

Standardisation in Liquid Scintillation Counting

Elizabeth Bush Mueller

706-208 S.W. 16th Avenue, Gainesville, Florida 32601, U.S.A.

The evolution of the commercial liquid scintillation counter can be seen as a response to some of the needs of its users as perceived by the manufacturers: for greater sensitivity of detection, especially of low-energy emitters; for greater precision, with long-term stability and reliability; for greater sample handling capacity, with versatility in accepting different kinds of samples; and for reduction in user time, attention and expertise required. As with many laboratory instruments this last need, for 'automation', came with the transformation from a specialist's research tool into a routine analytical instrument processing constant streams of samples. In addition to straightforward data-processing functions, capabilities for data-interpretation and decision-making have been incorporated into many models. The ideal instrument is often conceived of as a black box into which a sample in the required form can be inserted and from which the desired answer will then emerge without further manipulation by the operator. Such a box has a single control switch with two positions, 'on' and 'off', and no operating manual.

Efforts to substitute counter automation for user education have been accompanied, perversely, by an awesome proliferation of external counter controls. Each must be correctly set by the operator if the output data are to have meaning. Primarily this has resulted from the simultaneous need to satisfy the other goals, providing versatility while optimising the sensitivity, precision and counting time for each sample. In some cases one set of user choices has apparently been supplanted by another set which is presumed by the designers to be easier to comprehend. It is seriously questionable as to what extent this has succeeded in reducing the expertise required to extract accurate information from these instruments.

Conspicuous among the parameters which are being removed from direct operator control are the individual discriminator and gain settings. One is no longer forced to visualise the pulse height spectrum and its relationship to these parameters in order to select the counting channels. I want to present some of the reasons why I believe that a user — at least one who has occasion to count more than one type of sample — cannot afford to be unaware of the pulse height spectrum, or to be ignorant of the basic processes of liquid scintillation counting. There are

some problems with which the instrument is not yet equipped to cope. These are problems in the determination of counting efficiency, and they are at the same time problems of sample preparation.

I daresay that we might have achieved the goal of the black box if we had been willing to abandon the capability of counting many different forms of sample. I do not think this would have been a good trade-off, in the present state of sample preparation technology, because it would require too great a sacrifice either in sample size or counting efficiency or in convenience of preparation for many kinds of sample materials. But the alternative to limiting ourselves to one form of sample is to be able to provide an appropriate standard for each different sample form. We must also be able to recognise those forms of sample which are not acceptable because it is simply not possible to determine their counting efficiencies by any other method.

The meaning of sample 'form' as used here should become more explicit shortly. It may be defined as that collection of characteristics which must be reproduced in the standard with which the sample is to be compared. Some sample properties are not relevant to this comparison and they do not determine 'form'. Unfortunately, it is apparent that many users lack an adequate understanding of the required relationship between the samples and the standards.

In order to identify the determinants of form we may start with a simple expression for the magnitude of the electrical pulse produced in the counter by an event in the sample. This will be written for beta particles and their pulse height spectra explicitly, because the liquid scintillation counter is most often used to count betas. The implications of the following discussion for the counting of other high-energy radiations should be readily apparent, however.

$$\text{Pulse height} = E_{\beta} f_E e_E f_P e_P f_{pe} A_{PM} A_{amp}$$

$$1 \quad 2 \quad 3 \quad 4 \quad 5 \quad 6 \quad 7 \quad 8$$

- 1 E_{β} = energy with which β is emitted,
- 2 f_E = fraction of β -energy deposited in scintillator solution,
- 3 e_E = efficiency of conversion of energy $E_{\beta} f_E$ to photons of energies to which the photocathodes are sensitive (photons/keV),
- 4 f_P = fraction of photons collected at the photocathodes,
- 5 e_P = efficiency of conversion of photons to photoelectrons (pe/p),
- 6 f_{pe} = fraction of photoelectrons collected at the first dynode,
- 7 A_{PM} = gain of the dynode string,
- 8 A_{amp} = gain of the remainder of the circuit before pulse height analysis.

For simplicity in the following discussion it will be assumed that the counter has two identical photomultiplier tubes and employs linear amplification.

It may be noted that the expression does not contain a separate term for each energy-conversion step in the liquid scintillation counting process. Since it will be convenient to consider certain of these steps together, the corresponding variables have been combined, giving the seven terms labelled 2 through 8. Each of these terms may therefore be the product of several variables.

The expression is not linear in beta energy. Thus the shape of the pulse height spectrum is not the same as that of the beta spectrum. One source of this difference is the uncertainty associated with each of the variables in the pulse height equation: while an average value may be assigned to each variable, for any

particular sample and counter, that value is just the mean of a distribution of values which the variable takes for successive beta disintegrations. The final result is a distribution of pulse heights for any given beta energy, and a total spectrum which is the sum of these distributions. The relative breadth and the asymmetry of the pulse height distribution increase with a decreasing mean number of events (or participating quanta) per step of the liquid scintillation process. These statistical factors increasingly modify the shape of the spectrum as the beta energy decreases or the sample quenching increases, yielding fewer photoelectrons per disintegration.

Another source of discrepancy between the shapes of the beta- and the pulse height-spectra is the energy-dependence of Factors 2, 3 and 4 of the equation. Since each has its own form of energy-dependence, each will affect the shape differently: that is to say, different kinds of quenching produce different spectral shapes. Nevertheless, the pulse height spectrum should change in a reproducible way with any single variable affecting pulse height. That fraction of the spectrum falling within the fixed range of pulse heights selected by any counting channel should vary reproducibly with any other such fraction, as long as only one variable is allowed to change. Any portion of the pulse height spectrum should also vary reproducibly with the fraction of the total number of disintegrations which is not counted. This is the basis for the channels ratio method of standardisation. It is also a necessary condition for the method which measures the net counting rate of some portion of an external standard spectrum in order to determine what percentage of the sample disintegrations has been observed.

If two factors which affect the shape of the pulse height spectrum in different ways are allowed to vary independently, no unique relationship will be observed between chosen portions of a spectrum. The limits placed upon the form of the samples and their standards must be those which ensure that spectral shapes vary identically with efficiency.

