

## THE INFLUENCE OF THE USER ON INSTRUMENT DEVELOPMENT

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### ABSTRACT

The user very often influences the development of instruments for his needs. This has been, and surely will continue to be, the case with liquid scintillation counting systems. This paper will attempt to show only three areas where this has happened. These areas are 1) quench monitoring methods, 2) phase separation detection and 3) special data calculation software programs. Of course, there are many other areas that have been directly resultant from user desires and needs, but for the sake of brevity only these three will be discussed.

In the area of quench monitoring methods, several new methods have been developed recently which are much faster, more reliable, and apply over a much greater quench range. These new methods will be discussed as to how they work.

When the counting sample separates into two or more phases, it is impossible to determine the amount of radionuclide present because of unknown sample division between the two phases, which often count with different efficiencies. The user's samples have had to be physically examined after counting to detect phase separation. When working with plastic vials, this is often not possible. Some methods of detecting phase separation will be discussed.

Finally, the development of complete data reduction to final answers has been introduced into most commercial liquid scintillation systems. Single, dual and triple-label dpm programs are available. Histogram plotting with peak and peak counts or dpm is available. Radioimmunoassay final answers can be obtained. Half-life corrections can be made for short-lived radionuclides.

### INTRODUCTION

The needs of users of liquid scintillation systems have been some

of the driving forces in the many changes that have occurred in commercial liquid scintillation systems. Some of the changes have been due to the manufacturers' innovations as a direct result of desires and needs that were expressed by those using the systems. This paper discusses just three areas where such changes have occurred.

## 1. Quench Parameters

The measurement of the amount of a radionuclide in a liquid scintillation sample requires a knowledge of the counting efficiency of that radionuclide under those experimental conditions. Over the years, there have been many ways for obtaining the counting efficiency of a solution for a radionuclide. The methods have improved in accuracy, speed, range and reproducibility. A brief history of the methods of efficiency determination, along with a description of the present methods, will give an insight into the changes which were necessitated by the needs of users of liquid scintillation counters.

### Internal Standardization

One of the first successful methods of determining the counting efficiency of a radionuclide in a scintillation solution was the internal standardization method. A small, but known, amount of high specific activity of the radionuclide will be added to the sample to measure the counting efficiency of the radionuclide. First the sample is measured under known instrument settings to give the desired accuracy of the count rate, CPMs. Then a known amount of the radionuclide is added to the sample in an amount and chemical form such that the counting efficiency of the solution will not be changed. The combined count rate of internal standard ( $CPM_{is}$ ) and the sample ( $CPM_s$ ) is measured  $CPM_t$ . Usually the amount of radionuclide in the internal standard is chosen to be many times greater than the amount of radionuclide in the samples to minimize errors and measurement time. The internal standard count rate is obtained by subtraction:

$$CPM_{is} = CPM_t - CPM_s$$

and the counting efficiency is calculated:

$$\text{Eff} = \frac{\text{CPM}_{is}}{\text{DPM}_{is}}$$

where  $\text{DPM}_{is}$  is the disintegration rate of the radionuclide added in the internal standard. Finally, the amount of radionuclide in the sample is calculated:

$$\text{DPM}_s = \frac{\text{CPM}_s}{\text{Eff}}$$

The accuracy of this method depended upon the accuracy with which the  $\text{DPM}_{is}$  was known. Also, the addition of the internal standard must not change the counting efficiency of the solution. One major drawback to this method is that the sample can never be remeasured once the internal standard has been added.

In all subsequent methods, it is required that a relationship between the counting efficiency of the radionuclide and the parameter to be measured has to have been previously obtained by measuring a series of samples with a known amount of the radionuclide in each sample. The efficiency of the samples is altered by addition of different amounts of a chemical (commonly called a quencher) to each sample. This relationship is commonly referred to as the quench curve.

#### Sample Channels Ratio (SCR)

The distribution of pulse height responses (Figure 1) is due to the different number of photons produced in each scintillation event. The relative pulse height is proportional to the energy of the excitation particle. A beta-emitting radionuclide will emit a spectrum of energy events from zero to some maximum energy,  $E_{\text{max}}$ . Each energy event will produce a given number of photons (on the average). Quenching will reduce the number of photons produced for a given energy of excitation. A decrease in photon yield will cause a proportional decrease in the pulse height response. If the decrease is too great, the photon yield will be reduced below the threshold of

detection. Figure 1 shows the shift in the pulse height spectrum for a beta-emitting radionuclide as the counting efficiency changes.

