

EXTERNAL STANDARD METHOD OF QUENCH CORRECTION:  
ADVANCED TECHNIQUES

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ABSTRACT

The counting efficiency for a liquid scintillation sample varies with its composition. Impurities from solubilizers as well as the specimen itself typically quench the available energy, reducing both the pulse heights and the total number of detectable events. Counting efficiency as measured in a pulse height channel is therefore uncertain, particularly when assaying low-energy radionuclides or dual labeled samples.

An external source of gamma radiation is frequently utilized to quantitate the degree of quenching. This is done by analyzing the additional pulse height spectrum created from Compton electrons, which are produced in the sample by the radiation from the gamma source. The resulting data may then be used to correct for losses in pulse height and counting efficiency. The counts induced by the gamma source are usually measured in two pulse height channels and the ratio of the counts in these channels is used to quantitate quenching. This is called the external standard ratio (ESR). The fixed windows in these channels, however, limit the useful dynamic range of quench determination or may produce unacceptable statistical uncertainty in the estimated counting efficiencies of quenched samples.

A new method of quench determination based upon relative pulse height (RPH) is described. Applying this technique to the external standard Compton spectrum provides a means for quantitating quench in terms of RPH over a 30 to 1 change with less than 1½% standard deviation. By recognizing the quantum characteristic of weak scintillations, accurate pulse height restoration can be extended to quenched samples containing low energy radionuclides such as <sup>3</sup>H and <sup>14</sup>C. Isotope energy, window settings, and true disintegration rate can be computed from the RPH quench value of unknown samples without empirical curves.

## INTRODUCTION

Most commonly used methods for quantitating quench in a homogeneous liquid scintillation sample employ the use of an external gamma ray source. Each Compton electron induced in the sample by the penetrating gamma radiation transfers its energy to the solute in the same manner as an electron ejected from the nucleus of an atom undergoing beta decay. The net Compton spectrum is independent of the activity, energy, number and type of radionuclides that may be present in the sample. This universality largely accounts for the appeal of the method.

Gamma emitting radionuclides have been used as early as 1950 to measure the relative scintillation efficiency of organic solutes and solvents in liquid scintillation samples. Reynolds et al.<sup>1</sup> used the coincidence/non-coincidence ratio of Compton electrons induced in the test sample by a  $^{60}\text{Co}$  source to quantitate scintillation efficiency. Kallman<sup>2</sup> used the relative pulse height (RPH) as measured by the peak intensity from a radium source to quantitate relative scintillation efficiency.

The Compton spectrum induced in the liquid scintillation sample by a gamma ray source has also been correlated with the counting efficiency of beta emitting radionuclides. Kaufman et al.<sup>3</sup> combined the external standard technique with the sample channels ratio technique described by Baille<sup>4</sup> to correlate  $^{57}\text{Co}$  channels ratio with  $^3\text{H}$  efficiency. Fleishman et al.<sup>5</sup> correlated the count rate induced by a  $^{60}\text{Co}$  source with the counting efficiency of  $^{40}\text{K}$ . Similarly, Higashimura<sup>6</sup> correlated the count rate from a  $^{137}\text{Cs}$  gamma source with  $^{14}\text{C}$  counting efficiency. Horrocks<sup>7</sup> correlated the RPH of a  $^{137}\text{Cs}$  Compton edge as measured by its pulse height at half maximum intensity with  $^{14}\text{C}$  counting efficiency.

Quench measurements using either the ESR or RPH methods are unaffected by the half-life of the gamma source and also tend to produce congruent efficiency vs quench curves for samples differing in sample volume and electron density<sup>7</sup>.

Two advantages of the RPH method over the ESR method are 1) the useful range of quench is not bounded by fixed windows and 2) the RPH has physical significance for

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determining counting efficiency and quench corrected window settings<sup>8,9,10</sup>.

