CHAPTER 63

Quench Correction of Colored Samples in LSC

Kenneth Rundt, Timo Oikari, and Heikki Kouru

ABSTRACT

Liquid scintillation counting of weak β-emitters like tritium and ^{14}C, usually involves quench correction with a stored quench curve, which traditionally expresses the counting efficiency as a function of a quench monitor value. Generally, the quench curve is produced by measuring chemically quenched (uncolored) calibration standards; however, if the unknown samples contain color, the computed activity will systematically be in error when a basic quench monitor, like the isotope mean pulse height or the external standard endpoint, is used. The errors will be even more pronounced in dual label counting, as the shapes of the spectra depend on the relative amounts of color and chemical quench. In this chapter, we outline an improved quench correction method based on the dynamic determination of both the overall quench level and the amount of color quench. The counting efficiency is computed from a quench curve which in fact should be regarded as a surface. This solution was first implemented in the Wallac 1219 Rackbeta liquid scintillation counter. In a more general solution, applicable in multi-label counting, the shape of the spectrum can be considered a function of two quench parameters. This extended version has been implemented in the new Wallac 1410 liquid scintillation counter.

INTRODUCTION

Color quenching, or more generally, photon quenching, involves the absorption of photons by colored compounds, solid particles, or macromolecules suspended in the liquid scintillation solution. As the emitted light is mainly in the blue region, around 400 nm, yellow compounds are the strongest color quenchers. Color quenching differs from chemical quenching in that it depends on the geometry of the vial and on the spatial coordinates of the disintegration.[1,2] This difference in the modes of the two quenching mechanisms leads to a difference in the pulse height distribution; it becomes reflected in the quench index used for efficiency determination. Traditionally, there have been two ways of handling color: bleaching with a peroxide so that chemical quench prevails, or dividing the samples into two groups, chemically quenched and color quenched, and using two different quench curves. Bleaching is an extra laborious step that may not even reduce the amount of color completely. Furthermore, bleaching also increases the amount of chemical quench in the sample. Segregating the samples may sound very simple, but for two reasons this mode of operation is not satisfactory. First, it demands an extra operation by the user, and second, as it will be shown later, samples that have high amounts of both chemical and color quench should be treated as chemically quenched samples and not as color quenched samples. The second reason is the most difficult to handle, as the human eye cannot judge the amount of chemical quench in the samples.

The presence of color in the samples imposes certain demands on the quench correction feature of the instrument—the counter should have an automatic method to detect the presence of color and compensate for the difference between color and chemical quench. Several attempts have been made to improve counters in this respect. One solution that has been applied commercially is based on using the spectrum produced by the lesser of the two pulses from the photomultiplier tubes.[3] According to tests made by McQuarrie et al.,[4] the lesser pulse height method does indeed perform slightly better than other conventional methods.

In current literature there are a number of other proposed methods. For example, Ross[5] has proposed a method wherein the sample absorbance is determined by immersing a small glass ampule containing an unquenched scintillation solution into a given volume of uncolored scintillation solution and the colored unknown sample. The count rates of the uncolored solution and colored solution are denoted by C_0 and C_1, respectively. The counting efficiency for the colored sample is calculated by multiplying the counting efficiency derived from the calibration curve by the ratio C_1/C_0. This method is not applicable in an automatic LS counter, as the sealed light source must be inserted and removed manually, and wiped carefully between measurements. The counting time per sample is also prolonged.

Lang[6] has proposed a method based on using both the count rate induced by the external standard and the external standard channels ratio, ESCR. In this method, four calibration curves are needed: the counting efficiency as a function of the external standard count rate and the ESCR for both colored and uncolored standards. Lang's method could in general be applied in an automatic counter, but as the external standard count rate is dependent on the volume of the liquid as well, this method has not been commercially applied. Moreover, both the count rate and the ESCR are very dependent on the so called plastic vial wall effect, which is a result of penetration of solvent into the plastic wall.

Takiue et al.,[7] have proposed a method quite similar to Lang's method. Their method, which is based on using sample channels ratio, SCR, together with ESCR, also requires two sets of quenched standards and will result in four quench equations. This method is an analogy of the homogeneity monitor proposed by Bush[8] and is thus dependent on sample homogeneity. Also this method is sensitive to the plastic vial wall effect by the use of ESCR. Furthermore, the method is applicable only when the activity of the isotope is high enough, so that SCR can be determined with high accuracy.