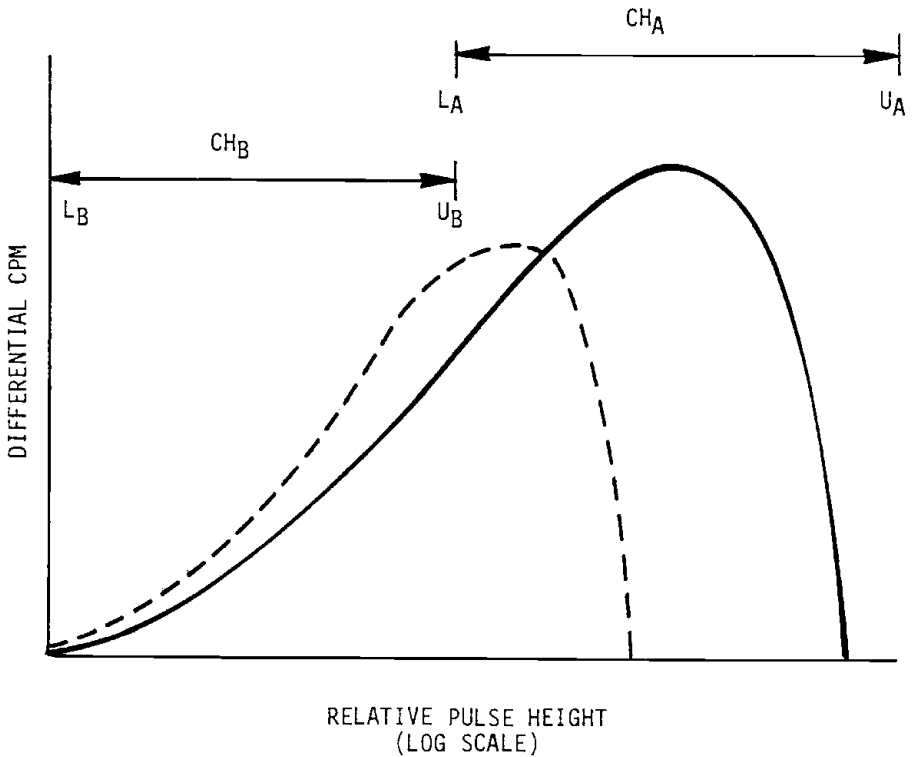


Figure 1. Pulse Height Spectra Showing a Selection of Counting Channels for Sample Channels Ratio (SCR).

The counting windows can be selected to be determined by a lower and upper pulse height value as shown in Figure 1. As the efficiency of the solution changes, the relative number of counts in the two counting windows also changes due to the shift in the pulse height spectrum. The ratio of counts in the two windows is called the Sample Channels Ratio (SCR):

$$SCR = \frac{\text{Counts or CPM in } CH_A}{\text{Counts or CPM in } CH_B}$$

Figure 2 shows several relationships between SCR values and counting efficiency for  $^3\text{H}$ . The different curves show different selections of window settings. This shows one of the limitations of this method; namely, a multitude of relationships for the same radionuclide counting efficiency. The other serious limitation of the SCR method is due to the sample count rate. When the count rate is low, long count times are necessary to obtain a statistically accurate ratio. Also at low sample count rates, it is necessary to correct for background before the ratio is calculated:

$$\text{SCR} = \frac{\text{CPM}_A - \text{BKG}_A}{\text{CPM}_B - \text{BKG}_B}$$

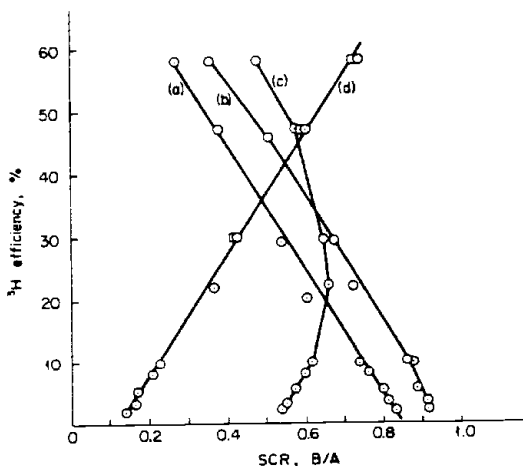


Figure 2. Tritium Efficiency vs. SCR Values for Different Sets of Window Selections.