When the spectrum shift due to quenching is extensive, part of the spectrum from one radionuclide may fall outside its fixed counting channel and may spill into another channel set to count another radionuclide. Two methods for automatically adjusting the counting windows for quenched samples have been investigated by Wang<sup>11</sup> and Herberg<sup>12</sup>. The objective of the method described by Wang, automatic quench correction (AQC), is to increase the electronic amplification to restore the sample's pulse height spectrum to the level it would have had were it not quenched. The degree of quenching is measured by the ESR. This method has two inherent difficulties. Wang demonstrated that separate correction relationships are required to restore the end points of  $^3\text{H}$  and  $^{14}\text{C}$ . Although the external standard windows can be tailored to approximate a linear response over a limited range of quench, the method is not linear over a wide range of quench. Secondly, although a single gain correction function may be adequate for moderately quenched samples containing high energy isotopes, it is inadequate for low energy isotopes or heavily quenched samples. Consequently, separate gain adjustments are required for different isotopes and clearly a single gain adjustment is not valid for all isotopes.

The automatic activity analyzer (AAA) described by Herberg uses ten sets of individually adjustable preset windows for each isotope corresponding to ten preset levels of quench. By electronically quenching each unknown sample to one of the preset levels of quench, the sample is adjusted to the preset window. This method creates three problems not present in the AQC system:

- 1) hours of operator time and skill are required to properly standardize the system for each isotope and counting condition;
- 2) each sample must be exactly quenched to one of the ten preset levels which takes in excess of three minutes;
- 3) since the sample is quenched further by the instrument prior to counting, it will be counted at less than the available efficiency, somewhat defeating the main objective of optimizing the settings to improve counting statistics.

A major advantage of the AAA method is that disintegration rate is obtained directly without curve fitting.

This paper describes a method for computing and setting pulse height analysis channels for any combination of isotopes at any level of quench without additional operator or machine time. Window levels are computed from mathematical relationships, RPH and the unquenched window settings.

### THEORY

RPH has been used to quantitate relative scintillation efficiency by many investigators<sup>1,2,7,8,9,13</sup>. However, relative scintillation efficiency, the relative number of photons generated per keV expended, is not always proportional to RPH because:

- 1) the number of photons emitted per keV of energy depends upon the energy of the primary beta particle<sup>10</sup>;
- 2) the emission spectra of the two solutions being compared may have different optical response at the photodetector<sup>13</sup>.
- 3) the pulse height at the output of a phototube is not proportional to the number of impinging photons for weak scintillations.

The latter case can be demonstrated by comparing the pulse height of a large 1000 photon scintillation with that from a weak single photon scintillation. Assuming a photocathode quantum efficiency of 0.30, the pulse height from each 1000 photon scintillation is statistically equal to 300 photoelectrons. However, the pulse height from a single photon scintillation, when detected, is equal to the pulse height of a single photoelectron. Although the relative photon input for the two cases is 1000 to 1, the relative pulse height output is only 300 to 1. The difficulty arises because of the finite light quanta and the less than 100% probability of detection. A single photon has a relative pulse height  $1/0.30$  greater than would be required for proportionality. However, since it is only detected 30% of the time, the charge delivered from 1000 single photon scintillations is statistically equal to that from a single 1000 photon scintillations. Therefore, on the average, the number of photoelectrons, but not necessar-

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ily the pulse height, is proportional to the number of photons per scintillation.

Pulse height shift with quenching are compared for  $^{133}\text{Ba}$  Compton,  $^{14}\text{C}$  and  $^3\text{H}$  spectra in figures 1 A, B, and C respectively. It can be readily seen that the pulse height shift for  $^3\text{H}$  is much less than for  $^{133}\text{Ba}$  and  $^{14}\text{C}$ . For spectra below about channel 10, count rate declines faster and pulse height shifts less rapidly with quenching due to the quantum threshold. This data compares with that of Wang<sup>11</sup>.

When analyzing relative pulse heights less than about 10 photoelectrons per scintillation, it is necessary to account for the quantum threshold and the dependence of pulse height upon the number of photoelectrons detected. Multiple phototube coincidence detectors further alter the pulse height distribution because the pulse height at one phototube is dependent upon both detection probability and pulse height from the second phototube. The method of combining the pulse heights from the detectors typically alters the pulse height distribution as a function of the number of photoelectrons<sup>14</sup>.