Let us look in more detail at some of the factors in the equation in order to see how they affect the spectral shape.

FACTOR 2

The fraction of the beta energy deposited in the scintillator will be reduced if any of the following occurs: escape of electrons at the periphery of the sample, energy loss in electron backscattering from the vial wall, electron energy loss due to self-absorption in undissolved sample material, or absorption losses in any other component of a second, non-scintillating phase which might be present in the sample. The wall losses should be negligible (less than 1%) in an homogeneous solution, if the beta energy does not exceed that of Carbon-14. This is not true for Phosphorus-32, however, whose maximum beta range is roughly two-thirds the radius of the conventional sample vial. Wall losses will be very significant if the labelled sample compound is adsorbed onto the wall or is precipitated onto the bottom of the vial. Counting geometry is then reduced from 4π to 2π . (In this case a reduction in Factor 4 of the equation also results, since light emitted close to the walls or bottom of the vial is less likely to be detected.)

Absorption losses may also be expected in a sample precipitate. If the loss is not severe, as may be the case for a high-energy emitter in a precipitate which is well penetrated by scintillator solution, any efficiency loss may be compensated to some extent by other effects of the precipitation, such as reduction of quenching by removal of the precipitated compound from solution, or perhaps a slight enhancement of light output due to light-scattering by the precipitate, with a consequent reduction of light-trapping in the vial. The resulting pulse height spectrum,

however, cannot be expected to have the shape of one produced by a dissolved sample material. Beta energy losses in backscattering and absorption are not linear with energy, and their effects on the shape of the pulse height spectrum are not the same as those of quenching of the scintillator solution.

If the labelled compound is partly in solution and partly in another phase, its pulse height spectrum will be a composite of that which is characteristic of the solution and that which has been degraded in energy. As an exercise one might try to treat this in the manner of a doubly labelled sample with superimposed spectra, and solve for the activity in each phase. A standard for each phase would be required. If solution efficiency were allowed to vary, the spectra would be required to vary in a fixed relationship as a function of quenching only, with the efficiency of the undissolved phase a known function of the efficiency of the solution. This would be possible only if the spectrum of the undissolved phase did not vary simultaneously with any other parameter, such as sample thickness. Alternatively, if solution quenching were held constant, the thickness of the undissolved phase could be allowed to vary. Efficiencies might be determined from the external standard ratio, in the first case, and from the sample channels ratio, in the second case (in which the solution efficiency would be known and constant). If, however, the solution efficiency and the value of Factor 2 vary independently, in general the overall counting efficiency cannot be determined from a combination of these ratios.

The requirement for two distinct standards, each of which is known to be present in one phase but not in the other, might be met. But the other requirements severely restrict the practical application of such a procedure. Ordinarily, when a second phase forms in a sample, neither the solution quenching nor the distribution of the solid phase is well enough controlled.

The prospects for accurate counting of a single undissolved species with negligible solubility in the scintillator solution are much better. One frequently used technique involves the precipitation onto a filter of a very small mass of sample. Usually the counting efficiency is determined by preparing a similar sample of a compound of known activity. Since most of the compounds which one wishes to measure will not be available in standardised preparations one is usually forced to compare one's unknowns with a standard sample prepared by precipitation of a different compound. There are certain obvious hazards in this method, such as the determination of chemical yields, problems of adsorption losses in manipulating very dilute solutions, and the possibility of partial leaching of either the sample or standard into the scintillator solution. But in addition there are less obvious problems, such as those which have been explored by Gill,¹ by Furlong,² and by Bransome and Grower.³ When using absorptive filters, such as paper, they found different counting efficiencies for molecules of different sizes, suggesting that these penetrate to different depths in the filter, and that the escaping betas suffer different absorptive energy losses. Gill found a significantly different ratio in the counting rates of large and small molecules according to whether they were precipitated onto paper filters or glass fibre filters. He advanced the very reasonable explanation that the large molecules stay on the surface in either case, but may suffer from self-absorption losses, while the small molecules show higher efficiencies when on the surface of the non-absorptive glass filter, but suffer from absorptive losses when embedded in the paper. Meyer and Keller⁴ also found different efficiencies on filter discs for the two compounds they wished to compare in their experiment: the precursor nucleoside triphosphate and the product DNA. Both Hydrogen-3 and Carbon-14 were used in all these experiments.

One straightforward way of resolving this problem is to determine the activity

of one sample of each species by an independent method in a preliminary experiment. Since samples of this kind often weigh a few milligrammes at most, it should usually be possible to get them into a true solution either by digestion in a basic solubiliser or by combustion, and to standardise using the usual solution standards. One may then proceed to establish the counting efficiency for each species on the kind of filter one wishes to use. One may also establish the way in which that efficiency varies with sample weight over the range of weights to be anticipated.⁴ It is often possible to derive a sample channels ratio curve to measure efficiency as sample thickness varies (for samples of less than infinite thickness). Thickness should be uniform across the sample support, and all variables other than sample weight should be held constant.

Partial leaching of the sample into the scintillator would result in a faulty method, as already discussed. It is easy enough to determine whether this is occurring by removing the filter from the vial and recounting the solution. One would normally expect that the scintillator used would consist only of toluene and fluor, since this provides high efficiency and minimum solubility for most of the compounds it is necessary to count in the solid phase. (If they are toluene-soluble to any appreciable extent they are probably best counted in solution.) Yet it is surprising to find reports in the literature of filters placed in dioxane or surfactant solutions for counting, with little or no evidence as to whether the samples have been eluted. It is not safe to assume that samples can be completely removed from filters in this way, and careful experimentation should be carried out to establish it. This is illustrated by some work of de Bersaques,⁵ who found incomplete, extremely slow, or non-reproducible elution from paper in several scintillator solutions. Similar problems are encountered when thin layer chromatogram media are immersed in scintillator solution.

Finally, there is another variable associated with counting on filters, one which properly belongs with the discussion of the fourth factor in the pulse height equation. The efficiency with which the scintillations are detected will depend upon the location of the filter in the vial, and upon its opacity. The seriousness of these problems with filters is, of course, a function of particle energy. When counting high-energy betas, such as those of Iodine-131 and Phosphorus-32, efficiency has been reported to be quite insensitive to optical or self-absorption effects.^{6,7} This undoubtedly depends upon the choice of a suitably wide counting channel, in which efficiency may remain quite constant despite small pulse height shifts.

