

New Method for Obtaining the Quench Correction Curve in Liquid Scintillation Counting

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INTRODUCTION

The use of a liquid scintillation counter to perform quantitative analysis requires that it be calibrated in absolute units. In addition, some scheme for making data corrections must be incorporated since the effects of quenching vary from sample to sample. No longer is there any problem in obtaining an instrument with a built-in computer, or feeding the counting data into such a computer. Instead, the difficulty presently encountered lies in the calibration of the scintillation counter. No real progress has occurred in this area, and the same old manual methods are still being employed.

Application of quench correction curves is usually required in order to make valid quantitative measurements with a liquid scintillation counter. Such curves are obtained by measuring a series of samples which have identical amounts of known radioactivity but increasing levels of quenching. Generally, the user either purchases a commercially available set or makes these standard quenched samples in his laboratory. Commercial sets are expensive, and the lifetime of these samples is not necessarily very long. The calibration can be made by pipetting an additional amount of quenching agent into the sample vial after every measurement. The preparation of standard samples demands the utmost care and accuracy in laboratory procedures, and whenever the composition of the scintillation liquid or sample is different, the corresponding calibration samples must be provided. Yet too many research workers calibrate their instruments against improper standards. Some even ignore the entire quench correction because they find the calibration procedures and associated data handling too difficult. An accurate, absolute radioactive standard is a requisite in this approach, however, and the maximum care is required to achieve reliable results. Storage of a standard solution requires close supervision to prevent changes in the standard sample or errors in its use. Solutions kept in containers in the liquid form can evaporate whenever the container is

opened. Also, volumetric dosing or weighing of the solution can be a source of errors.

PRINCIPLE

In order to simplify the calibration of a liquid scintillation counter, a new method has been developed, which permits counters to be readily manufactured and made commercially available to the largest number of users. This new approach is intended for counters having either external standardisation or channels ratio standardisation functions. With this system the user is able to freely select the scintillation solution, sample vial and volume of sample to suit his own particular needs.

Two basic components are employed within this system: (a) a standard capsule for the radioactive labelled compound (internal standard), and (b) an evaporation device for automatically adding the quenching agent.

The capsule, a small glass cup, contains an accurate amount of radioactive material which readily dissolves in the solvent. The compound containing the isotope is secured to the inner wall of the capsule in the form of a thin film, permitting the safe handling of capsules with either the hands or pincers (Fig. 1). Capsules are packed in plastic, press-thru strips, each strip containing 10 capsules, for example.

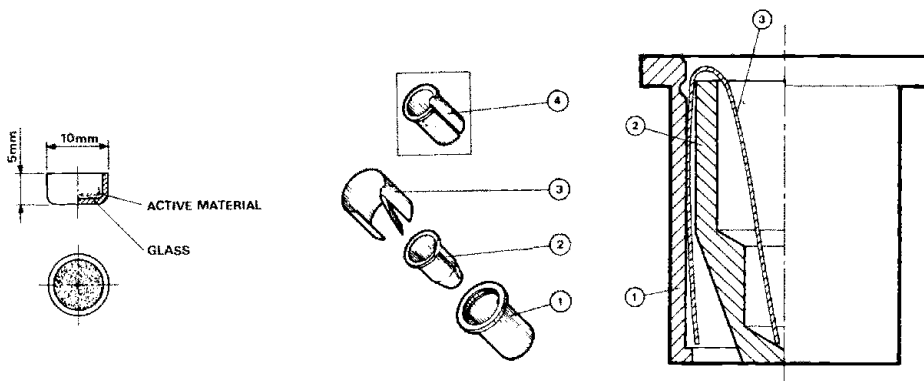


Figure 1

Figure 2

Figure 3

Fig. 1. The internal standard capsule.

Fig. 2. Construction of the evaporation device. 1, Outer body. 2, Quench agent container. 3, Chromatography paper. 4, Paper fixed around container.

Fig. 3. Construction of the evaporation device. 1, Outer body. 2, Quench agent container. 3, Chromatography paper.