Ring et al.,[9] have suggested a method based on two parameters calculated from the external standard pulse amplitude spectrum. One parameter, called the quench index (QI), is proportional to the mean pulse height, the other parameter, called the color index (CI), is equal to the pulse height (channel number) below which the number of pulses is a constant fraction of the total number of pulses in the spectrum. An example of such a parameter is the median, which divides the spectrum into two parts having the same number of counts. In this method, two equations are needed: one expresses the counting efficiency of chemically quenched samples as a function of QI, and the other expresses the ratio between the counting efficiency of a colored sample and an uncolored sample, as a function of both QI and CI. By applying these two equations, the correct counting efficiency can be determined. Also this method depends on the plastic vial affect through the color index CI. The method is also affected by chemiluminescence in the solution as the CI is calculated using all pulses from channel one upwards.

The methods by Lang, Takiue et al., and Ring et al., require at least two sets of quenched standards to be prepared by the user. Generally, the correction can be performed two ways. Either the counting efficiency is corrected or the quench index is corrected. If P denotes the total quench index, equal to, e.g., the endpoint of the external standard spectrum, and R denotes a color index, then the counting efficiency E of any sample can be written as a product of two functions:

$$E = E(P) \cdot F(R,P)$$

E(P) is the function representing the overall quench curve, while F(R,P) is a function giving the correction to E(P) needed for a colored sample. More generally, it may be a better solution to try to combine R and P into a single parameter, Q, the actual quench index seen by the user. This means writing

$$E = E(Q) = E(Q(R,P))$$

which means that the quench curve is a function of Q, which is a function of both R and P. This is the solution taken by Wallac[10] in the Rackbeta series of instruments (Rackbeta 1219, 1214, and optionally 1209). Our external standard quench index, SQP(E) (Sample Quench Parameter of External standard), is based on two parameters: P which is the channel number below which 99% of the external standard spectrum is located, and R, which is described in more detail in this article. In the new 1410 LS counter, the actual quench curve is not a curve but a surface, i.e.,

$$E = E(R,P)$$

The SQP(E) value printed out by this instrument is equal to P, and the Color Index, that can be printed out as well, is equal to R. This solution was selected because the 1410 counter stores the shape of the spectra, as well as the counting efficiency, as functions of both P and R.

The parameter R is also computed from the external standard spectrum. The method implemented in Rackbeta instruments and in the new LS counter 1410 can best be described by using Figures 1 and 2. Figure 1a and 2a show three-dimensional plots of the ^{152}Eu external standard Compton spectrum. Similar plots for the beta-isotope dissolved in the scintillation liquid have been produced by Laney earlier.[11] The sample that produced Figure 1 was purely chemically quenched, and the sample that produced Figure 2 was purely color quenched. The counting efficiency of both samples was nearly the same (about 30% for tritium). Figure 1b shows a projection of the three-dimensional spectrum on a plane perpendicular to the pulse height plane in Figure 1a. The instrument does not actually record the spectra in these two figures, but the summed pulse height spectra of the two regions A and B are recorded. This is accomplished by using the left/right comparator and dual MCA technology. Dual MCA technology generally means that a pulse can be directed to one of two MCAs, depending on a certain criterion, which can be selected through software. When the external standard is measured, all pulses for which PHR/PHL > 1.5 or PHL/PHR > 1.5 (PHL and PHR are the linear pulse heights) are directed to the MCA-B while all other pulses are directed to the MCA-A. The resulting spectra are shown in Figure 3a and 3b. After the external standard has been measured, the P-value (equal to the 99% endpoint of the total spectrum A + B) and the color index R are calculated. R is equal to the ratio of counts (A + B)/A in a certain pulse height window.

EXPERIMENTAL

A set of 64 tritium samples was prepared for this work. The counting efficiency of the samples ranged from 3 to 62% and the color index R ranged from 1.0 to 6.5. The scintillation cocktail used was OptiScint HiSafe (Pharmacia-Wallac), a proprietary, high-flashpoint cocktail for organic samples. Standard 20 mL glass vials with 10 mL of cocktail were used. The quenchers used were nitromethane and Sudan 1 (yellow). The samples were prepared as one set of purely chemically quenched samples, one set of purely color quenched samples, and several sets of samples with a varying mixture of chemical and color quench. Figure 4a shows the amounts of quenchers and Figure 4b shows the recorded color index and total quench index for all the samples. The samples were measured in a prototype of the 1410 LS counter, with ^{152}Eu as external standard. The plastic vial effect was assessed by using a traditional toluene based cocktail in standard plastic vials.