External Standard Count Rate (ESCPM)

A gamma ray source will produce Compton scattered electrons in a media by collisions with the electrons of the media imparting part of the gamma ray energy to kinetic energy of the struck electrons. When the media is a liquid scintillator solution, these energetic electrons will cause excitations resulting in scintillation events with the number related to the efficiency of the solution. The measure of the

number of counts per minute produced by a source placed near a sample was used as a measure of the efficiency when compared to a calibration curve, as shown in Figure 3. However, the many limitations of this method, as listed in Table 1, led to the early demise of this method.

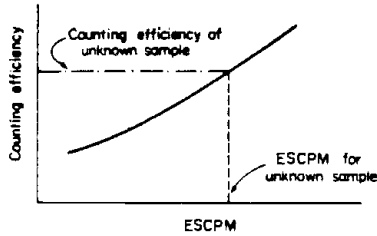


Figure 3. External Standard Count Rate, ESCPM vs. Counting Efficiency Plot.

Table 1. Limitations of the ESCPM Method for Quench Measurement.

Factor	Effect
Position of gamma source relative to sample	The source position has to be exactly reproduced or changes in the counts will be observed which will not reflect quench changes
Volume of sample	The measured count varies as the volume of the sample changes, because the number of $\gamma$ -rays, which are scattered, depends on the amount of material available with which the $\gamma$ -rays can interact
Half-life of the $\gamma$ -ray source	The count rate has to be expressed on a relative basis or corrected for decay from the time elapsed between when the quench curve and samples were measured. This is more critical for measuring very slight quench changes
Changes in electron density of the sample and/or surroundings	The number of scattered electrons will be different if the number of electrons (electron density) in the $\gamma$ -ray flux changes. The sample itself can alter the electron density, as can the thickness of the vial walls, possible quenches, etc.

### External Standard Channels Ratio (ESCR)

The ESCR method combined the SCR and external standard methods. The Compton scattered electrons were counted in two pulse height ranges and the ratio of the counts was the ESCR value:

$$\text{ESCR} = \frac{\text{ESCPM (in Range A)}}{\text{ESCPM (in Range B)}}$$

If the sample also produced counts in the two selected pulse height ranges, it was necessary to make an additional measure to be able to subtract sample counts:

$$\text{ESCR} = \frac{\text{Total CPM (in Range A) - Sample CPM (in Range A)}}{\text{Total CPM (in Range A) - Sample CPM (in Range B)}}$$

The use of different radionuclides as the external gamma ray source and the selection of different pulse height ranges led to widely different values of ESCR for the same quench level. Table 2 lists three of the most commonly-used radionuclides and some of their nuclear decay properties. There are two situations where the ESCR method has been shown to give errors. These are: when measuring the ESCR for samples in plastic vials<sup>1,2</sup> and when measuring the ESCR for different volumes of samples<sup>3</sup>. The diffusion of scintillator material into the plastic vial causes changes in the ESCR value which do not reflect an actual change in counting efficiency. Smaller volumes can produce changes in the ESCR value due to the range of the Compton scattered electrons. This range effect is more serious for the higher-energy gamma ray sources (i.e., <sup>226</sup>Ra).

### Average Pulse Height - Center of Gravity

The amount of quench in a sample has also been related to a measure of the average pulse height, or center of gravity, of the Compton electron spectrum produced by the external gamma ray source. The weighted centroid of the spectrum (or a part of the spectrum) will vary due to the level of quench. Calibration of the change of the centroid with counting efficiency will enable the calibration of any

Table 2. Relative Abundances for Gamma-Ray Energies from Different Sources.

Source	$\gamma$ -ray Energy (MeV)	Relative Abundance (%)
$^{133}\text{Ba}$	0.356	67.6
	0.301	18.2
	0.383	7.4
	0.276	5.4
	0.162	1.4
$^{137}\text{Cs}$	0.662	100.0
(0.032 Ba X-rays not energetic enough to be detected)		
$^{226}\text{Ra}$ + daughters	0.609	50.0
	1.130	5.0
	0.769	3.0
	1.760	3.0
	Others (0.05-2.43)	39.0
	all > 0.6	(~15)

unknown sample. The weighted centroid,  $C_w$ , calculated by the following equation:

$$C_w = \frac{\sum_{i=L}^{i=U} (X_i) (i)}{\sum_{i=L}^{i=U} X_i}$$

where:  $i$  is the channel number,  
 $L$  is the lower limit of the channel number,  
 $U$  is the upper limit of the channel number,  
and  $X_i$  is the counts (or counts per minute) in channel  $i$ .