The foregoing discussion has been concerned with small solid samples, but larger weights — up to one or more grammes — can also be successfully measured under properly controlled conditions. The best efficiencies are usually achieved by dispersing the solid particles in a gel scintillator. Fortunately, in this case it appears that reproducible counting efficiencies can be expected if the average particle size and the size distribution are held within the limits obtainable by normal chemical techniques for precipitation. This permits standardisation by comparison with similar samples of compounds of known activities. Such standard compounds must be like the unknowns in physical form (particle size and optical transparency) and in beta absorption coefficient. Counting efficiency varies with the total mass of the suspended sample material, due in part to variations in Factor 4 of the equation, such as absorption and scattering of light by the sample particulate. A common problem in obtaining reproducible efficiencies is that of controlling sample geometry, or spatial distribution. Too thin a gel permits settling out of the sample, and excessive viscosity makes uniform dispersion difficult. If reproducible geometry can be achieved, the sample channels ratio

can often be used to correct for changes in efficiency due to varying self-absorption and transparency. The standard set is prepared using a compound of like physical form, varying only that property which is allowed to vary in the samples (usually weight).

The most popular of all types of two-phase samples is undoubtedly the emulsion. It is popular because there are available some very efficient and inexpensive emulsifying agents which can incorporate relatively large amounts of polar compounds into the best alkylbenzene solvents. Because the technique is so versatile, and because it often provides a high figure of merit (the product of the sample weight and the counting efficiency), it is extraordinarily tempting. A number of detailed studies have been done, especially on Triton surfactants in toluene or xylene, to find compositions suitable for the incorporation of various sample solutions. For the Triton systems and for a number of others there are at least three identifiable kinds of mixtures one can produce by varying the relative proportions of surfactant, alkylbenzene, and aqueous solution: one type appears to have the properties of a true solution, being fluid and clear; one is quite apparently an emulsion, being fluid and hazy or cloudy; and one is a gel, viscous and clear-to-hazy. This latter may be used to suspend solids, as previously mentioned. The following discussion will concern the counting of aqueous and other polar liquids.

We may eliminate from consideration any formulation whose properties are not stable with time and with the changes in temperature the sample must undergo before counting. This probably eliminates most of the very cloudy fluid emulsions, as well as those gels which have a tendency to settle slowly, showing separation of a liquid after a day or so. Accurate assay of unstable samples cannot be expected because the radioactive sample may be undergoing a change of phase and/or the solution efficiency may be changing with time.

Assuming a stable composition, how are we to determine counting efficiency in these preparations? Which ones are true solutions, and which ones must be treated as heterogeneous? The literature of liquid scintillation counting is marked by considerable confusion in the terminology it applies to these mixtures, and little information as to their actual molecular arrangements. The lack of such information, however, is not an obstacle to correct standardisation. Only a knowledge of those properties which can be measured by liquid scintillation counting is necessary. Visual examination of samples can supply clues which are valuable in the prediction or interpretation of counting rates.

The following operational definition of a true solution is suggested as appropriate and useful for classifying samples in liquid scintillation counting: a solution is a mixture of components in which any one of those components is counted with the same efficiency, if tagged with a given radionuclide. Thus, if a mixture containing Triton, toluene and water exhibits the same counting efficiency for tritiated water as for tritiated toluene, it behaves as a solution. It can then legitimately be compared with solution standards, providing, of course, the samples and standards have the same values for Factors 3 and 4 of the pulse height expression. By this definition, all true solutions have the same value for Factor 2 (except for any difference caused by varying the surface-to-volume ratio, which is trivial except for high-energy emitters). This criterion can be applied to both the fluid and the gel samples. It can also be applied to the cocktails employing basic solubilisers, such as Hyamine hydroxide, NCS and Soluene. The microstructures of these mixtures are not known, but they are irrelevant to counting if the mixtures behave as true solutions according to the definition.

Standardisation is much simpler for true solutions than for other kinds of samples. The solution is to be preferred unless there is an important advantage

to be gained in convenience or figure of merit by using a heterogeneous sample. The heterogeneous preparations of surfactants may be expected to present the same problems as do the partially precipitated samples already discussed: if the radioactive material is distributed in an unknown way between the phases, and if both the self-absorption and quenching factors are allowed to vary, the counting efficiency is not easily determined. If it is necessary to use such an emulsion it is most prudently limited to the counting of sample material containing a single labelled compound. A standard of this same compound should be available for efficiency determination by preparing duplicate standard samples, or by internal standardisation with care that the phase distribution of the sample is not altered by the addition of standard.

If solution behaviour can be established for a preparation, by comparing its counting efficiencies for appropriate standardised lipophilic and hydrophilic compounds, a channels ratio method may be used for counting samples. For reasons to be discussed in connection with Factors 3 and 4, it is best to prepare a special standards set, using the same surfactant system quenched with unlabelled material like the sample material to be assayed. Experience in the author's laboratory with a variety of surfactant systems, including proprietary preparations sold exclusively for liquid scintillation counting, indicates that both the gelled and the fluid compositions are more likely to behave as solutions if optically clear. Unfortunately, this is not an infallible criterion, nor is it well-defined: there is often a gradual transition from clarity to haziness and translucence as composition is varied. The user is advised to test any composition range he expects to use by counting appropriate internal standards.

To sum up the methods of determining counting efficiency for non-homogeneous samples, it is necessary, first of all, to have available a compound of known activity which is very similar to the unknown. The similarity should be in physical characteristics, if the sample is insoluble, and in chemical characteristics, if the sample is soluble in some non-scintillating phase. Comparison with the standard can then be carried out by one of three methods: preparing a duplicate standard sample, internal standardisation (for soluble emulsion samples), or use of a sample channels ratio curve, if one pertinent parameter can be varied while others are held constant.