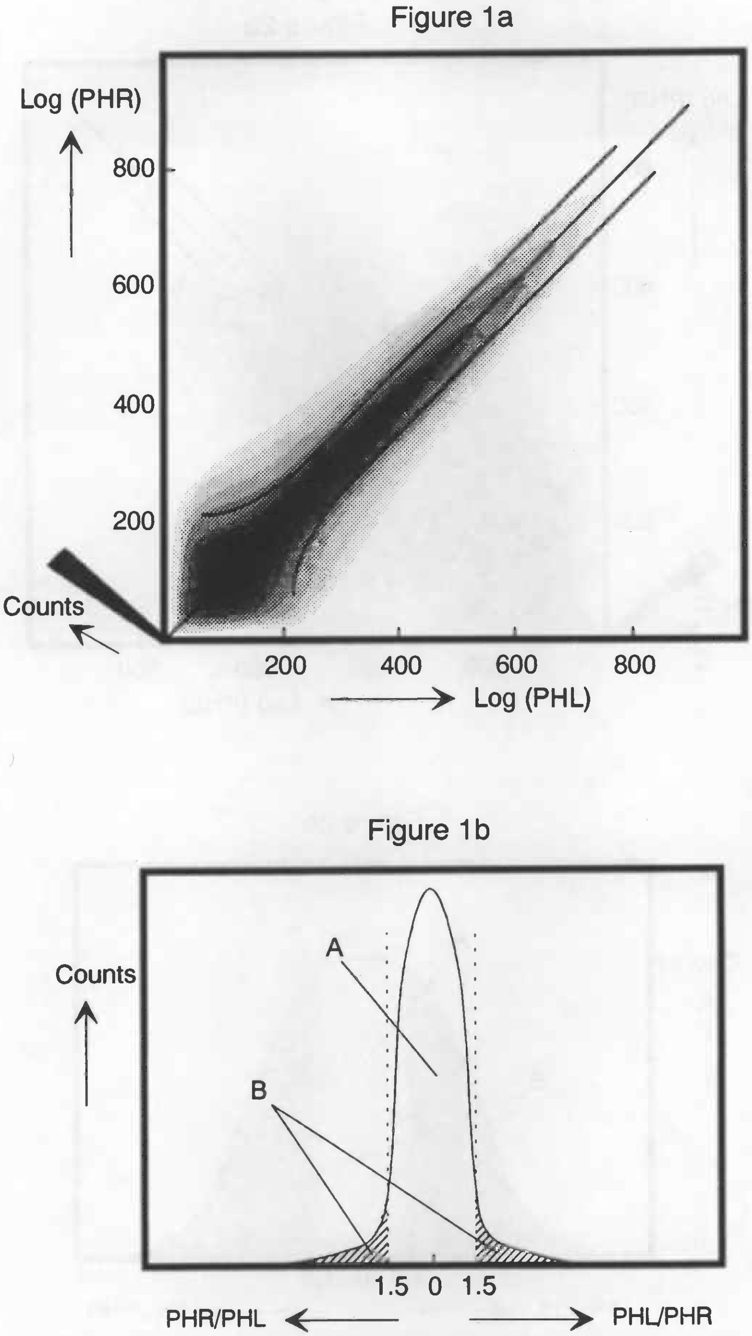

Figure 1. Left/right pulse height distribution for a chemically quenched sample (sample 'C' in Figure 4). a) three-dimensional plot showing the full distribution and the comparator limits (1:1.5). b) two-dimensional projection of the distribution in a). The color index R of the sample is equal to the ratio of counts (A + B)/A. For this sample R = 1.08.