This method has been used, in slightly different forms, to measure the quench of samples in two commercial systems. The Tracor Analytical (formerly Searle) instruments use the term ESP<sup>4</sup> which

relates the centroid of the total  $^{133}\text{Ba}$  Compton electron spectrum of the sample to the centroid for the total  $^{133}\text{Ba}$  Compton electron spectrum of the reference (unquenched sealed standard). Generally the ESP values for experimental samples are  $> 1.000$  where an ESP value of 1.000 is the calibration value for the reference.

The Packard instruments, using the term  $\text{SIE}^5$  calculate the centroid of that part of the  $^{226}\text{Ra}$  and daughters Compton electron spectrum above a lower threshold (between the  $^3\text{H}$  end-point, 18.6 KeV, and the  $^{14}\text{C}$  end-point, 156 KeV) and the maximum threshold (2 MeV). The centroid value is related to a calibration value of 1000 for the reference (unquenched sealed standard). The SIE values of samples are fractions of the 1000 value.

#### Compton Edge, H#

Measurement of the change of pulse height response produced by a given energy has been used to monitor the quench of a sample. The Compton electrons produced by a monoenergetic gamma ray will have energies from zero to some maximum energy,  $E_{\text{max}}$ , given by the equation:

$$E_{\text{max}} = \frac{2E_{\gamma}^2}{2E_{\gamma} + 0.51}$$

where  $E_{\gamma}$  is the energy of the gamma ray in MeV. Thus the electrons of energy  $E_{\text{max}}$  are a known, invariant energy. Measuring the pulse height response produced by the Compton electrons of  $E_{\text{max}}$  energy can be used to relate the quench of the sample. This is the basis for the Beckman Instruments method called H#<sup>6</sup>. Figure 4 shows the Compton spectra for a reference (unquenched, sealed standard) and a sample. The difference between the pulse heights for the two Compton Edges gives the value of the H#. An H# value of zero would be for the reference.

#### Factors Affecting Quench Parameter Values

There are several factors which can affect the pulse height distribution measured for an external gamma ray source irradiation of a sample and sample container. The H# does not seem to be influenced by these effects:

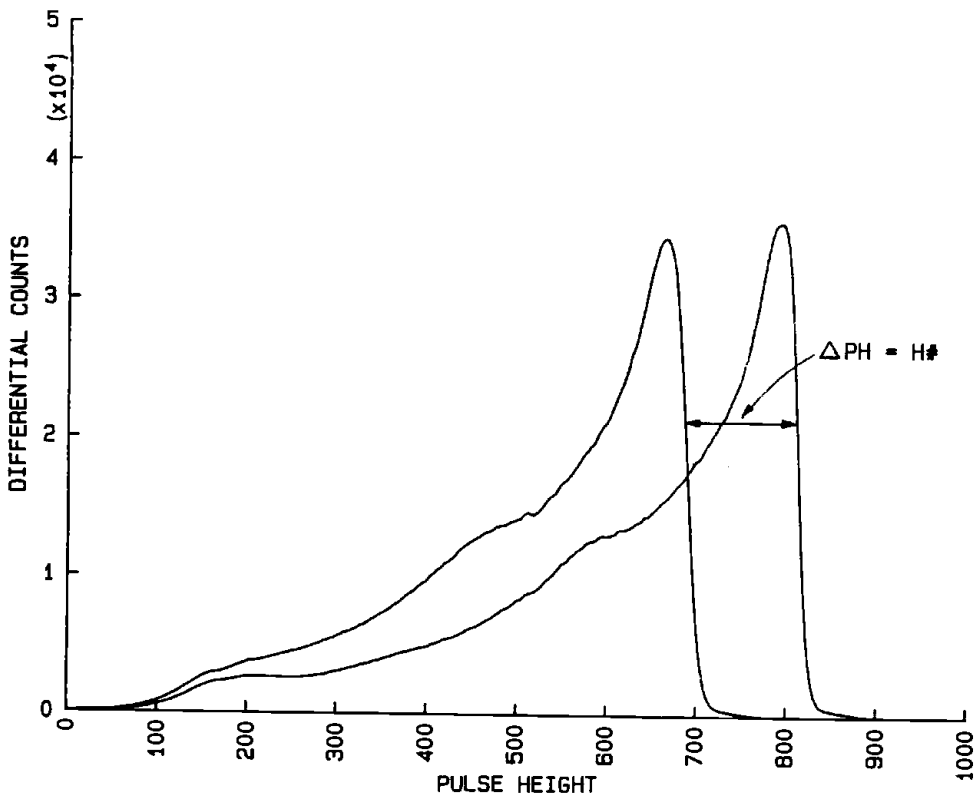


Figure 4. Pulse Height Spectra Showing Compton Edge Shift ( $H\#$ ) Between Unquenched and Quenched Sample.