As previously discussed, there are many non-homogeneous samples for which the determination of counting efficiency is not feasible. It is necessary to be able to recognise such samples when they occur accidentally. Bush⁸ has discussed various methods for detection of phase separation, including a 'double ratio' technique, which requires a set of standard quenched samples known to be homogeneous. An interesting extension of this technique has been described by Glass,⁹ who routinely counted all samples in this fashion. By monitoring both the sample channels ratio and the external standard ratio, he was in effect providing an independent check on the expected output of each of the three counting channels. He was thus able to detect discrepancies from various sources, such as chemiluminescence, contamination by another nuclide, and counter drift or other malfunctioning, in addition to revealing phase separation in samples. In his laboratory, liquid scintillation counting data were reduced by computer. This represents an imaginative use of the capability inherent in automatic data processing to overcome the threat that it presents in alienating the experimenter from his data. I anticipate that we shall see an expansion in the use of software to perfect the black box counter.

The need to monitor and stabilise instrumental functioning has been met by the manufacturers with improved hardware. At least one counter automatically

monitors itself for changes in gain and makes any adjustment necessary to return itself to a calibrated state, so that channel efficiencies and ratios should be stable indefinitely. A start has been made towards monitoring the sample as well: I believe there are at least two counters with devices designed to recognise and discriminate against chemiluminescence or other processes which produce single photons at a rate high enough to cause significant accidental coincidence counts. We may hope that future automation will include ways of utilising that information in the signal which reveals how counting efficiency is related to sample form.

FACTOR 4

Among the variables that determine what fraction of emitted photons reaches the photocathode are absorption in and total internal reflection at the vial wall, both varying with vial material. Reflection losses are greatest at the external surface of a vial with a clear, smooth wall, like the glass vial, and are decreased by roughening the outside wall or by using a diffusing wall material such as polyethylene. Light trapping may also be broken up by a scattering phase within the sample. The increases in absolute counting efficiency achieved by these means are usually only a few percent. The pulse height spectra of scattering samples have slightly different shapes than those of clear samples, and produce slightly different channels ratio curves.

The position of the photon source in the vial affects the probability for escape of the photon, and for its chance of ultimately reaching the phototube face at a favourable angle. Thus the value of Factor 4 depends upon the scintillator volume and the location of the radioactive source in that volume (whether distributed throughout or on a surface somewhere within it). The internal dimensions of plastic and glass vials are sufficiently different to produce observable efficiency changes due to this geometry factor. The pulse height spectrum of the external standard is even more dependent on scintillator volume, which determines the probability for interaction in the scintillator of a gamma ray, as a function of gamma energy. For all these reasons, the same kind of vial should be used for the standard samples as for the unknowns. If polyethylene vials are used, the standards should be prepared at the same time as the unknowns, since the vials will swell and the samples change in efficiency as time passes. Samples containing light-scattering materials, such as Cab-O-Sil or other gelling agents, should not be compared with clear standards. Sample volume should be held within the limits determined for any given counter to yield a single calibration curve.

Finally, light absorption in a coloured sample not only decreases the number of photons escaping but changes the way in which the shape of the pulse height spectrum varies with efficiency. In 1971 ten Haaf¹⁰ presented a simple model for colour quenching which, while it was only one-dimensional, established a basis for understanding the difference in shape of the spectra of colour-quenched and chemically quenched samples. The colour-quenched spectrum is broader because the dispersion of pulse heights is greater for events of any given energy. In addition, according to this model, coincidence losses vary with energy in different ways for the two types of quenching.

Since the first study of the channels ratio method was published in 1960¹¹ we have known that different calibration curves were required for coloured and colourless samples. Modern counters provide considerably better spectral resolution, and one now finds that for precise work different curves are needed for different colours, and for various admixtures of chemical and colour quenching. Most coloured samples are also chemically quenched to some degree.

When the two quenching factors vary independently in a set of samples, no single channels ratio curve will fit all the points. Fortunately, in many cases one type of quenching predominates, so that reasonable accuracy can be obtained with a single set of standards. These should be prepared so as to duplicate the samples as nearly as possible. For example, in our laboratory we found it necessary in assaying muscle tissue in various solvent systems to prepare a separate set of standards for each system, using not only the same solvent and the same kind of muscle from an unlabelled animal, but a piece of muscle which had been washed until it had approximately the same colour as the sample to be assayed.

A recent innovation called 'colour restoration' is now available on one commercial counter. Its function is to obviate this problem in choosing standards. For the calculation of sample channels ratios, pulse height analysis is performed on a spectrum consisting of the smaller of the two pulses produced in coincidence by the two tubes, rather than on the spectrum of summed pulses. The shape of this 'lesser pulse height spectrum' was found to be less sensitive to the kind of quenching than was that of the summed pulse height spectrum. Very few results with this system have yet been published. While both the manufacturer's literature^{1 2} and an independent report^{1 3} indicate that the 'lesser spectra' are not identical for chemically and colour-quenched samples, a very substantial reduction is reported in the discrepancy between the channels ratio curves for the two types of samples. For tritium samples, which have relatively short quenching ranges and thus lower maximum colour intensities, the discrepancy between the two curves may be within normal curve-fitting errors over the usable range of efficiencies.

FACTORS 6 AND 7

These variables have been factored out explicitly in writing the expression for pulse height because they are employed by different manufacturers in their systems of instrumental response to quenching. As the average pulse height decreases with quenching, it is highly desirable to 'follow' the spectrum with the channels in which one is measuring it, so as to maintain optimal discrimination between spectral regions. Either the channel discriminator settings or gain can be varied to keep the spectrum in a channel. These procedures ensure that all pulses corresponding to a chosen beta energy range will be counted, but they cannot maintain a constant efficiency. Total efficiency drops with quenching because of losses below the threshold for detection, while efficiency in any given range of the spectrum may change with quenching because of change in the spectral shape. The spectrum of a quenched sample cannot be made identical with that of an unquenched sample by increasing the gain. For pulses produced by small numbers of photoelectrons (such as those typical of Hydrogen-3 and Carbon-14 counting) a change in the mean number of photoelectrons per pulse results in a significantly different pulse height distribution than does an equivalent change in mean pulse height produced by Factors 7 or 8.