Since detection efficiency ultimately depends upon the number of photoelectrons liberated from the phototransducer, relative pulse height corrected for the quantum threshold can be used to monitor changes in overall system efficiency. The calculated relative pulse height,  $\text{RPH}_i$  of a spectrum with measured average lesser pulse height  $\text{PH}_i$ , relative to a reference spectrum with average lesser pulse height  $\text{PH}_R$ , corrected for the single photon intercept, is approximated by:

$$1) \text{ RPH}_i = \frac{\text{PH}_i - \text{PE}_R}{\text{PH}_R - \text{PE}_R}$$

Some of the methods for quantitating the pulse height of a spectrum include: the channels ratio<sup>3,4</sup>, the pulse height at the peak intensity<sup>2</sup>, the pulse height of the upper edge<sup>7</sup>, and the average pulse height.

The average pulse height of the Compton lesser spectrum from  $^{133}\text{Ba}$  was selected as the method for quantitating RPH for the following reasons:

1. Relative pulse height can be measured directly without empirical relationships.

2. High statistical precision is obtained from the average because of the high count rate obtained using all, rather than part of, the spectrum.
3. The average pulse height of the lesser spectrum is a good analog of the average efficiency of detection over a spectrum of pulse heights which may be distorted due to energy dependent quench mechanisms.
4.  $^{133}\text{Ba}$  was selected for its relatively low energy which more closely approximates that of low energy beta emitters.

An ideal external standard gamma source for assaying beta emitting radionuclides with maximum energy less than 200 keV should have a Compton electron spectrum extending to about 200 keV: high enough to track large changes in quench, low enough to track energy dependent quench mechanism and low enough so as not to produce instability from phototube fatigue. Ideally, the Compton spectrum should overlap the same energy range as the isotope being assayed, so that energy dependent quenching mechanisms<sup>10</sup> will alter the Compton spectrum in the same manner as the beta spectrum and thus produce a change in the external standard pulse height, which is analogous to that of the sample spectrum. Under these conditions, the external standard response will tend to track energy dependent changes in efficiency from different solvent-solute-quencher systems.

Gamma sources which produce most of their Compton electrons beyond 200 keV are equally capable of monitoring changes in scintillation efficiency which are not energy dependent, but do a poor job of tracking energy dependent quenching of low energy beta emitters such as  $^3\text{H}$  and  $^{14}\text{C}$ .

High energy gamma sources also tend to cause phototube drift. Phototube fatigue occurs at high anode current. A high activity gamma source sufficient to produce  $10^5$  to  $10^6$  counts per minute is normally employed to achieve a low statistical uncertainty in the quench measurement in less than one minute. The higher the energy count rate product of the external standard source, the greater the anode current and consequently phototube amplification is more unstable. It is therefore important to use a gamma source with energy sufficiently high to produce a statistically significant pulse height shift over wide ranges of