1. Plastic effect<sup>1,2</sup> - diffusion of solvent, solut(s), sample, etc. into the plastic walls, resulting in scintillations from the plastics;
2. Volume effect<sup>3</sup> - the shape of the pulse height spectrum can change with changes in the volume of the sample, especially for multi gamma ray sources (i.e.,  $^{133}\text{Ba}$  and  $^{226}\text{Ra}$  and daughters);
3. Range of Compton electrons<sup>3</sup> - the higher-energy electrons can reach the wall or edge of the scintillator solution before depositing its entire energy, resulting in a response (scintillation event) equivalent to only the energy deposited in the solution.

Figure 5 shows the plastic effect on the shape of the Compton

electron spectrum as a function of the time the scintillator solution has remained in the plastic vial. Note that the position of the Compton Edge (H#) has not changed. Figure 6 shows the effect of volume changes upon the Compton spectra for  $^{226}\text{Ra}$  and daughters. Since the probability of each energy gamma ray (there are 18 different energy gamma rays) to undergo Compton scattering is different and changes at different rates with volume, the shape of the spectra are very volume-dependent. Thus, parameters which measure a property of the spectra shape (ESCPM, ESCR, ESP, SIE) will change with volume changes.

Figure 7 shows the effect of electron range on the shape of the pulse height spectrum. The  $^{32}\text{P}$  spectrum is shown at different volumes and geometries in maxi- and mini-size scintillation vials. Note the very different shapes of the spectra for the small volumes.

## 2. Two-Phase Detection

Since the first investigators tried to add aqueous samples to the predominantly organic scintillator solution, it has been a desire to have a means of monitoring the stability of the counting sample - did the sample separate into two different phases? Modern detergent-containing cocktails allow for the inclusion of much more aqueous sample into scintillator solutions with a greater stability against phase separation. However, in many cases, the emulsion will become unstable and the sample will separate into two or more phases. It is impossible to utilize counting data from these two types of samples because each phase will have its own counting efficiency (often quite different) and because the amount of sample in each phase is unknown.

Early phase detection depended upon visual inspection. However, this could not confirm phasing at the time of counting unless the inspection took place at the time the sample was counted (actually just prior or after counting). In nontransparent plastic vials, visual inspection was impossible (or at least more difficult).

The double ratio technique<sup>7,8</sup> has been used, with some limitations, to detect phase separation. A plot of the sample channels ratio (SCR) versus the external standard channels ratio (ESCR) was obtained for totally miscible samples of a radionuclide in