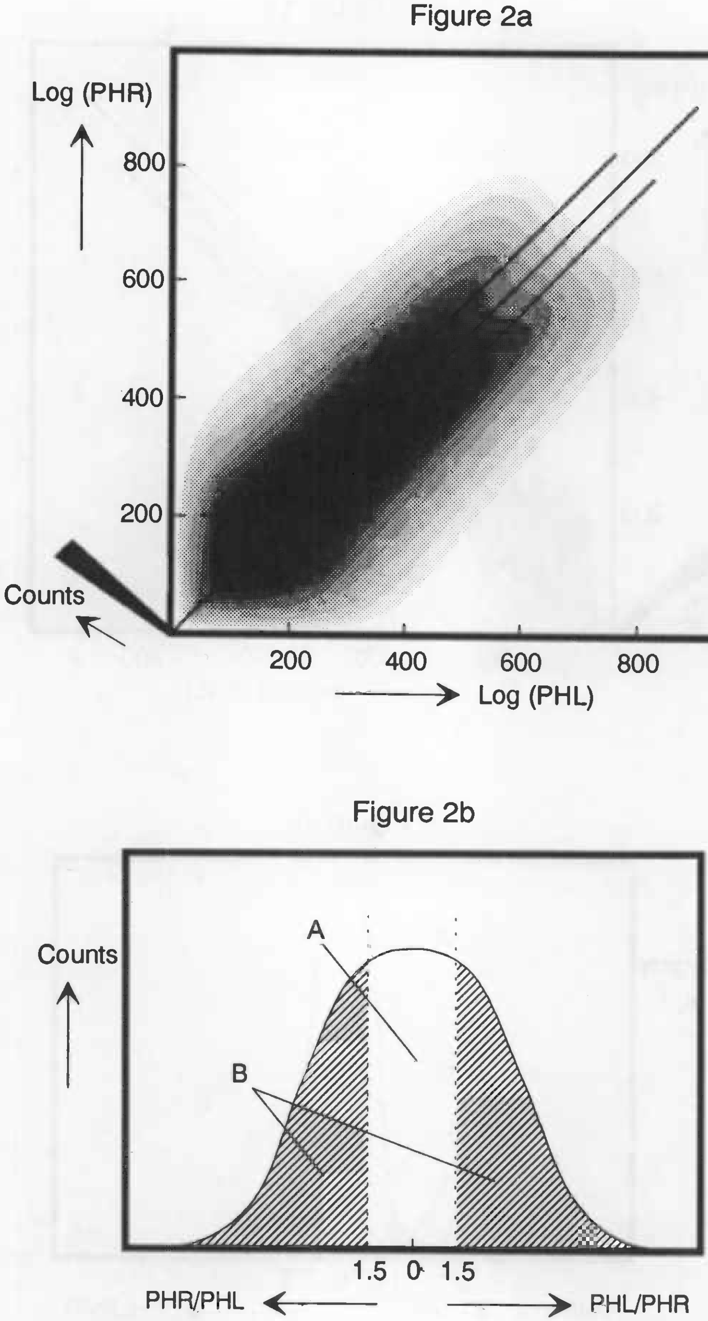

Figure 2a

Figure 2b

Figure 2. Left/right pulse height distribution for a chemically quenched sample (sample 'Y' in Figure 4). a) three-dimensional plot showing the full distribution and the comparator limits (1:1.5). b) two-dimensional projection of the distribution in a. The color index *R* of the sample is equal to the ratio of counts (A + B)/A. For this sample *R* = 2.68.

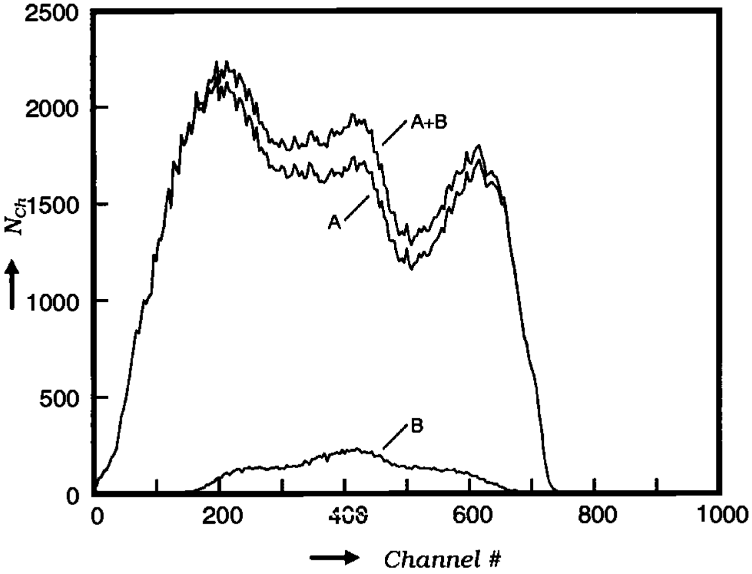

Figure 3a. Dual-MCA spectra of a chemically quenched sample "C". A is a spectrum comprising pulses within the two comparator limits and B is a spectrum comprising pulses outside the two comparator limits. A + B represents the normal spectrum.

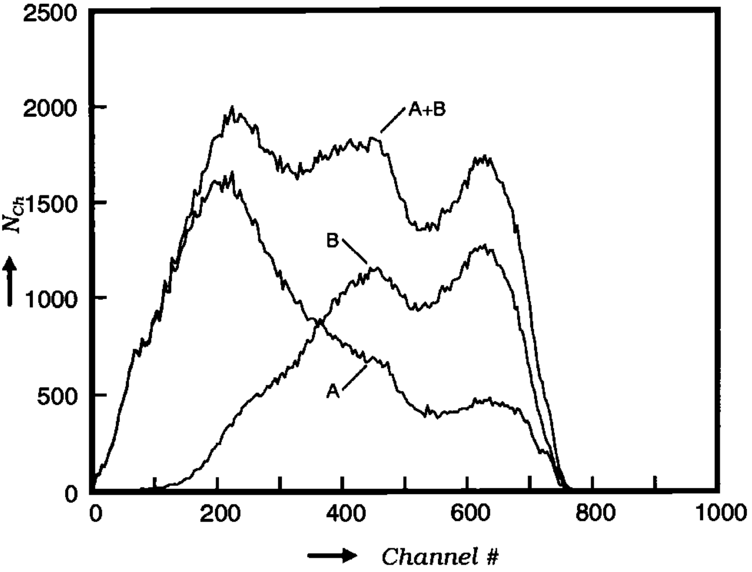

Figure 3b. Dual-MCA spectra of a color quenched sample "Y".

