

## STANDARDIZATION IN LIQUID SCINTILLATION COUNTING

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In the almost 25 years of liquid scintillation counting, the convenience and engineering sophistication of counting instruments has increased remarkably. This laudable progress has not however resulted in an equivalent increase in accuracy, because there is still no easy road either to the preparation of samples suitable for liquid scintillation counting, or to devising appropriate known standards. The use of inappropriate standards (or none at all), removes one of the most important criteria of adequate sample preparation.

By definition, the introduction of an unknown sample into a scintillation vial containing properly selected scintillators and solvent is equivalent to the introduction of an impurity and therefore a certain degree of quenching: attenuation of the radioactivity itself and of the photoelectrons resulting from interaction with scintillators (1).

The LS counting literature is already replete with expositions of how to employ the three approaches to monitoring quenching by:

1. Internal standardization of each sample
2. Plots of sample channels ratios vs counting efficiency
3. Plots of external standard channels ratios vs counting efficiency.

Recent short books by Kobayashi (2) and Horrocks (3) can be consulted by the novice. Dr. Peng discusses chemiluminescence - the other bane of sample preparation - in this volume (4).

The loss of radioactivity and decreased fluorescence quantum yield are my concerns in this brief discussion of how one decides whether a "quench correction" algorithm is appropriate for a specific sample. For standardization to be valid, the samples must be both "homogeneous" and similar to the series of standards used to construct quench-correction curves.

### Homogeneity of the Samples

A homogeneous sample can be defined as one in which the composition of the volume traversed by emitted radiation is the same everywhere (5). True solutions are of course included in this definition, but it encompasses any situation in which the sample is evenly dispersed in a four  $\pi$  system. Absorption or adsorption of samples results in a  $2\pi$  system. None of the three techniques of standardization are capable of monitoring loss of counting efficiency when a sample is in  $2\pi$  configuration. Dr. Wigfield (6) and our own laboratory (7,8) have been concerned with this problem. Counting samples on solid supports is thus unacceptable.

Gel or emulsion counting may qualify if the droplet or particle sizes are small compared to the length of the pathway of the  $\alpha$ ,  $\beta$  or  $\gamma$  radiation. A sample "homogeneous" for one isotope may therefore not be so for one of lesser energy. E. B. Muller has proposed a fairly rigorous basic test of "homogeneity" in surfactant systems: that the efficiency of counting  $^3\text{H}_2\text{O}$  and  $^3\text{H}$ -toluene be identical (9). A somewhat less sensitive but more generally applicable test is the "double ratio plot": comparison of a quench correction curve derived using sample channels ratios to one obtained with external standard channels ratios. If the two are discordant for quenched samples the sample is not "homogeneous" (10). It is also obvious but probably important to mention that a significant portion of energetic radioactivity may escape altogether from the counting vial. In such a situation quenching may result in an apparent increase in counting efficiency (11).

### Similarity of Standards and Samples

It may seem obvious in the conduct of an international conference on liquid scintillation counting that quenched standards should imitate the composition of the samples to be counted. In practice however, they usually do not. Kalbhen and Rezvanie have shown that different problems were encountered with each of 17 cocktails, 3 solubilizers and 2 sample oxidation methods (12):

1. Chemiluminescence
2. Effects of sample volume on counting efficiencies
3. Phase separation.

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Little and Neary have shown that for each sample type and solvent, there is a different optimum concentration of scintillator (13). There are significant differences between color and "chemical" quenching, the magnitude of which depends on the energy of the isotope, the counting equipment, the counting system, the degree of quenching, the electron density of the samples and the colors involved (14).

If surfactants are used they may act as scintillators with  $^{14}\text{C}$  or more energetic isotopes; standards not containing similar amounts of the surfactant may therefore be extremely inapropos (15). If the phase separation of an emulsion is unstable or if the sample precipitates out of solution over time (a significant problem with inorganic samples) a  $4\pi$  system becomes a  $2\pi$  system and no series of standards will be valid. Finally, differences in vials may invalidate quench correction curves obtained with external standardization (16).

### Conclusions

- a) the quenched series of sealed samples commercially available should seldom be used for anything but calibration of counting instruments.
- b) sample "homogeneity" for the isotope being counted should be established, with the caution that changing amounts of the sample may also affect the relationship of the sample counting efficiency to the quench correction curve obtained from a set of standards.
- c) low energy radionuclides in heterogeneous systems (eg solid supports) are often not amenable to standardization. The effects of adsorption etc. on pulse height spectra are often unpredictable.
- d) standards for quench correction curves should be as similar as possible to the unknown samples to be counted.
- e) if sample composition is unknown, or cannot be reproduced for standards, the best tactic is if possible to resort to combustion in sample preparation. Then standards which will approximate unknown samples can easily be devised.

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