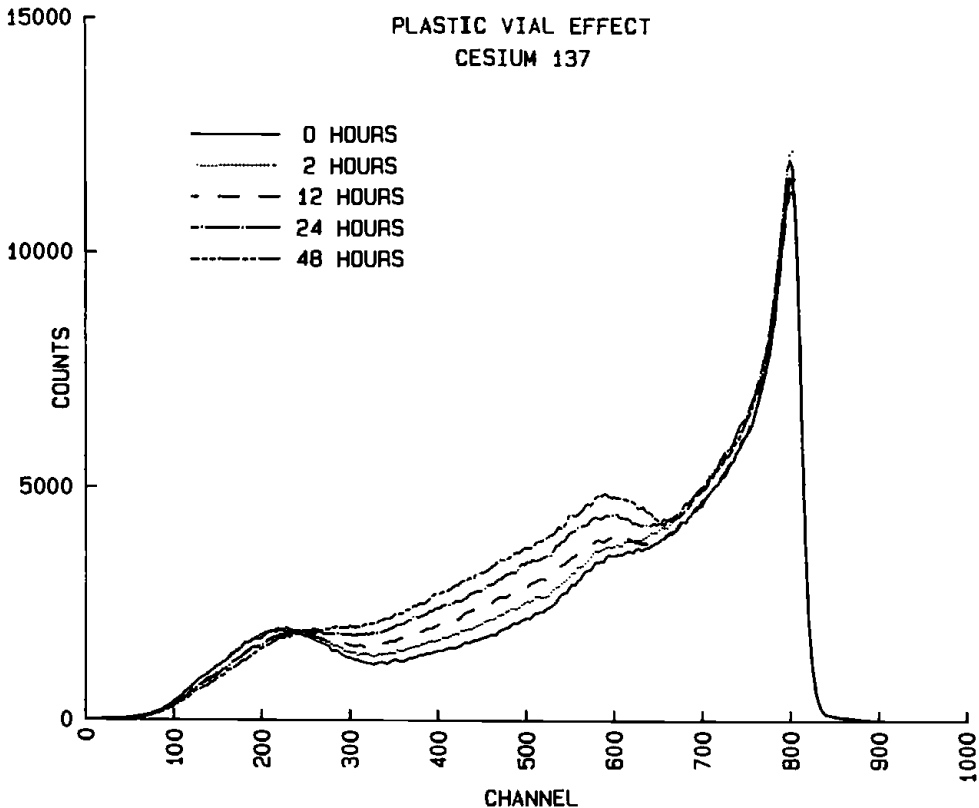


Figure 5. Effect of Diffusion of Scintillator into Plastic Vials on the Pulse Height Spectrum at Different Times.

a scintillation cocktail. Samples which had separated into different phases showed variations in the SCR and/or ESCR value which led to points on the double ratio plots much different than samples which were totally dissolved. This technique was not valid for high-energy radionuclides (i.e.,  $^{32}\text{P}$ )<sup>8</sup>. Also long counting times were required to obtain statistically accurate values of SCR for low count rate or highly quenched samples.

Recently<sup>9</sup> a new method has been developed for detecting phasing in counting samples. This method is based upon the Compton Edge method of quench monitoring (H#). When emulsion-type cocktails with aqueous samples separate into two or more phases, each phase contains solvent, emulsifier, solutes, aqueous and sample. The amount of each component in each phase is, of course, variable. But each phase has all the

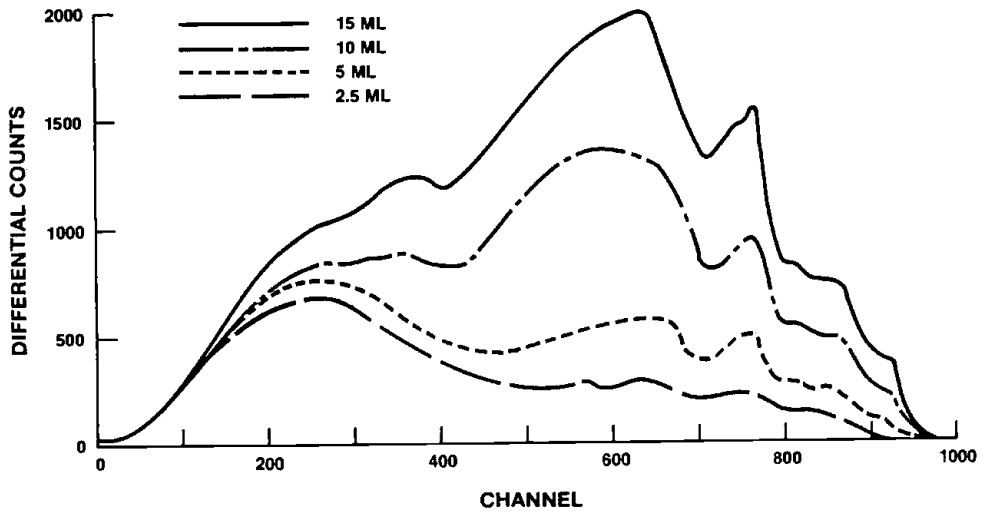


Figure 6. Changes in Relative Pulse Height Spectrum for  $^{226}\text{Ra}$  and Daughters Compton Electrons as Function of Volume of Liquid Scintillator.