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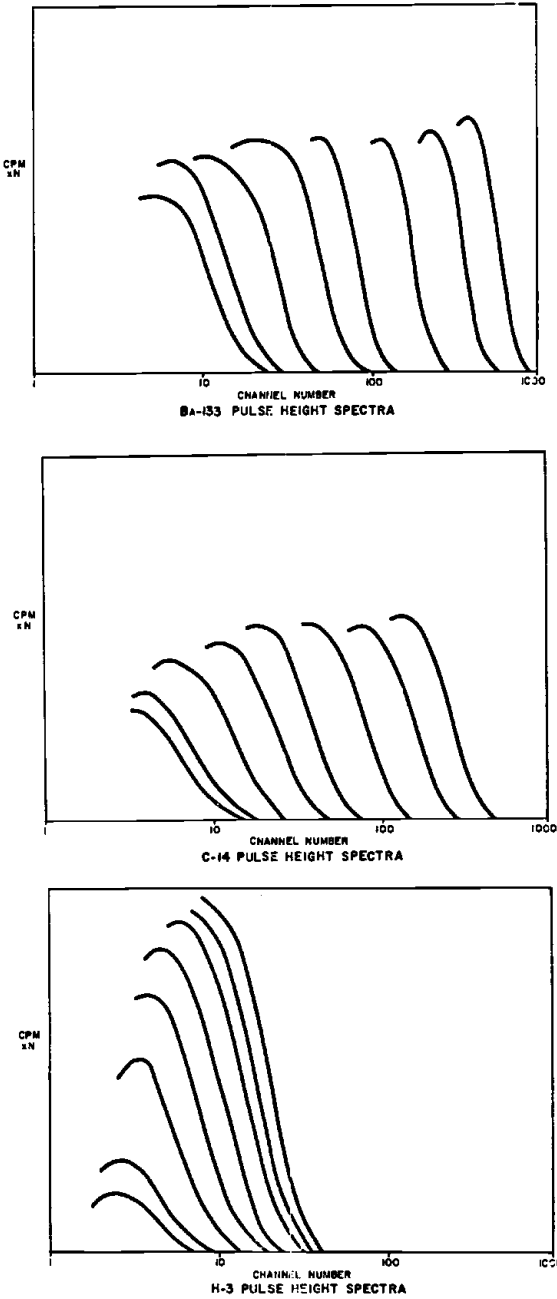


Figure 1 Pulse Height Spectra of  $^{133}\text{Ba}$ ,  $^{14}\text{C}$  and  $^3\text{H}$

quench, yet not so energetic as to produce instability in the measurement.

Thus,  $^{133}\text{Ba}$  with a peak Compton energy of 207 keV is an almost ideal gamma source for assaying low energy beta radionuclides.

Once the RPH is known, the energy of the beta spectrum can be estimated and the absolute disintegration rate DPM of the sample can be determined without quench curves.

The procedure for estimating the isotope energy is as follows: 1) the pulse height of the sample is accurately measured by accumulating 200,000 counts, 2) the pulse height of the sample spectrum is quantitated by computing the average pulse height. The pulse height energy,  $\text{PHE}_i$ , of the sample is computed from the measured pulse height of the sample,  $\text{PH}_i$ , and the RPH determined with the external standard as follows:

$$2) \quad \text{PHE}_i = \frac{\text{PH}_i - \text{PE}}{\text{RPH}_i} + \text{PE}$$

$\text{PHE}_i$  is the estimated pulse height of the sample at the reference quench at a  $\text{RPH} = 1$ . PE is the average pulse height intercept. It has a value of 1.35 channels as determined from quench  $^3\text{H}$  with lesser pulse height analysis. Beta energy in keV can then be calculated using the correction for ionization quenching shown by Gibson<sup>10</sup> and a single energy calibration.

## MATERIALS AND METHODS

All measurements were performed with a Searle Analytic Mark III liquid scintillation spectrometer. Nine microcuries of  $^{133}\text{Ba}$  hermetically sealed in a stainless steel pellet is mechanically positioned beneath the center of the sample. EMI type 9750QB phototubes are operated with linear dynode voltage distribution at a nominal voltage of 1300 volts (a block diagram of the spectrometer is shown in Figure 2). The pulse heights from each of the phototubes are simultaneously applied to the coincidence circuit and two 12-bit analog to digital convertors. The coincidence circuit, through the bus interface, initiates the simultaneous conversions from both phototubes. Upon completion of the digitizing of the pulse heights from both phototubes, the numerical value corresponding to the linear pulse height from each of the phototubes is transmitted to a micro-