Some counter manufacturers have provided for automatic monitoring of the pulse height and for appropriate adjustment of some channel parameter(s) to follow the desired portion of the spectrum as it is shifted by quenching. Monitoring is performed by making a measurement of an external standard ratio for each sample as it arrives at the counting station. The measurement is always taken at the same gain and in fixed channels. The observed value of the ratio determines the adjusted channel settings at which the final sample counting will be done. The efficiency under these counting conditions must be determined from some empirical relationship which has been established by counting a quenched standard set.

In one type of counter the system gain is adjusted by changing the high voltage applied to the photomultiplier dynode string (Factor 7).¹⁴ The voltage on the first dynode remains constant. Discriminator levels remain fixed. The efficiency is taken from a calibration curve of the external standard ratio measured at a constant gain versus efficiency at the adjusted gain.

In another type of counter the discriminator levels are varied, but not as a continuous function of the external standard ratio. Ten discrete settings are provided, and over the narrow range of quenching for which the spectrum falls into one of these windows an attempt is made to keep the spectral shape constant. This is done by varying Factor 6, through application of a magnetic field external to the photomultiplier tube. The change in the statistical distribution of electron numbers at the first dynode caused by a small decrease in Factor 6 closely resembles that which results from a reduction in the number of photons arriving at the photocathode. The efficiency at each of the ten settings is determined by counting standards.¹⁵

FACTOR 3

I should like to return now to Factor 3 of the pulse height expression. This is the one whose value is affected by chemical quenching and fluor self-quenching. In the past we have not had to concern ourselves much with the particular kind of Factor 3 quencher when using the sample channels ratio, because they were all observed to produce the same kind of change in the sample pulse height spectrum. With the advent of external standardisation the chemical nature of the sample became important because the electron density of the sample determines the gamma absorption cross section. This not only determines the counting rate for the external standard, but may also affect the shape of the electron spectrum produced by the gammas. The cross section for gamma interaction affects the geometric distribution of electrons produced within the sample volume, and this will affect the pulse height spectrum. For these reasons, more stringent limitations on composition and volume must be observed when comparing samples and standards by external standardisation.

Progress, however, has not stopped here. We have arrived at the point where we may now have to consider the effect of sample chemistry on the internal sample pulse height spectrum. I believe that this is the result of improved photocathode efficiency, and the resulting improvement in spectral resolution. Earlier such improvements had similar consequences in the counting of coloured samples: in the early 1960's we were not able to distinguish a chemically quenched from a colour-quenched spectrum for tritium samples, principally because for those small pulses the statistical broadening at the photocathode obscured such differences. Now that our tritium efficiency is about twice as great, we can see those differences in the tritium pulse height spectra, just as we could then in the Carbon-14 spectra.

Five or six years ago in our laboratory we began to notice unexpected differences between toluene and dioxane scintillators. The apparent activity of a tritiated water sample, as determined by a sample channels ratio method, depended upon whether it was counted in dioxane or in toluene—ethanol. The same difference was observed for a sample of tritiated toluene. In each case the comparison was made with a scaled standard set containing toluene quenched with acetone. The most quenched of these standards contained a rather large volume percent of acetone. Finally, by carefully preparing complete quenched sets of the three types of solutions we discovered that we had three slightly different channels ratio curves. This posed a real and unexpected problem in standardisation, as well as

a challenge to our curiosity.

In seeking an explanation, we first noticed that the indices of refraction of dioxane and acetone were lower than that of the glass vial, while the toluene index was higher. Small differences in light-trapping within the various samples seemed possible. We already had a simple model for colour quenching which qualitatively explained the shape of the pulse height spectrum in terms of dependence of pulse height on the position of an event in the vial, although it was not developed as quantitatively as ten Haaf's model. It seemed reasonable that light-trapping differences might affect the shape of the pulse height spectrum in an analogous way. We therefore investigated a number of solvents with different refractive indices. We also put solvents which gave different channels ratio curves into vials in which light-trapping was reduced, such as polyethylene, nylon and frosted glass vials. We found similar differences between the solvents in all vials. We were not able to correlate these differences with the refractive indices of the solvents.

In the original comparison of toluene and dioxane solutions we had observed deviations of up to about 4% in apparent d.p.m. as determined by the sample channels ratio: i.e. if the toluene samples were used as the standards, an efficiency taken from their channels ratio curve would lead to a calculated d.p.m. for a dioxane sample which differed by as much as 4% from the true d.p.m. In expanding our range of solvents, we found considerably larger deviations. We also found a dependence on quenching agent in some cases. Figure 1 shows some typical deviations. These are plotted horizontally, and represent the error in d.p.m. which results from using a sample channels ratio curve of a particular set of standards to determine efficiencies of different kinds of samples. In this case the standards contain butyl-PBD in toluene and are quenched with carbon tetrachloride. The scale shows negative deviations to the right of zero simply because most of the deviations we observed were negative (as are those due to colour), and in plotting graphs we were accustomed to placing the origin at the left. We therefore adopted this convention. The true (internal standard) efficiency of each sample is plotted vertically. Each curve on the graph represents a quenched sample set in which one component is varied to change the counting efficiency while others are held constant. The standards are represented by a vertical line at zero error. The distance of any curve from this axis can also be expressed as the relative efficiency difference at a given channels ratio between the channels ratio curves for the 'standards' and the 'samples'. Such a graph thus shows how various channels ratio curves diverge or otherwise deviate as a function of quenching.

Note that in Fig. 1 the units of the abscissa are comparable in magnitude with the experimental error. The uncertainty in the internal standard activity was believed to be less than $\pm 1\%$ (at the 68% confidence level), as was that of the channels ratio. The error in determining the position of the channels ratio curve is somewhat larger, however. The data were usually reduced by a computer program which fitted a curve to the standards data points. It was necessary to use care in the spacing of these points in order to avoid gyrations in the curve, particularly at the ends. If the lines on the graph zigzag in unison, this is assumed to be due to the shape of the standards curve. When we were in doubt about the position of the standards curve we either prepared new standards, used one of the sample sets as standards in order to get a better spacing of points, or hand-fitted a curve for comparison with the computer-derived curve. When the deviations we were measuring were comparable in magnitude with experimental error we often replicated the samples or repeated the counting using a different channels ratio. The remaining figures present smoothed curves which may be the averages of such composite data; in Fig. 1 actual data points are shown to indicate their