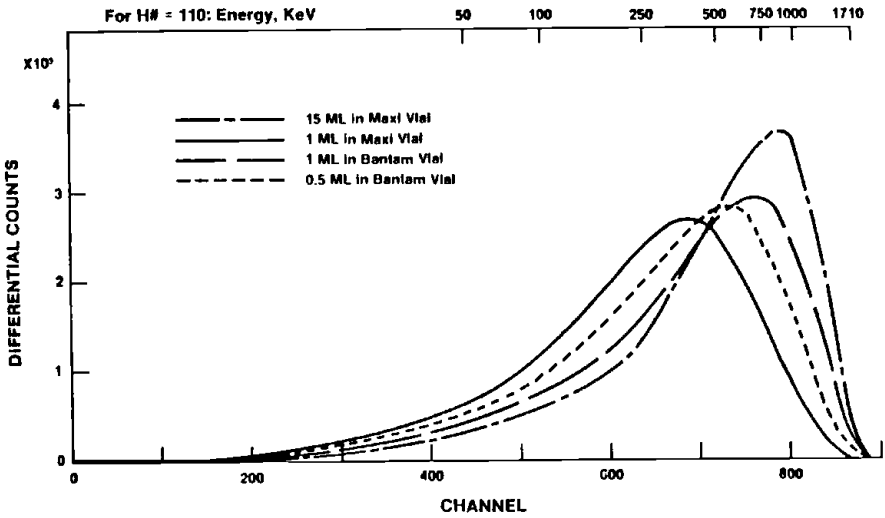


Figure 7. Effect of  $^{32}\text{P}$  Beta Energy (Range) on Pulse Height Spectrum for Volume and Geometry Changes.

components necessary to produce scintillations when excited by ionizing radiation. Figure 8 shows the Compton electron spectrum produced by  $^{137}\text{Cs}$  source irradiation of a sample which has separated into two phases. Each phase produces a Compton spectrum with pulse heights determined by the scintillation efficiency of the phase. The total spectrum is the sum of the spectrum of each phase. When the efficiencies of the two phases are different, there will appear in the pulse height spectrum as two Compton Edges, as seen in Figure 8.

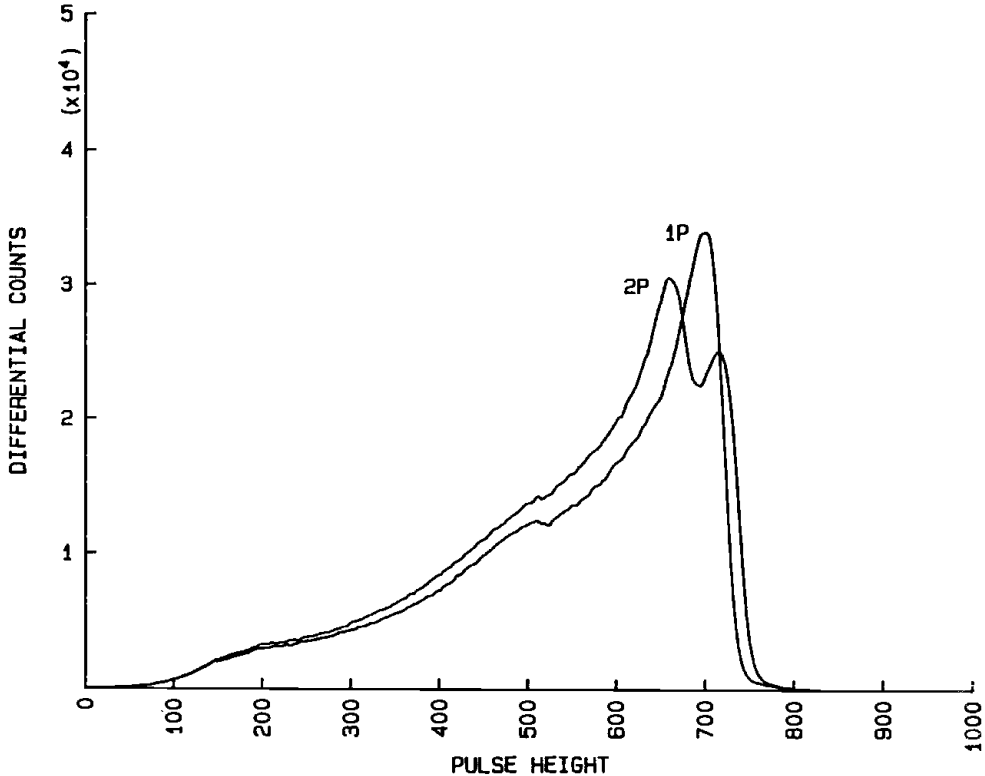


Figure 8. Compton Electron Spectrum (for  $^{137}\text{Cs} - ^{137m}\text{Ba}$ ) on Sample Which has Separated into Two Phases.

Analysis of the Compton electron pulse height spectrum by the use of microprocessor techniques allows the instrument to compare the spectrum for an unknown sample to the spectrum of a single-phase sample of the same quench level (H#). Differences in the spectra can be used as a means of flagging those samples which have separated into phases at the time the sample has been counted. Further, a second check can be done at the end of the sample count to be sure the sample did not separate during the counting time.