The active material, dissolved in a suitable solvent, is dosed into the capsules by using an automatic pipetting machine. The solvent is then dried. For organic solvent systems, ^3H -tristearate and ^{14}C -tristearate have been used as labelled compounds. ^3H -sucrose and ^{14}C -sucrose have been used for water solvent systems. The initial activity has been 400,000 d.p.m. for the Hydrogen-3 capsules and 100,000 d.p.m. for the Carbon-14 capsules.

The capsule can then be inserted into the sample vial by simply pressing the plastic, press-thru strip. Once the capsule has been added to the vial, it must be

turned so that the active film is facing upwards. This is accomplished by agitating the vial. The scintillation solution can be added immediately to systems with organic solvents. Tristearate dissolves immediately, and activity is homogeneously distributed throughout the scintillation solution by the motion of the vial. However, when using scintillation systems with water soluble samples, the capsule must first be dissolved in a small amount of water in the counting vial, the quantity of water being approximately the same as the water content of an actual sample. Finally, the scintillation solution is added.

The plastic evaporation device (Figs. 2 and 3) contains a small container for the quenching agent and a filter-paper evaporation element. Carbon tetrachloride, a quenching agent which evaporates quite rapidly, is pipetted into the container, being absorbed into the filter paper and passing to the evaporation surface outside the container. The shape of the evaporation surface is such that the evaporation rate increases as a function of time, permitting complete quenching of the sample within a reasonable period. The quenching agent is distributed evenly by convection in the vial, provided the viscosity of the scintillation solution is substantially low. Carbon tetrachloride has been chosen as the chemical quencher; no colour quencher has been selected yet.

200 μ l of quenching agent is generally the quantity pipetted into the evaporation device. The amount of quenching liquid used is not critical, and the pipette accuracy can be $\pm 20\%$. After being filled, the evaporation device is immediately placed on the vial in place of the lining, and the vial is closed with its normal cap. Finally, the vial is moved to the liquid scintillation counter for repeated counting. The evaporator automatically increases the quenching level of the sample, and the counter begins its tabulation.

An automatic liquid scintillation counter must be set so that it measures the sample repeatedly, printing out the channels ratio and c.p.m. values after each count. The channels ratio can be determined by using either the 'channels ratio' method or the 'external standard channels ratio' method. Counting should be continued until a desired quenching level is exceeded. In practice, it will take approximately 15 min to get observation points from 50% to 5% efficiency with 10 ml tritium samples. The total number of measuring points per curve depends upon both the counting time set for one measurement and the sample volume. For example, the values of the preset count and sample volume shown in Table 1 produce approximately 30 measuring points within a Hydrogen-3 efficiency range of 50% to 5%.

Table 1. Recommended Preset Parameters:

| Counting preset count | Standardisation preset count | Sample volume (ml) |
|--------------------------|---------------------------------|-----------------------|
| 10,000 | 10,000 | 2 |
| 15,000 | 15,000 | 3 |
| 20,000 | 20,000 | 4 |
| 25,000 | 25,000 | 5 |
| 50,000 | 50,000 | 10 |
| 75,000 | 75,000 | 15 |

Since the counting of sample activity does not occur simultaneously with the measurement of the external standard channels ratio, a mean value of two

consecutive external standard ratios must be calculated. This mean value represents, with sufficient accuracy, the simultaneous observation of the external standard ratio and the count (Fig. 4).