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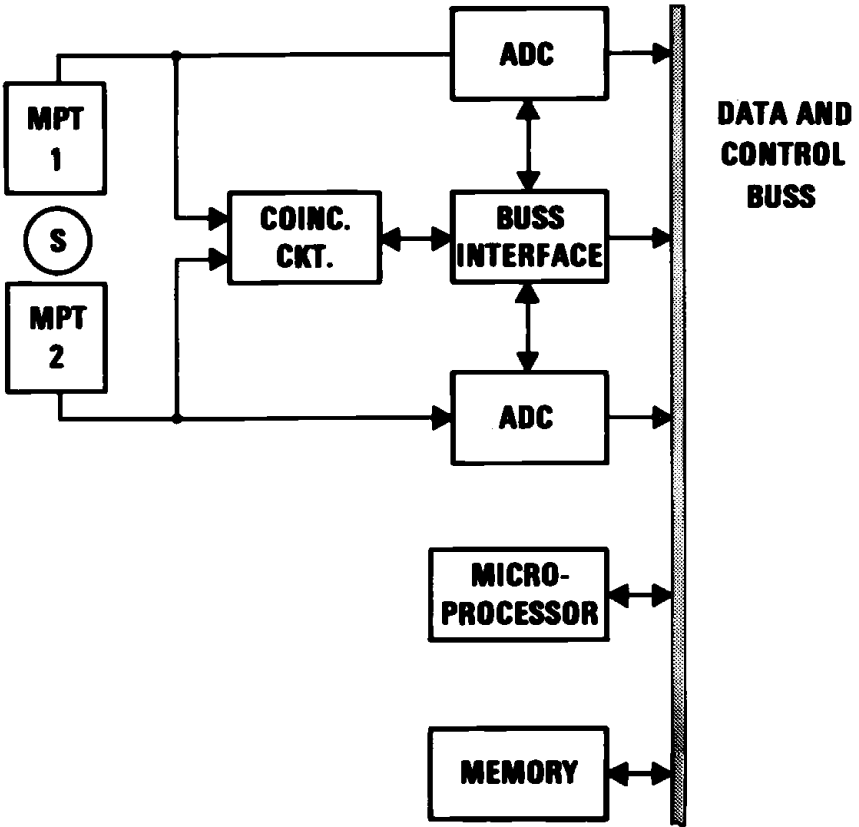


Figure 2 Pulse Height Analyzer Block Diagram

processor via the data bus. A test is made to insure that the two pulse heights are properly correlated<sup>15</sup> and then the smaller of the two numerical numbers corresponding to the lesser pulse height<sup>14</sup> is then analyzed and stored.

In one mode of operation, data is collected in the memory as it would be in a multi-channel analyzer and decisions about pulse height analysis are made subsequent to the collection of all sample data. This mode is used for storing the spectra of standards, and for external standardization. In another mode, when assaying unknown samples, quench corrected windows are numerically determined prior to sample counting, and pulse height analysis is determined in real time.

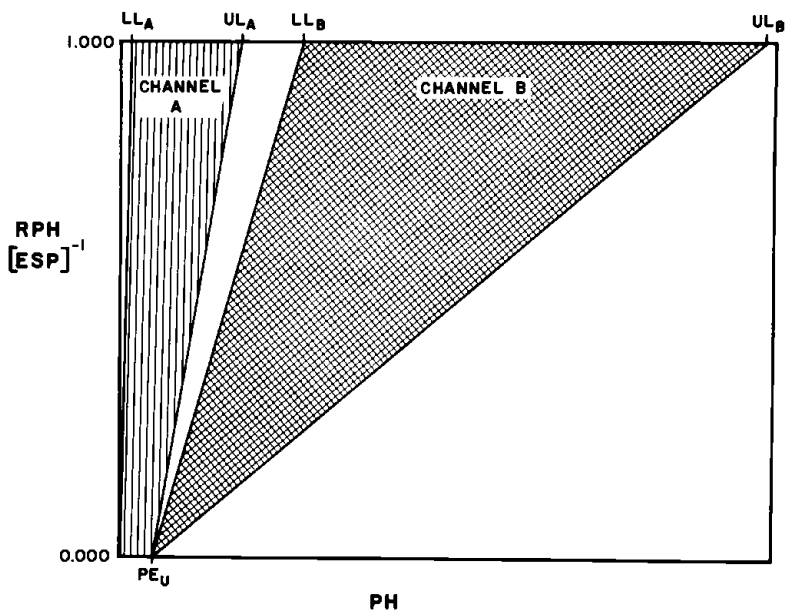
The procedure for determining the RPH from the external standard, is as follows: first, the <sup>133</sup>Ba external standard is positioned beneath the sample; the gross external standard spectrum is accumulated in the multi-channel mode for a period of 0.4 minutes. Then, the external standard is removed to its shielded storage location and the sample spectrum is accumulated for 0.4 minutes and subtracted from the gross external standard spectrum. Finally, RPH is computed from the net external standard spectrum.