### 3. Data Reduction

Most modern liquid scintillation systems will convert the counting data obtained for a sample into final answers. These final answers are determined by several special programs which are designed into the system. Also, most systems have means for outputting the raw data to an off-line computer for special user-designed data reduction programs.

#### DPM Calculations

Systems are now available which will calculate the disintegrations per minute (dpm) of single, two or even three radionuclides in a single sample. These systems will store the quench curve(s) (counting efficiency versus quench parameter) in memory so that it is no longer necessary to rerun the quench curve(s) every time a set of samples is to be measured. Further, some systems allow for half-life correction of each radionuclide. A background quench curve can be stored for each counting window for automatic background subtraction based on the quench of the unknown sample (very important for low counting rate samples).

Table 3 shows some data for the dpm determinations of  $^3\text{H}$ ,  $^{125}\text{I}$  and  $^{14}\text{C}$  in the same sample at different quench and radionuclide ratios.

#### Percentage of Reference

In certain experiments, it is desirable to know the ratio (or percentage) of the radionuclide in a sample relative to a reference sample. These programs calculate the "% of reference" based upon the cpm (constant quench) or dpm (variable quench). The "% of reference" can also be calculated for two radionuclides in the same sample with each radionuclide referenced to its own reference value. These

Table 3. Calculated DPM as Percentage of Added DPM for Different Ratios of the Three Radionuclides.

DPM Ratio $^3\text{H} : ^{125}\text{I} : ^{14}\text{C}$	H#	% DPM Recovery		
		$^3\text{H}$	$^{125}\text{I}$	$^{14}\text{C}$
1:2:1	104	99.81	103.67	99.41
	157	104.66	101.41	100.71
	214	101.87	103.91	100.29
	270	95.76	103.16	100.99
	306	104.18	101.60	100.10
2:2:1	104	98.82	103.46	96.30
	157	101.37	101.08	101.68
	215	104.92	102.29	99.72
	271	101.97	102.66	98.38
	307	103.28	103.90	99.46
1:3:3	104	90.12	109.60	92.85
	158	109.91	98.37	101.82
	218	105.29	99.08	102.45
	272	92.00	102.94	99.17
	308	88.44	99.35	99.70

programs also provide half-life correction for each radionuclide.

#### Histogram Plots and Peak Integration

In some experiments, fractions have been collected which contain the radionuclide(s). Some of the LS systems will count the set of samples and plot a histogram of sample cpm or dpm as a function of sample fraction. The total cpm or dpm is calculated for the set. Then various routines are used to determine the fraction of the total that is present in each of the identifiable peaks of the histogram. These systems use various criteria for determining the start and end of a peak and some can identify shoulders.

These histogram calculations can also be done for each of two radionuclides in the same sample. Separate or overlapping plots are obtained for each radionuclide. The data can be expressed as cpm (constant quench) or dpm (variable quench) and half-life corrections

can be made for each radionuclide.

#### Individual Normalization

In some experiments, each sample contains a different amount of material; i.e., volume, weight of tissue, etc. It would be desirable to express the final answer per the same weight or volume. The weights or volumes of each sample can be stored and recalled as each sample is counted for normalization of the final answers.

#### RIA Programs

Some systems provide final answers for radioimmunoassays (RIA). The complete standard curve, reference, blanks (NSB) and unknowns are measured and final answers of dose value of patient samples are calculated. The RIA routine involves the plotting and curve-fitting of a standard dose versus a selectable parameter based upon measure of the amount of radionuclide in a sample. The user can select the curve fit routine, the x-axis, y-axis and y-axis scale from the choices given in Table 4.

Bad data points for the standards can be edited manually or automatically in the RIA programs. The limit of rejection of outliers can be selected by the user and the rejected points are still plotted on the graph, but not included in the calculation at the standard dose response curve.

#### CONCLUSION

In these three areas the instrument manufacturers have responded to the needs of the users by designing new features into their systems. What future changes will be made will depend in great part upon the future needs of those using liquid scintillation systems.

Table 4. Selectable Parameters for RIA Data Calculation of Standard Curve.

PARAMETER			
Curve Fit	Y-Axis	Y-Axis Scale	X-Axis
Linear Regression	Bound	Percent	Dose
Weighted Linear Regression	Free	Linear	Log Dose
	Bound or Free/Total	Log	
Spline	Bound or Free/ $B_{max}$	Logit	
	$1/\text{Bound}$		
	$1/\text{Free}$		
	Total/Bound or Free		
	$B_{max}/\text{Bound or Free}$		

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