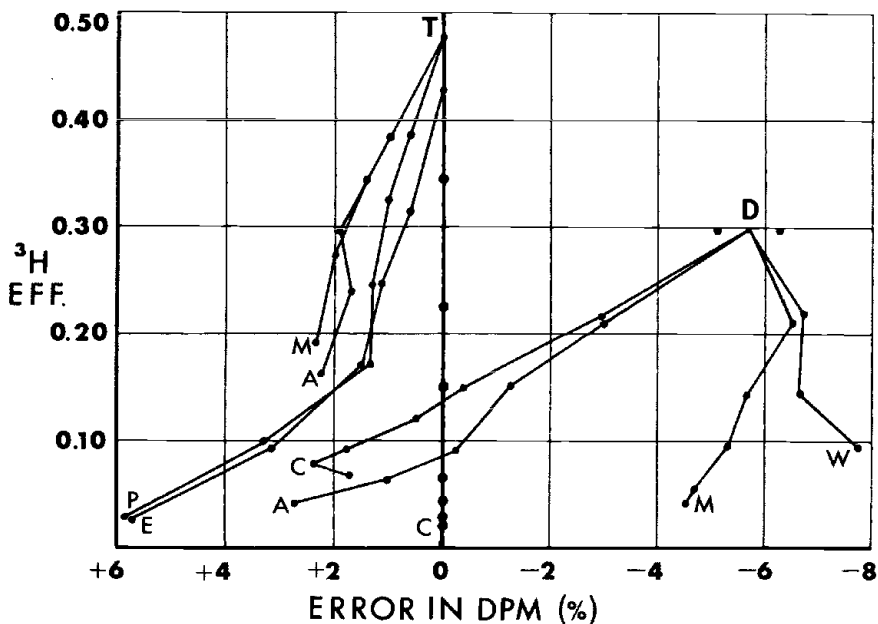


Fig. 1. Relative error due to deviations in channels ratio curves for ^3H samples as a function of quenching agent and of solvent
Standards: Butyl-PBD in toluene + CCl_4 , 0–2.8% by vol.

Samples: (T) Toluene + butyl-PBD 6.1 g/litre, (M) CH_3OH to 40%, (A) CH_3COOH to 30%, (E) $(\text{C}_2\text{H}_5)_2\text{NH}$ to 4%, (P) $\text{C}_6\text{H}_5\text{OH}$ to 11.5%, (D) Dioxane + butyl-PBD 25 g/litre, (A) CH_3COOH to 12%, (C) CCl_4 to 1.4%, (M) CH_3OH to 53%, (W) H_2O to 15%.

Quencher concentrations in volume percent. For all figures in this chapter, the fluor concentrations given are those in the final counting samples, and all samples were labelled with 1% toluene- ^3H or ^{-14}C .

precision. The two points representing unquenched dioxane samples are indicative of the reproducibility for replicate samples. There was a small difference in their channels ratios in this case, although their efficiencies in a wide channel were essentially identical. The values for these two points were averaged and this average was used in drawing the curves.

These data show that the nature of the chemical quenching agent may have quite a significant effect on the shape of the pulse height spectrum. These and a limited number of results with other solvents suggest that such effects are larger in aliphatic than in aromatic solvents. However, the extent to which curves for different quenchers will diverge depends upon factors other than whether the solvent is aromatic or aliphatic, such as the amount of quencher present at a given efficiency. This of course depends on the inherent solvent efficiency and its resistance to quenching as well as on the fluor and its concentration. In any case, one should be careful in choosing a quenching agent for a standards set. For instance, it appears from Fig. 1 that carbon tetrachloride might be a particularly bad choice for many toluene samples, since it deviates from the other quenchers which were examined.

Figure 2 shows another characteristic difference between aromatic and aliphatic solvents. In these samples there is no quencher, but the fluor concentration is

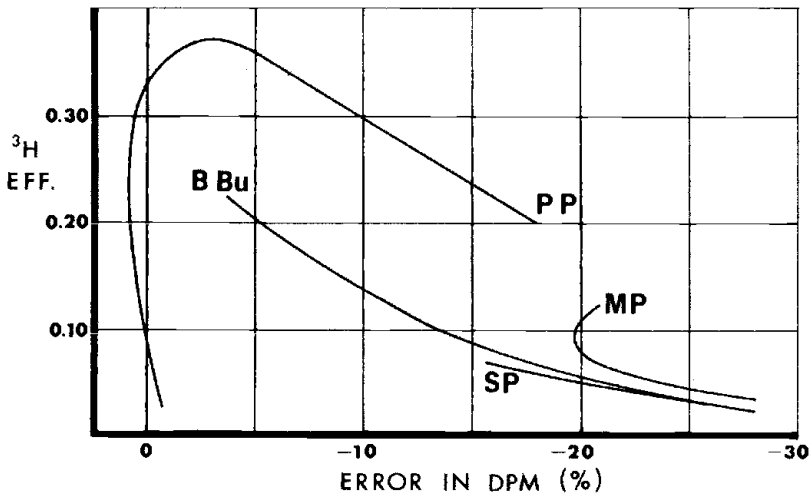


Fig. 2. Relative error due to deviations in channels ratio curves for ^3H samples as a function of fluor concentration, and of solvent
 Standards: 7 g/litre butyl-PBD in toluene + CCl_4 .
 Samples: (PP) PPO 0.25–100 g/litre in phenylcyclohexane, (BBu) Butyl-PBD 0.2–5 g/litre in bicyclohexyl, (MP) PPO 1.25–30 g/litre in mineral oil, (SP) PPO 8–20 g/litre in spinacane.

varied. (The particular fluor used is irrelevant to this comparison). The fluor concentration was extended approximately to the limit of solubility in each solvent. Efficiency was still increasing with concentration except in the case of phenylcyclohexane, in which the fluor was much more soluble (to 100 g/litre) and showed marked self-quenching. Three features of the phenylcyclohexane curve were typical of those for the aromatic solvents we examined: at low fluor concentrations there is not much difference between the channels ratios of samples which have low fluor concentrations and those which are quenched with carbon tetrachloride (the standards for this graph); near the concentration of maximum efficiency (about 8 g/litre in this case) ratios begin to differ from those of the standards; the deviation then increases as efficiency drops with increasing fluor concentration. The aliphatic solvents all showed strong deviations from the aromatics at low fluor concentrations. If the fluors can attain high enough concentrations in the aliphatic solvents their curves also show increasing deviations after reaching maximum efficiency: this is beginning to happen in the mineral oil at about 6 g/litre, but at the maximum concentration of 30 g/litre the efficiency has not yet begun to drop. Figure 3 shows a similar curve for dioxane containing butyl-PBD, which reaches a concentration of 100 g/litre. Here the pulse height spectrum does deviate sharply at high fluor concentration, although the fluor suffers only a small loss in efficiency due to self-quenching.