$$3) \quad RPH_i = \frac{ESPH_i - ESPH_\infty}{ESPH_R - ESPH_\infty} = \{ESP\}^{-1}$$

The reference external standard pulse height,  $ESPH_R$ , is a constant determined from a measurement of the external standard pulse height with a sealed nitrogen flushed toluene standard using PPO and POPOP as primary and secondary fluors. This constant has a value of 135 channels and varies from one instrument to another within a range of  $\pm 8$  channels. The external standard pulse height at infinite quench, ( $ESPH_\infty$ ) is a constant having a value of 1.75 channels.  $ESPH_i$  is the external standard pulse height measured on any unknown sample  $i$ .  $RPH_i$  is the true relative pulse height for sample  $i$  relative to the reference standard, corrected for the single photoelectron intercept. The inverse of the relative pulse height is defined as the ESP. Efficiency vs quench curves are typically more linear when plotted vs. the inverse relative pulse height, ESP.

Quench corrected window settings are computed from their unquenched value  $L_R$  and the relative pulse height of individual samples as follows (Figure 3): all lower levels

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QUENCH CORRECTED DUAL LABEL WINDOW SETTINGS

Figure 3 Quench Corrected Dual Label Window Settings

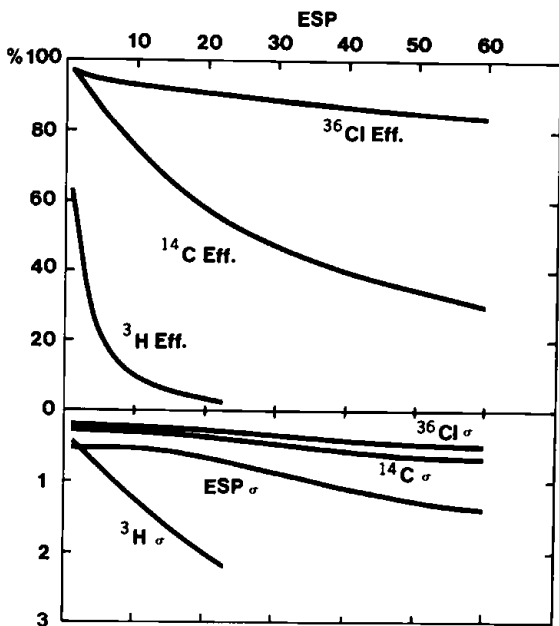


Figure 4 Efficiency And Standard Deviation vs ESP

except for dual label, high energy isotope windows are computed as follows:

$$4) \quad L_i = (RPH_i) L_R$$

All upper levels and dual label high energy isotope lower levels are corrected for the single photoelectron intercept as follows:

$$5) \quad L_i = (RPH_i)(L_R - PE_U) + PE_U$$

$L_R$  is any level setting for an unquenched sample, i.e., when the relative pulse height is equal to 1.0.  $PE_U = 4.70$  channels, determined from measurements of quenched  $^3H$ . Since all pulse height information is lost below the single photoelectron level, discriminator settings for isotope separation below this level are meaningless. Therefore, the lower level of the high energy isotope in a dual-labeled experiment approaches this level as the RPH approaches zero. In this way, only statistically significant data is maintained in the high energy channel.

For single labeled isotopes, the lower level and upper level discriminators converge to zero and  $PE_U$  respectively as the relative pulse height approaches zero.

