

Chapter 13

Critical Remarks about Current Trends in Liquid Scintillation Counting

D. A. Kalbhen

Institute of Pharmacology, University of Bonn, W. Germany

In the development of instruments and techniques in liquid scintillation counting within the past 15 years one can observe that this very useful counting technique becomes more and more complex and may sometimes be quite confusing for beginners. The instrumentation has become more sensitive and delicate (although more automated) and the number of sample preparation techniques, solvents, solutes and solubilisers has increased enormously. There are about 100 to 200 different scintillation solutions which are described in the literature and nearly every researcher uses his own 'modified' version. For each variation in solvent, solute, and sample preparation technique, it is necessary to recalibrate the liquid scintillation counter to optimal counting conditions. It has become quite impossible to quantitatively compare counting results from one laboratory with those from another.

This pattern of variation and alteration in counting conditions is mainly due to the fact that tracer samples, especially those of biological origin, have so many different physico-chemical properties and need therefore special methods of sample preparation. The principal factors which influence counting conditions and efficiency are the following:

1. Background (liquid scintillation counters cannot automatically handle background values in quenched samples).
2. Phosphorescence.
3. Chemiluminescence.
4. Properties of counting vials.
5. Quenching: colour and/or chemical.
6. Instability of samples: phase-separation, precipitation, chemical alteration.
7. Instability of instrumentation.
8. Application of non-suitable quench-correction-curves for the calculation of d.p.m.

To cope with these various factors, and to handle all kinds of tracer samples, the manufacturers of liquid scintillation counters have developed more and more sophisticated

instruments with higher efficiencies and sensitivities, and with quite elegant data calculating systems (the latter reach from mechanical calculators to off and on-line computers). Besides being very expensive, the running of modern liquid scintillation counters with in-built or external computers requires intelligence, understanding and working experience.

In the course of this symposium and in regard of the current status of liquid scintillation techniques it becomes quite clear that the development of instruments and counting techniques is oriented only to the task of handling the many different tracer samples. Since about 90% of all samples counted by the liquid scintillation technique contain carbon-14 or tritium from biological specimens or biochemical experiments, it appears to be reasonable to standardise the sample preparation techniques in order to obtain a more or less uniform counting sample.

I would like to suggest that all users of the liquid scintillation technique, as well as all manufacturers of liquid scintillation counters, put more effort into the elaboration of standardised sample preparation methods and instruments, which (for instance by oxygen combustion) convert all biological materials into the same combustion products. Prototypes of this kind of instrument are already made by Packard Instruments. Although the final stage of development in this direction is not yet reached, these methods and instruments may become most useful and versatile. It is quite possible that an automatic sample preparation instrument can be developed which will handle not only carbon-14 and tritium samples but also sulphur-35, phosphorus-32, calcium-45 and others. In combination with only one scintillation solution automatically and uniformly prepared tracer samples can be counted in standardised liquid scintillation counters without the complicated data calculating processes which are necessary today.

DISCUSSION

L. Schutte: What is the effect of halogens, or other elements, on quenching or oxidation yield in the general combustion procedure? (e.g. CCl_4 cannot be oxidised).

D. A. Kalbhen: Only large amounts of halogens and other elements with quenching properties will interfere with counting efficiency. In biological specimens variations are rarely too broad (regarding e.g. halogen content, etc.). The counting of CCl_4 will always be problematic but calculable.

D. S. Glass: I would like to take issue with the point that a standard method should be used for all liquid scintillation counting to accommodate the large majority of samples counted which are of biological origin. To reduce liquid scintillation counters to a basic instrument for measuring a standard scintillation mixture ignores the important property of the instrument, that it can be an extremely accurate and rapid analytical instrument for concentration measurements. This property apparently is not fully exploited, and instrument manufacturers should remember this when turning their attention to their biochemical users.

D. A. Kalbhen: I also support the idea to use the counters for other analytical (photometric) purposes, as I mentioned in my paper about chemi- and bio-luminescence in liquid scintillation counting. But for this the instrumentation can be quite simple and reduced (e.g. single photomultiplier).

C. P. Bond: We have measured quite a lot of sulphur-containing compounds by 'oxygen-bag' combustion and never noticed any extra quenching problems over those of carbon-containing compounds.

D. A. Kalbhen: I agree with your remarks. We, too, had no problems counting sulphur-containing samples (using the wet-combustion technique by Mahin and Lofberg [see chapter 1, ref. 18]). $\text{H}_2\text{}^{35}\text{SO}_3$ or $\text{H}_2\text{}^{35}\text{SO}_4$ formed by oxidation can easily be counted by liquid scintillation with minimum quench observed.