Figure 3 also shows the effects on the channels ratios of varying the concentrations of four different fluors in toluene. A small deviation from the carbon tetrachloride-quenched standards appears at low fluor concentration (low efficiency). A small difference between fluors is just measurable at low concentrations: the long-wavelength emitters are slightly displaced from the short. In trying to verify this we repeated the experiment a number of ways, and I believe that this is a real difference. In dioxane these same four fluors had slightly

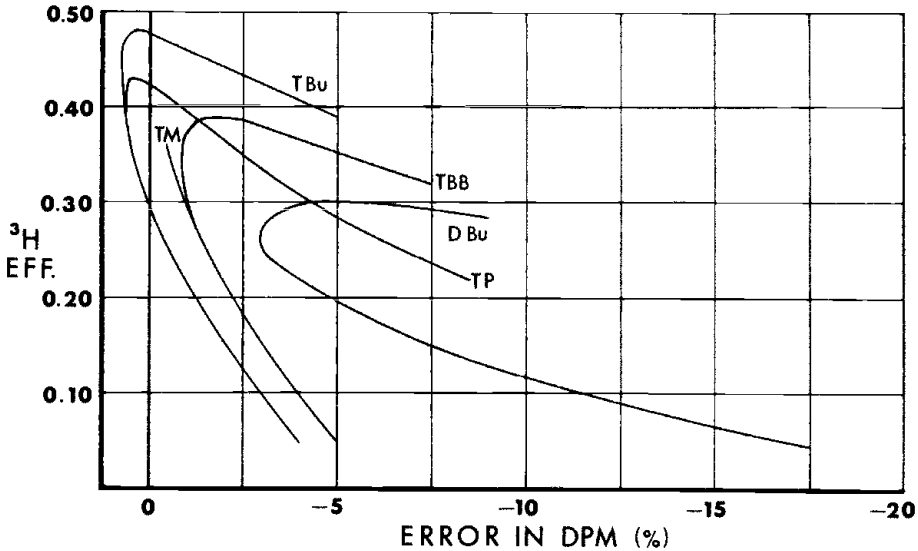


Fig. 3. Relative error due to deviations in channels ratio curves for ^3H samples as a function of fluor and its concentration, and of solvent Standards: Butyl-PBD 7 g/litre in toluene + CCl_4 . Samples: (TBu) Butyl-PBD 0.1–100 g/litre in toluene (maximum efficiency at ~ 7.5 g/litre), (TP) PPO 0.075–100 g/litre in toluene (maximum efficiency at ~ 5 g/litre), (TBB) BBOT 0.15–25 g/litre in toluene (maximum efficiency at ~ 8 g/litre), (TM) Dimethyl-POPOP 0.1–2.5 g/litre in toluene (maximum efficiency at 2.5 g/litre), (DBu) Butyl-PBD 0.3–100 g/litre in dioxane (maximum efficiency at ~ 25 g/litre), minimum error at ~ 5 g/litre).

different curves but they were not ordered by wavelength. A very interesting thing happened when the counter was operated in the unsummed mode, i.e. when pulse height analysis was performed on the output of one phototube only while using the output of the other tube simply to operate the coincidence detector. The curves for the long-wavelength fluors were then displaced relative to those for PPO and butyl-PBD, so that they were closer to the axis. A definite separation then appeared in dioxane, and a reversal of position for the two curves at low concentration in toluene.

Pulse summation might be expected to affect the relative difference between the shapes of the pulse height spectra of coloured and colourless samples. Although none of these samples was coloured in the usual sense, there is significant self-absorption of fluor emission at all these concentrations, the amount differing with the fluor. Light absorption losses outside the solution are also wavelength-dependent.

In an analogous experiment with Carbon-14 labelled samples counted in the summed mode, the channels ratio curves of the same four fluors were not resolved. It might have been expected that a greater difference would be observed than with Hydrogen-3, since the counting efficiency was higher for a given fluor concentration, and lower concentrations were reached. (These samples were not counted in the unsummed mode.) Figure 4 shows typical Carbon-14 curves for varying fluor concentration in toluene and in dioxane. Although the maximum fluor concentrations were the same in these samples as in the Hydrogen-3 sets, the effect

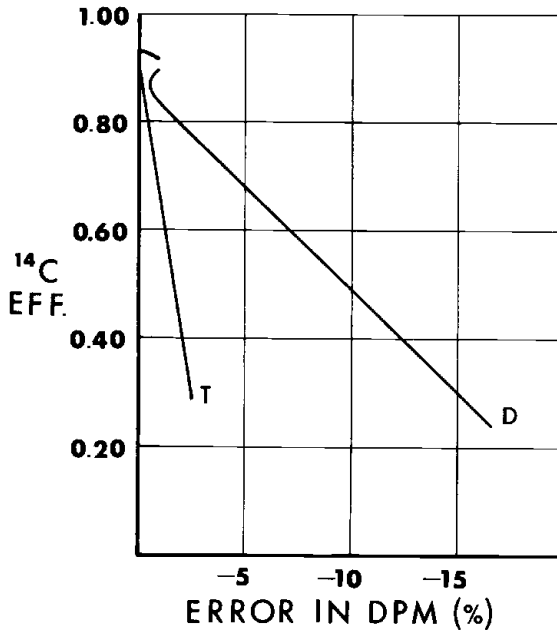


Fig. 4. Relative error due to deviations in channels ratio curves for ^{14}C samples as a function of fluor concentration, and of solvent.

Standards: Butyl-PBD 7 g/litre in toluene + CCl_4 .

