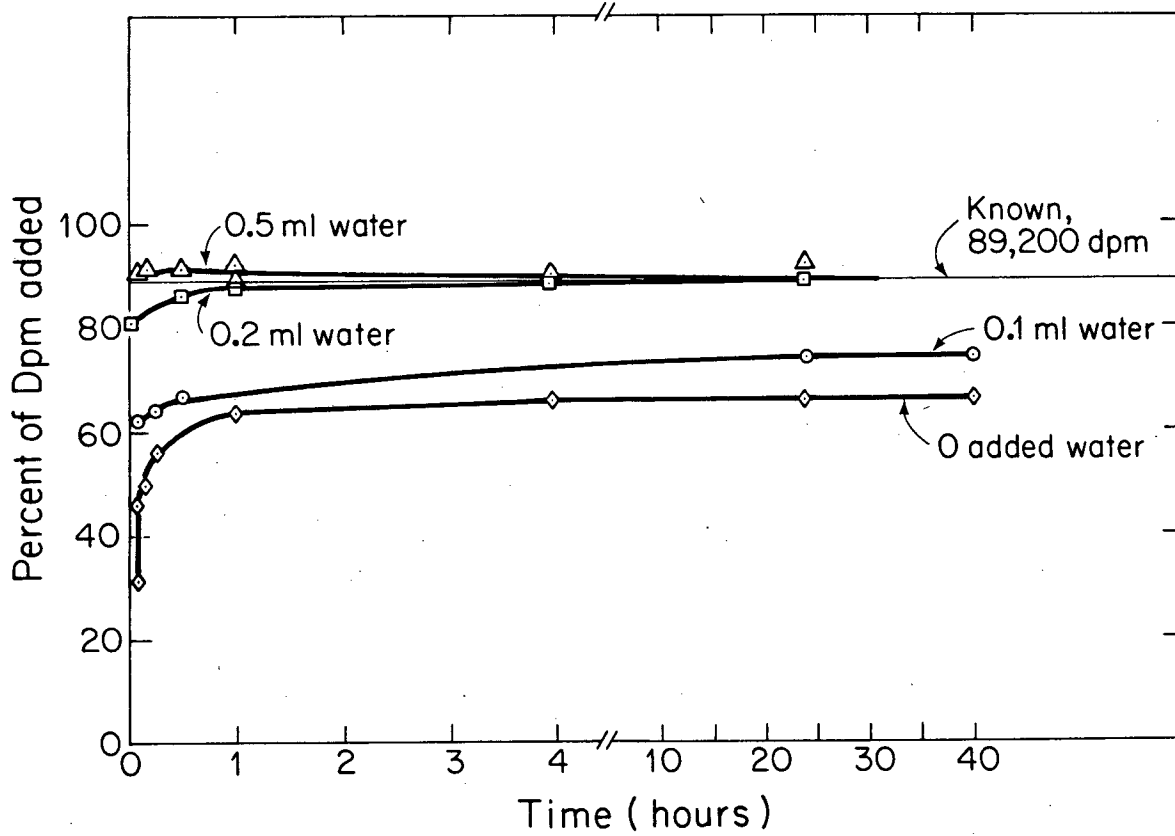
The widely applicable internal standard method of determining liquid scintillation counting efficiency has become much more convenient with the availability of encapsulated standards of carbon-14 and tritium, the latter being available in oil and water soluble forms.

Accurate determination of counting efficiency in any of a variety of cocktails whether as solutions, gels, or colored solutions, has been demonstrated. The tritiated sucrose standard is not compatible with a detergent cocktail (Aquasol-2) unless a minimum amount of water is present (1.3% for Aquasol-2). Above this concentration results are accurate and precise. Other surfactant cocktails may have other water requirements.

The preparation of a quenched standard set in any cocktail is rendered much simpler so that the ultimate user can easily prepare a variety of standard sets covering the cocktails in use in his laboratory.

Bibliography

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Academic Press (1974) p. 217.



Rate of solution of HW standard in 15 ml AQ-2

— Figure 1 —

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