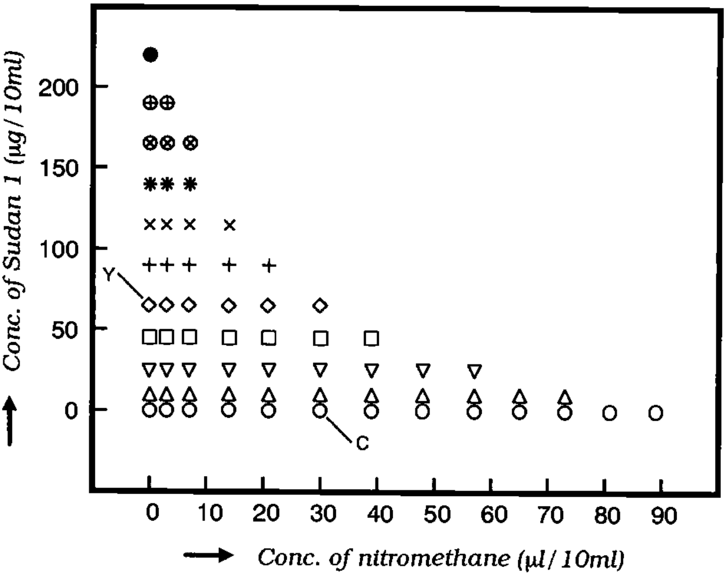

Figure 4a. The composition of the 64 tritium samples used in this work. The symbols are the same in each set of constant color quench but increasing amount of chemical quench.

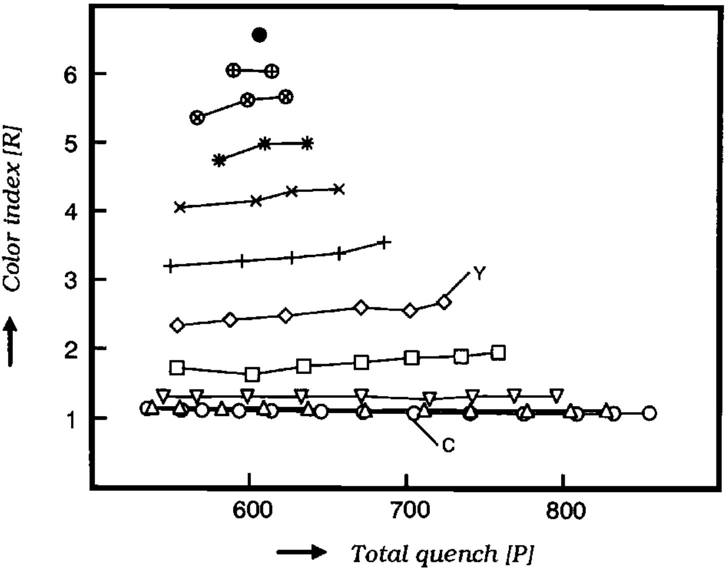

Figure 4b. The color index and the total quench of the 64 samples. In both figures, sample "C" is a purely chemically quenched sample "Y" is purely color quenched. Both have counting efficiency near 23%.

RESULTS

The need for color correction becomes obvious when looking at Figure 5. This figure shows the relative error in the calculated activity when calculating the activities of all the 64 samples with a traditional calibration curve, *without any color correction*. Especially when the counting efficiency goes below 10%, the error increases rapidly. Similar behavior when using the mean pulse height of the tritium spectrum as quench monitor has been documented by one of the authors elsewhere.[12] Figure 5 also shows that adding chemical quench to a color quenched sample makes the deviation from the chemical quench curve noticeably smaller (observe, e.g., the sample set marked by the symbol +). This means that it does not suffice to correct for pure color quench only, but that the instrument must be able to determine the relative amounts of color