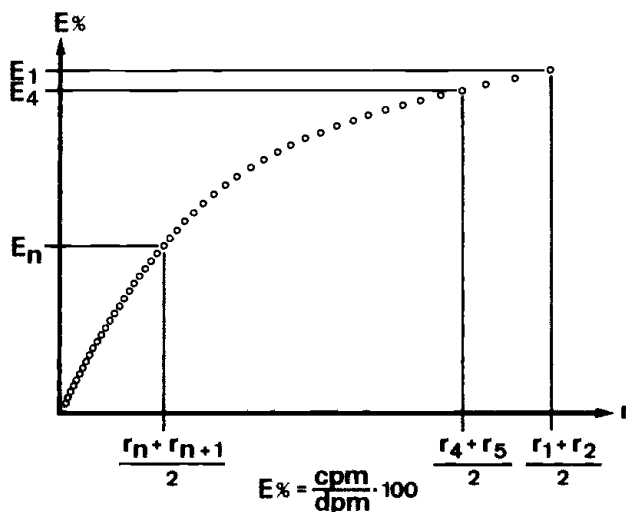


Fig. 4. Mean values of two consecutive external standard ratios are to be calculated. This mean value represents the simultaneous observation of external standard ratio with counting result.

The quench correction can be performed by either a computer contained within the liquid scintillation counter or with a separate calculator. The absolute activity, A , of a sample is obtained from Eqn. (1), where N is the measured c.p.m. and E is the counting efficiency:

$$A = \frac{N}{E} \quad (1)$$

It is convenient and practical to feed values of E into the computer according to Eqn. (2), which is an equation representing the quench correction curve. The coefficients, a_0 , a_1 , a_2 , a_3 , have been obtained by using the least square polynomial method, given the observation points (r , c.p.m.):

$$E = a_3 r^3 + a_2 r^2 + a_1 r + a_0 \quad (2)$$

A sufficient number of observation points can be selected within the quenching range where the actual measurements of the sample will be taken. If there is little change in the level of quenching of the unknown samples, the quench correction can be represented sufficiently by two points lying on the straight line described by Eqn. (3). Of course, it is assumed that the curve is quite linear in that region:

$$E = a_1 r + a_0 \quad (3)$$

Coefficients a_1 and a_0 can easily be determined manually. However, the coefficients of Eqn. (2) should be calculated with a computer, using an adequate number of observation points. All commercial computers, and the best of the desk

calculators, are furnished with ready-made library programs for '3rd-order curve-fitting'.

Finally, the absolute activity of an unknown sample can be determined with a built in computer, or separate computer, using the previously determined coefficients along with Eqns. (1) and (2).

ACCURACY AND RELIABILITY OF THE METHOD

The determination of the absolute activity of standard capsules is based on a comparative measurement with a Tritium-standard No. 4947 ($C_6H_5CH_3$) and Carbon-14 standard No. 4222 ($C_{16}H_{34}$) of the National Bureau of Standards. The Bureau lists the accuracy for Tritium-standard No. 4947 as $\pm 1\%$ which is the estimated maximum inaccuracy, which was assigned to the tritiated water standard of NBS, the standard deviation in the comparative measurement being less than 0.1%.¹

The accuracy of Carbon-14 standard No. 4222 is listed as $tS_m + \Delta = 3\%$, where $tS_m = \pm 0.5\%$ is the computed standard error at the 99.73% confidence level (3.23σ) of comparison measurements and $\Delta = \pm 2.5\%$ is the linear sum of the systematic errors.²

The estimated limits for absolute accuracy of the standard capsules set by comparison measurements with reference standards of NBS are for tritium capsules $\pm 1.2\%$ and for carbon capsules $\pm 3.2\%$.

Deviation in activity between different capsules depends upon the accuracy of the automatic pipetting machine. Experience has shown that the machine in use doses the isotope with a standard deviation of $\pm 0.2\%$.

The stability of the activity of the capsules depends upon the purity of the carrier compound and ambient conditions. The tristearate used during the preparation of capsules was found to contain so many light components that chromatographic purification had to be performed prior to dosing. Stability was also improved when the non-active carrier compound was added, resulting in a low specific activity of the capsule. With regard to ambient temperature, the capsules have been found to be stable for several months in an environment between room temperature and $-20^\circ C$. The long-term stability is still being evaluated, so results are not yet available. Dosing of the quenching agent with the evaporation device is satisfactory for toluene, xylene and dioxane-based scintillation systems. The equilibrium obtained in scintillation solutions has been so good that no significant differences have been observed when comparing quench curves with manually prepared quencher additions. With increasing viscosity of the scintillation solution, however, the quenching agent becomes less evenly distributed, and the resulting curves begin to diverge from each other. This means that the quenching agent must be added manually for samples with high viscosity as emulsion samples. Figures 5, 6, 7, 8, 9 and 10 illustrate quench correction curves obtained for different scintillation solutions, either done manually or with automatic evaporation.