Unquenched  $^3H$  lower and upper discrimination levels in program 8, channel B are typically 0.8 and 25.0 respectively. Unquenched  $^{14}C$  lower and upper levels in program 8, channel B are typically 10.1 and 321 respectively. Program 9 window settings for unquenched dual labeled  $^3H$  and  $^{14}C$  are typically 0.8 to 9.1 and 25 to 321 in channels A and B respectively. Program 1, channel B has unquenched levels to 0 to 26 for assaying  $^3H$ . Program 2, channel B has unquenched levels of 0 to 315 for assaying  $^{14}C$ . Window settings may be computed at any RPH using equations 4 and 5.

The method for determination of absolute disintegration rate (DPM) is as follows: the instrument is standardized for a given isotope by counting a series of quenched standards and the total multi-channel spectrum of each standard is permanently stored along with the known DPM. To assay an unknown, the RPH is determined using the external standard, the spectrum of the unknown isotope is computed from the stored spectra at that RPH, efficiency is computed from the constructed standard spectrum by integrating the count rate within the boundary of the window used to assay the unknown sample, and the calculated efficiency is divided

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TABLE I

SINGLE LABEL  $^{14}\text{C}$  EFFICIENCY  
WITH QUENCH CORRECTED WINDOW SETTINGS

RELATIVE PULSE HEIGHT (ESP) <sup>-1</sup>	FIXED WINDOW EFFICIENCY %	CORRECTED WINDOW EFFICIENCY %	RELATIVE INCREASE %
0.83	87	89	2
0.26	63	84	33
0.15	43	78	81
0.111	27	72	170
0.083	15	63	320
0.050	3	42	1,400
0.037	0.5	29	5,800

TABLE II

DUAL LABEL  $^{14}\text{C}$  EFFICIENCY  
WITH QUENCH CORRECTED WINDOW SETTINGS

RELATIVE PULSE HEIGHT (ESP) <sup>-1</sup>	FIXED WINDOW EFFICIENCY %	CORRECTED WINDOW EFFICIENCY %	RELATIVE INCREASE %
0.83	68	71	4
0.59	58	70	20
0.40	45	68	52
0.26	28	64	132
0.15	8.0	56	600
0.11	1.8	48	2,600
0.08	.1	39	39,000

into the count rate from the unknown. In other words, a standard spectra is computed at the same quench as the unknown sample and integrated in the same window used to count the unknown sample in order to determine the efficiency for counting the same isotope, at the same level of quench and in the same window as the unknown.

RESULTS

Fixed and quench corrected window settings for single labeled  $^{14}\text{C}$  are compared in Table I. Fixed window settings in program 8 were set at RPH=0.83. The improvement in counting efficiency for the moderately quenched samples

having  $RPH = 0.111$  is 170%, whereas, the improvement in the heavily quenched sample having  $RPH = 0.050$  is 1400%.

The improvement in counting efficiency with pulse height restoration is even more dramatic when narrower counting channels are used, as when required for assaying dual labeled samples in program 9. Table II shows the improvement when assaying  $^{14}\text{C}$  in the presence of  $^3\text{H}$ . At a  $RPH = 0.11$ , the improvement in counting efficiency is 2600%. Heavily quenched samples are uncountable in the fixed counting windows, whereas, the quenched corrected windows gave counting efficiencies in excess of 30%.

The ability to track different energies as a function of quench, was determined by counting  $^3\text{H}$  and  $^{14}\text{C}$  separately as the low energy isotope in program 9. In this program, the lower level of the B channel is set to the same value as the upper level of the single label program 8. Table III shows the excellent tracking of the upper level for both  $^3\text{H}$  and  $^{14}\text{C}$  below 6% relative scintillation efficiency for  $^3\text{H}$  and below 2% relative scintillation efficiency for  $^{14}\text{C}$ .