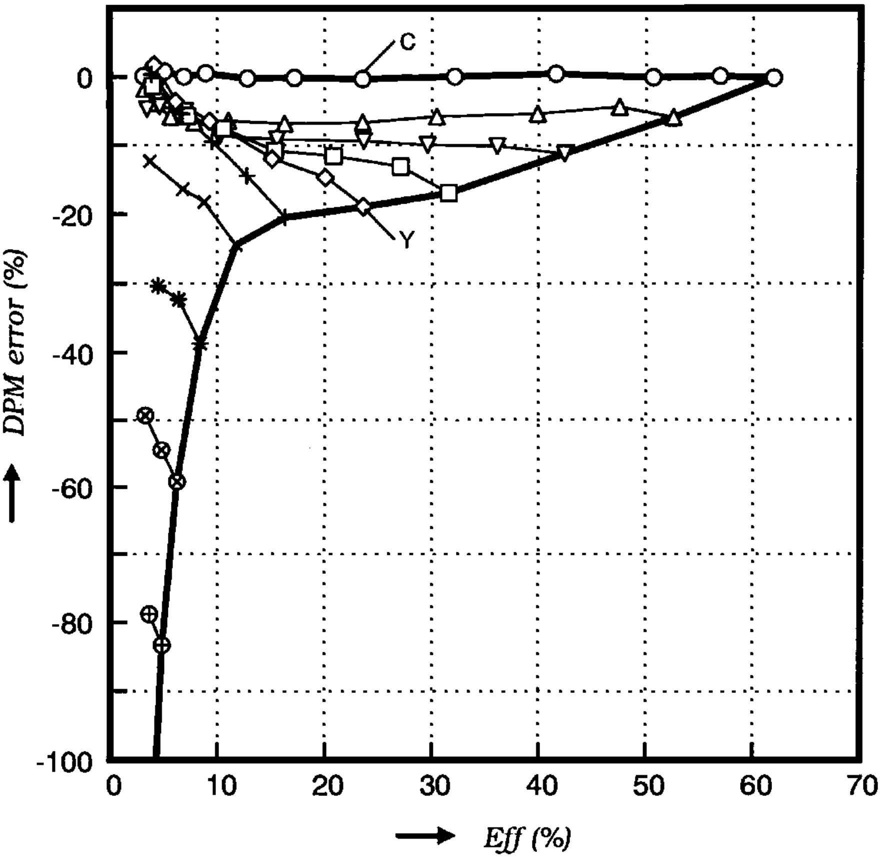

Figure 5. The relative error in tritium activity for all samples when a traditional quench curve involving no color correction is employed. The different symbols refer to the sample sets as shown in Figure 4.

and chemical quench. This is important as real samples mostly have both types of quench.

A natural question is whether the color index R and the total quench index P are good enough to describe the behavior of all kinds of samples. In order to give an indication of how well P and R work, the 64 samples were used to prepare a counting efficiency surface. The function selected to describe the surface was a linear combination of P and ln (R) having 13 parameters. This function was fitted to all the 64 points on the surface by using the method of least squares; thereafter, the function was used to predict counting efficiencies for all the samples. The result is shown in Figure 6. Notice the difference in ordinate scale between Figure 5 and Figure 6. Generally, for samples having a counting efficiency above 10%, the relative activity error is negligible, and for samples with counting efficiency between 3 and 10%, the error is below 5%.