The preceding discussion has presented a method for determining quench correction curves in liquid scintillation counting. The main significance of this technique is that it simplifies and expedites practical procedures in preparing the correction curves. The observation points obtained by means of the evaporation device accurately correlate with results of other methods. In addition, this evaporator approach produces many points over the entire quenching range and, depending upon the type of sample and investigation, any desired number of these points can be selected.

As a final note, the use of an internal standard capsule might be widened to other fields of practical isotopic work, such as standards and means of control. This requires, however, that the manufacture of capsules is developed for industrial production and that a reliable quality control is established.

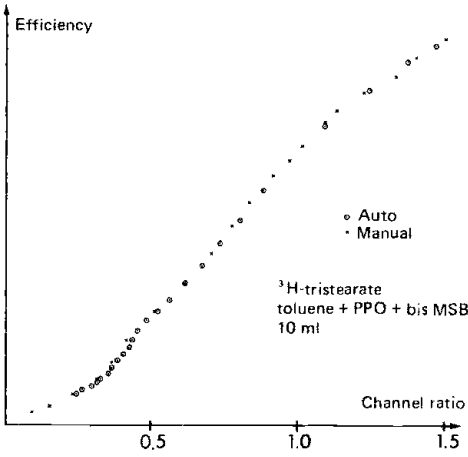


Figure 5

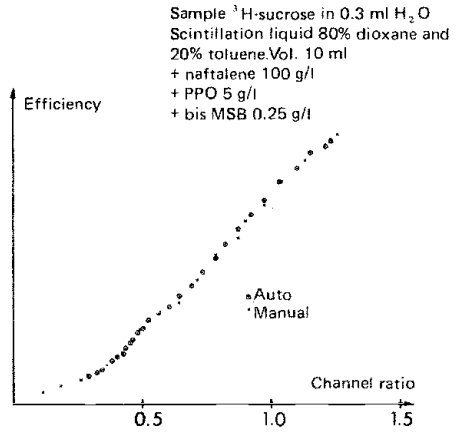


Figure 7

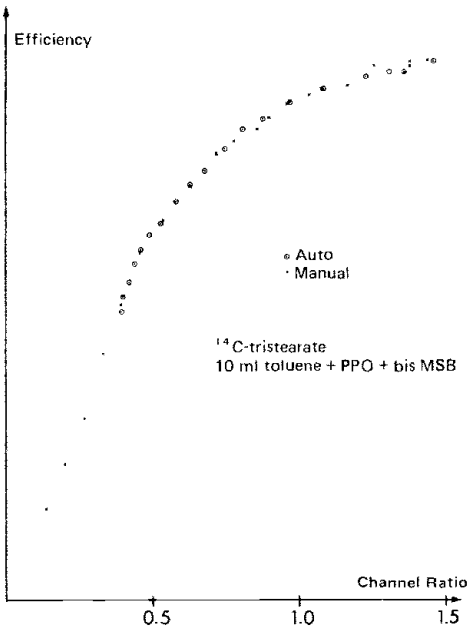


Figure 6

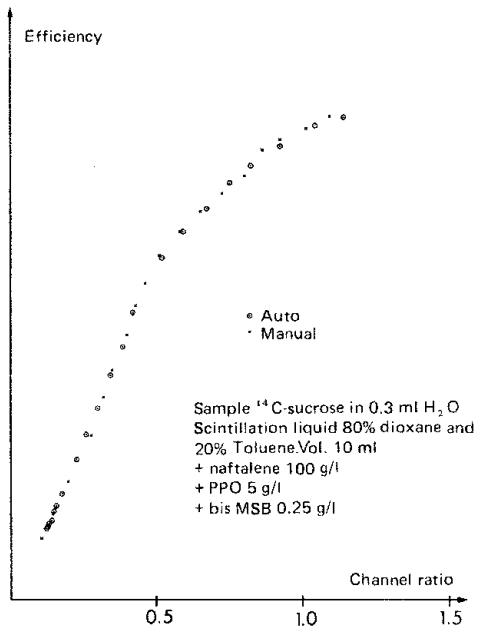


Figure 8

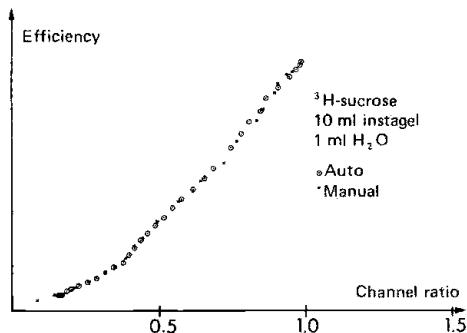


Figure 9

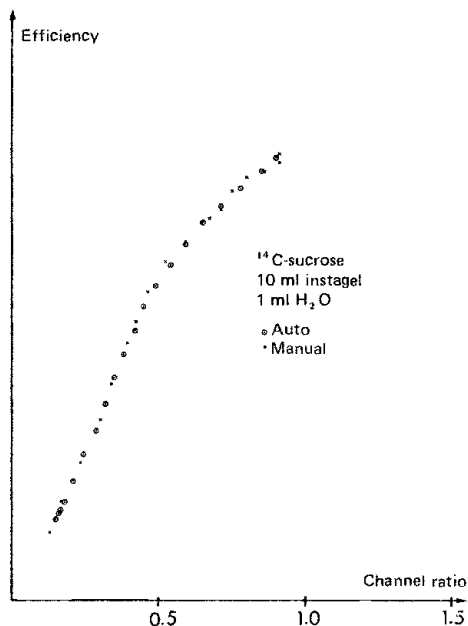


Figure 10

SUMMARY

A new method for quench correction in liquid scintillation counting has been described. The active internal standard, in dry form, is added in a glass capsule. The quenching agent is added by means of a special evaporating device while the counter is repeatedly tabulating the counting results of c.p.m. and channels ratio. The reproducibility of the standard capsules has shown a standard deviation of 0.2%. The absolute accuracy depends mainly on the accuracy of the reference materials used. The evaporation device is suitable when using low viscosity scintillation mixtures.