Samples: (T) Butyl-PBD 0.01–100 g/litre in toluene. (D) Butyl-PBD 0.01–25 g/litre in dioxane.

of self-quenching is hardly evident in these curves. Little or no loss of efficiency was measured at high concentration, although the sample channels ratios did show sizeable changes indicative of decreasing pulse heights. This is explained by the fact that efficiency was measured in an integral channel at high gain, and that it changed only very slowly with channels ratio at high efficiency. The flatness of the channels ratio curves would tend to obscure deviations between them. A different choice of channels would be needed to measure differences in the spectra at high concentration.

It is tempting to attribute the changes in shape of the Hydrogen-3 pulse height spectra at high fluor concentration to self-absorption, since these changes are in the same direction as those due to colour. It is also very difficult to explain the large deviations at low fluor concentration in aliphatic solvents on the same basis! Changes in self-absorption might also result from the effects of solvents and quenchers on the emission and absorption spectra of the fluors. Such spectra were examined, and some small shifts were seen. We were unable to correlate these with the counting results on the basis of any simple assumptions. However, our data were certainly not sufficient to prove that differences in solution transparency did not contribute to the observed differences in the shapes of the pulse height spectra.

In an effort to get independent information on the relevance to these phenomena of geometry factors, i.e. the factors responsible for the change in shape of the colour-quenched spectrum, we looked at some different sample configurations. We sealed solutions labelled with tritium and containing varying fluor concentrations into capillary tubes which could be suspended in an upright position inside

an ordinary counting vial. The inside diameter of the tubing was 1 mm, and the depth of filling corresponded to a 20 ml filling of the vial. The tubes were then surrounded with 20 ml of various unlabelled solutions. They were counted together with an ordinary quenched standard set of bulk-labelled solutions, and the channels ratio curve of that set was used to calculate their activities. These were compared to the known activities. By this means we hoped to get some measure of self-absorption effects on efficiencies and channels ratios for sources at various locations in the vial.

When the tubes were centred in the vials and surrounded by solutions of their own respective compositions their percent deviations in d.p.m. followed the same order as for the same compositions in ordinary bulk samples, and were close to being numerically the same in many cases. When the tubes were surrounded by pure toluene, approximately the same deviations were observed, although they were slightly smaller in a few cases. The pulse height spectrum of the 1 mm thick source thus had very nearly the same characteristics as the spectrum of a 20 ml bulk source of the same solution.

The efficiency for a tube sample surrounded by its own scintillator was considerably higher than the efficiency for 20 ml of the same solution labelled in bulk. This would be expected when the tube is centred, since light collection from the centre of the vial is relatively good. When tubes containing PPO solutions at concentrations of 0.075–4 g/litre were transferred from vials containing pure toluene to vials containing their own scintillators, respectively, there was very little change in their counting efficiencies. It appears that these 1 mm sources must be considered as 'thick' with respect to self-absorption at their own concentrations. When they were placed in a solution of 100 g/litre PPO their counting rates dropped by 20–45%, and there was a small loss in relative d.p.m. as would be expected for a 'colour' effect.

The tubes were also counted at other positions in the vials, surrounded by a (yellow) coloured solution. The changes in efficiency and calculated d.p.m. were in general agreement with what we expected from our simple model of colour quenching. We concluded from these experiments with capillary tubes that fluor self-absorption along a multiplicity of light paths can cause a shift in the pulse height spectrum like that due to colour, but that this does not adequately explain the differences in the shapes of the pulse height spectra of the unquenched, 'colourless' solutions.

We considered the possibility that the scintillation time decay curves might be different in these solutions, and that variation in the ratio of fast and delayed decay components might lead to variable discrimination between large and small pulses. The expected magnitudes of the fast and delayed components, however, made it seem unlikely that this effect would be observable.

No temperature dependence was observed for the deviations over the range accessible in the counter (–10 to +20°C). This was true for a non-viscous solvent, toluene, as well as for a viscous one, benzyl alcohol, whose viscosity varied significantly over this range. The benzyl alcohol was quenched with a variety of strong quenchers in small volumes, and diluters of low viscosity in large volumes. The pulse height spectra of these tritiated solutions showed only small variations, as was typical of aromatic solvents. In our dioxane samples the observed deviations were not correlated with solution viscosity.

The data presented here were obtained with a Nuclear-Chicago Mark I counter. Similar results were seen when samples were counted in a (higher efficiency) Mark II. The differences in the channels ratio curves for Hydrogen-3 samples were observable with any choice of channels. They could also be seen with a

Barium-133 external standard ratio. At large pulse heights, as for high efficiency Carbon-14 samples, errors in efficiency determination due to differences in curves may be minimised in the same way as for spectral changes from other causes (such as quenching or phase separation): by counting in a channel in which efficiency is nearly constant and not sensitive to spectral shape.

Our investigation was terminated without having produced an explanation of the observed variations in pulse height spectral shape among colourless solutions. We found no clear relation with 'geometry factors'. I am reporting this exploratory work not only because of the practical implications of these phenomena in standardising, particularly as counter resolution improves, but in the hope of stimulating further investigation by others. My present feeling is that a complete quantitative description of the optics of the liquid scintillation counter is long overdue. I hope that Dr. ten Haaf and others are continuing their work in this area. We may not know to what extent these effects are optical or geometric until we get an adequate model.

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DISCUSSION

B.W. Fox: A mixture of a hydrocarbon solvent and an aqueous solution will in most cases be two phases. If a surfactant is added, it only disperses these two phases as a colloid or emulsion. Thus if any mixture contains surfactant it must be regarded as heterogeneous and not a true solution. Most commercial mixtures appear to be based on mixtures which are situated between the solvents, detergent margin and the centre and these are fortuitously similar in counting efficiency for tritiated toluene and tritiated water standards. At lower detergent, the toluene efficiency is higher than water, and at low toluene levels, the efficiencies from H_2O-^3H are higher (although the absolute efficiencies are much lower).

E. Mueller: It was to avoid such a controversy that I offered an 'operational' definition of a solution — if the efficiencies of ^3H -toluene and $^3\text{H}\text{-H}_2\text{O}$ are the same, the mixture behaves as a solution from the point of view of counting.

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