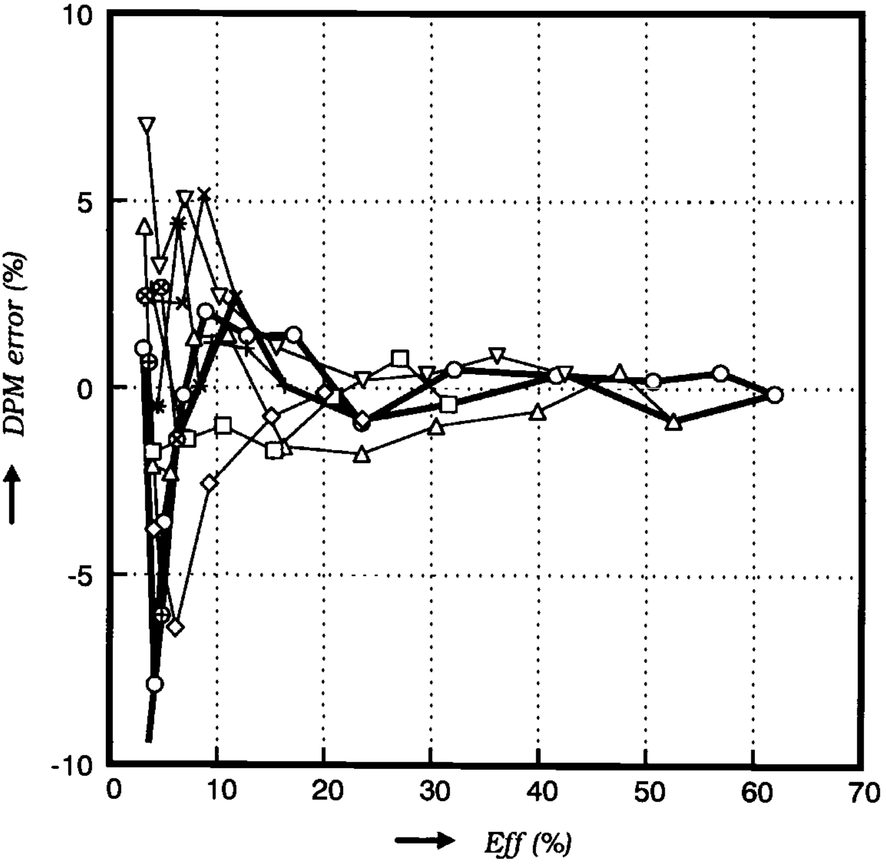

Figure 6. The relative error in tritium activity for all samples when a quench surface involving both total quench and color quench is employed. The different symbols refer to the sample sets as shown in Figure 4.

Even better results can be accomplished by selecting a more elaborate surface function.

Another important point of concern is the shape of the isotope spectrum. It has been noticed that the shape of the spectrum is different when comparing chemically quenched and color quenched samples having the same counting efficiency.[2] This is once again verified in Figure 7, which shows the tritium spectra of the two samples 'C' (pure chemical quench) and 'Y' (pure color quench). For single label counting, the spectrum shape is not very critical, but for dual label counting, the accuracy of, e.g., tritium is directly dependent on how well the spectra of the calibration standards describe the spectra of the samples. In Rackbeta counters, the "Three-Over-Two" method was equipped with an algorithm to correct the relative intensity curves for color. In the new 1410 counter, using Digital Overlay Technique (described elsewhere in this publication[13]), the single isotope spectra fitted to the unknown composite spectrum are retrieved from a spectrum library, wherein the spectra are stored

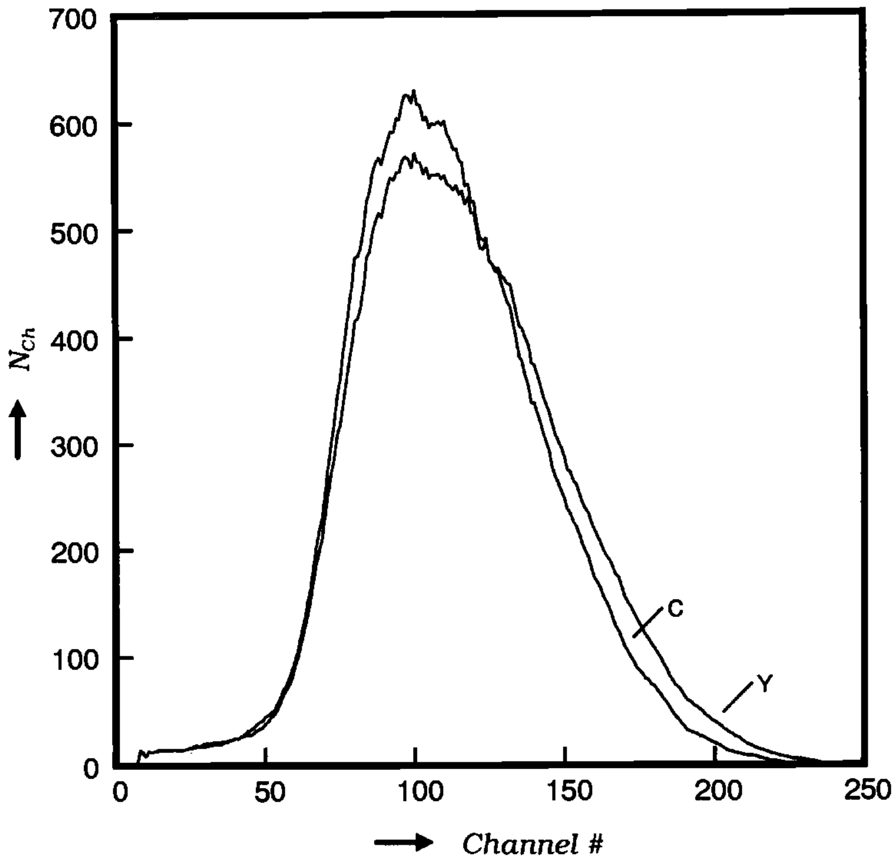

Figure 7. The tritium spectra of the two samples 'C' and 'Y'.