REFERENCES

- 1 S.B. Garfinkel, W.B. Mann, R.W. Medlock and O. Yura, Intern. J. Applied Radiation and Isotopes **16**, 27 (1965).
- 2 W.B. Mann, R.W. Medlock and O. Yura, Intern. J. Applied Radiation and Isotopes **15**, 351 (1964).

DISCUSSION

F.R. Jacobsberg: You suggested that your evaporator could be used for colour quench; presumably involving involatile materials. How does this work?

E. Soini: This is a problem. We have used a number of compounds but these will also involve chemical quenching.

P. Johnson: You intrigued people, I think, with your implication that you may have thought of ways of applying your method to colour quenching, but obviously you have not done so. As you realise, many of us who are using biological samples are unable to apply a single chemical quench curve, such as you obtain with carbon tetrachloride, in some of the quenching situations which we encounter. Your elegant method would obviously have greater general interest if it were applicable

to such problems, but obviously it is restricted to volatile quenching agents such as carbon tetrachloride.

E. Soini: Yes, it is true that it is only applicable to volatile quenching agents; it is difficult to find suitable coloured quenching agents.

J.A. Heslop: How do you obtain the efficiency of counting and correlate this with an external standard channels ratio, as the counting efficiency is changing with time? What errors are introduced?

E. Soini: The efficiency is taken as the mean of two consecutive counts. As the rate of evaporation of quencher is slow, no large error is introduced.