The precision of the DPM measurement of quenched samples was determined by computing the standard deviation (S.D.) from 30 conveyor cycles in the  $^3\text{H}$  single labeled program 1 and the  $^3\text{H}$  dual labeled program 5 and for  $^{14}\text{C}$  in the single labeled  $^{14}\text{C}$  program 2 and the dual labeled  $^{14}\text{C}$  program 5. The S.D. is less than 1½% for both isotopes under both single and dual labeled counting conditions quenched down to a  $RPH$  of 11%. For  $^{14}\text{C}$ , the reproducibility of the disintegration rate measurement is maintained at less than 1½% down to a  $RPH$  of 2%. For  $^{14}\text{C}$ , the S.D. increases to about 2½% at an  $RPH$  of 7%. However, since the counting efficiency for  $^3\text{H}$  at this level of quench is only 4%, this corresponds to an absolute efficiency uncertainty of only 0.1% (table IV)

The efficiencies and standard variances are shown in Figure 4. As expected, high energy isotopes can be assayed more accurately than low energy isotopes. High energy isotopes have flatter quench curves and are less effected by variations in the quench parameter. It is interesting to note that the % S.D. in the ESP value is greater than % S.D. in the disintegration rate DPM derived from the ESP as shown for  $^{14}\text{C}$  and  $^{36}\text{Cl}$ . Measurements of the S.D. of an external standard quench parameter alone are not very meaningful. Variations in sample geometry, vial thickness and dirt accumulation may cause changes in the counting efficiency

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and corresponding changes in the quench parameter. Since both measurements are correlated, a component of the variance is eliminated in the DPM calculation.

TABLE III  
TRACKING OF ESP CORRECTED LEVELS

<u>TRITIUM EFFICIENCY (%)</u>			<u>CARBON 14 EFFICIENCY (%)</u>		
RPH	L TO U	U TO $\infty$	RPH	L TO U	U TO $\infty$
0.71	55	0.5	0.71	86	0.2
0.55	50	0.5	0.40	84	0.3
0.38	41	0.5	0.34	84	0.3
0.18	23	0.4	0.28	84	0.3
0.13	17	0.3	0.044	65	0.4
0.086	11	0.2	0.026	52	0.5
0.056	6.9	0.1	0.020	43	0.5

TABLE IV  
PRECISION OF DPM MEASUREMENT

<u>TRITIUM % VARIANCE</u>			<u>CARBON 14 % VARIANCE</u>		
RPH (ESP) <sup>-1</sup>	SINGLE LABEL	DUAL LABEL	RPH (ESP) <sup>-1</sup>	SINGLE LABEL	DUAL LABEL
0.77	0.4	0.3	0.77	0.2	0.2
0.67	0.4	0.3	0.40	0.1	0.3
0.50	1.1	1.1	0.17	0.2	0.4
0.34	0.5	0.6	0.09	0.3	0.6
0.22	0.9	0.3	0.05	0.3	1.1
0.11	1.1	1.2	0.031	0.7	0.9
0.07	2.3	2.5	0.017	0.7	1.4

## CONCLUSIONS

The average pulse height of the lesser spectrum from a  $^{133}\text{Ba}$  Compton spectrum has been used to reproducibly quantitate quench in a liquid scintillation system in terms of RPH. The method is useful for quantitating quench over a 60 to 1 change in RPH. Pulse height response is non-linear for weak scintillations; the non-linearity is dependent upon whether a single phototube or two in coincidence are employed and upon how the signals from each are combined. By correcting the pulse height response to weak scintillations for the single photon intercept, RPH can be used to quantitate relative scintillation efficiency assuming the photon emission spectrum probability is constant.

Quench corrected window settings can be accurately determined from the external standard RPH for both  $^3\text{H}$  and  $^{14}\text{C}$  without operator intervention, calibration curves, loss in counting efficiency or additional time. Counting efficiency is substantially improved particularly for heavily quenched and dual labeled samples. The method of pulse height restoration should be applicable for all beta emitting isotopes.

The disintegration rate can be reproducibly determined for single or dual labeled  $^3\text{H}$  and  $^{14}\text{C}$  from the RPH of the external standard without quench curves.

The beta energy of unknown isotopes can be estimated from the measured pulse height of the sample spectrum and the external standard spectrum assuming the photon emission spectrum is constant.

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