Table 1. The Effect on Color Index *R* and Total Quench Index P of Diffusion of Toluene into the Plastic Vial Wall

Time/hour	R	P
0	1.30	699
5	1.32	698
28	1.37	698
46	1.38	699
75	1.38	698

as a function of the two quench parameters P and R. This guarantees that the correct spectrum shape is always fitted to the sample spectrum.

Volatile liquids in plastic vials give rise to the so called plastic vial effect that significantly affects external standard quench indices based on the channels ratio or mean pulse height. The influence of the plastic vial effect on the color index *R* was tested with one chemically quenched sample having 10 mL of a toluene based cocktail. The initial value of the color index is as high as 1.3, although no color has been added. The color index is normally slightly greater in diffuse plastic vials than in clear glass vials. The result of the measurement (Table 1) indicates that there is a small but negligible increase in the color index with time as a result of diffusion of toluene into the plastic material. As the quench level increases, the plastic vial effect becomes more pronounced, but it is always at quite a moderate level.

SUMMARY

An automatic quench correction method based on determining both the amount of color quench and chemical quench in liquid scintillation samples has been described. The method depends on using the external standard spectrum and pulse amplitude comparison for determining two quench parameters: the total quench level and a color index. Using two parameters instead of one means that quench correction must be based on quench surfaces instead of quench curves. In the simplest case, the two parameters are used to retrieve a counting efficiency value from such a quench calibration surface. In a more elaborate structure, the two quench parameters are used to retrieve model spectra for the isotopes of interest when using Digital Overlay Technique for resolving multi-labeled samples.

REFERENCES

1. ten Haaf, F.E.L. "Colour Quenching in Liquid Scintillation Coincidence Counters," in *Liquid Scintillation Counting, Vol 2*, M.A. Crook, P. Johnson, and B. Scales, Eds. (London: Heyden & Son Ltd, 1972), pp 39–48.
2. Neary, M.P. and A.L. Budd. "Color and Chemical Quench," in *The Current Status of Liquid Scintillation Counting*, E.D. Bransom Ed., (New York: Grune & Stratton, 1970), pp 273–282.

3. Laney, B.H. "Method and Apparatus for Determining Efficiency in a Liquid Scintillation Counting System," U.S. Pat. No. 3,725,657 (1973).
4. McQuarrie, S.A., C. Ediss, and L.I. Wiebe, L.I. "Some Performance Characteristics of Modern Liquid Scintillation Counters," *Int. J. Appl. Radiat. Isot.*, 34(7):1009–1012 (1983).
5. Ross, H.H. "Color Quench Correction in Liquid Scintillator Systems Using an Isolated Internal Standard," *Anal. Chem.*, 37(4):621–623 (1965).
6. Lang, J. F. "Chemical vs. Color Quenching in Automatic External Standard Calibration. Application of Empirical Observations in a Computer Program," in *Organic Scintillators and Liquid Scintillation Counting,* D.L. Horrocks and C.T. Peng, Eds. (New York: Academic Press, 1971), pp. 823–833.
7. Takiue, M., T. Natake, and M. Hayashi. "Double Ratio Technique for Determining the Type of Quenching in Liquid Scintillation Measurement," *Int. J. Appl. Radiat. Isot.*, 34(10):1483–1485 (1983).
8. Bush, E.T. "A Double Ratio Technique as an Aid to Selection of Sample Preparation Procedures in Liquid Scintillation Counting," *Int. J. Appl. Radiat. Isot.*, 19:447–452 (1968).
9. Ring, J.G., D.C. Nguyen, and L.J. Everett. "Liquid Scintillation Counting from Gross Counts to Spectral Analysis," in *Liquid Scintillation Counting, Recent Applications and Development, Vol.1,* C.T. Peng, D.L. Horrocks, and E.L. Alpen, Eds. (New York: Academic Press, 1980), pp. 89.
10. Oikari, T. and K. Rundt. "Method for Determining Counting Efficiency in a Liquid Scintillation Counting System," US Patent 4,700,072, 1987.
11. Laney, B.H. "Two-Parameter Pulse Height Analysis in Liquid Scintillation," in *Liquid Scintillation Counting, Vol 4,* M.A. Crook and P. Johnson, Eds. (London: Heyden & Son Ltd, 1977), pp 74–84.
12. Rundt, K. "The Effect on Quench Curve Shape of the Solvent and Quencher in a Liquid Scintillation Counter," in *New Trends in Liquid Scintillation Counting and Organic Scintillators,* 1989.
13. Kouru, H. and K. Rundt. "Multi-Label Counting Using Digital Overlay Technique," in *New Trends in Liquid Scintillation Counting and Organic Scintillators,* 1989.