

Applications of Quench Monitoring Using Transformed External Standard Spectrum (tSIE)

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ABSTRACT

The transformed external standard spectrum is an effective quench monitor in quantitating samples in liquid scintillation analysis. It uses the Compton spectrum from the gamma source, ^{133}Ba , to monitor sample quench. This technique uses tSIE as an accurate method of quantitating the DPM in radiolabeled samples; it is shown to produce accurate DPM.

The use of tSIE is an accurate method of monitoring quench in a sample even under the following conditions:

1. a large dynamic range of tSIE (quench)
2. a large range of sample volumes
3. independence of wall effect, cocktail type and quenching agent
4. DPM determination without substantially increasing the count time of the sample

The use of tSIE parameter provides an accurate method for quench correction in the liquid scintillation analyzer. In addition, an automatic method (AEC) for adjusting the counting regions assists in DPM determination for both dual and triple labeled radioisotope samples.

INTRODUCTION

To understand the use of external standards for quantitating DPM in a radiolabeled sample, it is important that the basic principles of liquid scintillation process be understood. The entire process is to convert the energy from the beta particle into photons of light. This conversion must be efficient, and the intensity of the photons of light must be directly proportional to the energy of the beta particle. This process is illustrated in Figure 1.

The process of liquid scintillation involves the transfer of the energy of the beta particle to the solvent. Most all solvents used today for the liquid scintillation process contain an aromatic ring structure with π electrons. The solvent is chosen because it efficiently transfers the energy from the beta particles to the electrons of the solvent. The second step in the process is to transfer the energy from the activated solvent to a scintillator. This scintillator molecule becomes

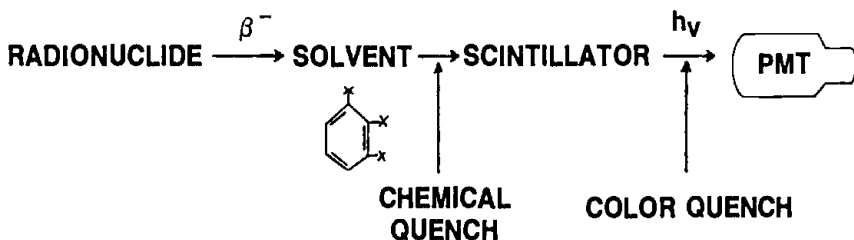


Figure 1. The basic liquid scintillation process.

excited, and when it returns to the ground state, it emits photons of light which are directly proportional to the energy of the beta particle. The photon (intensity of light) is then converted to a voltage pulse by the photomultiplier. The intensity or pulse height will be dependent on the number of photons entering the photomultiplier. These voltage pulses (analog in nature) are then converted to a digital pulse for direct analysis by a multichannel analyzer (4096 channels) over the analysis time of the sample. The entire energy range which is analyzed, is 0–2000 keV. This is the energy range of all beta-emitting radionuclides.

The beta particle decay is a process in which a neutron from the nucleus is converted into a photon, an electron (beta particle), and a neutrino (mass-like particle). The energy loss in the decay is distributed between the beta particle and the neutrino. Thus, the beta particle can have any energy from zero to the maximum energy of the beta particle. For example, for ^{14}C , an energy from 0–156 keV can be seen in the spectrum. This is illustrated in Figure 2; it is a typical energy spectrum of an unquenched ^{14}C spectrum using the Spectralyzer of the Packard 2500TR liquid scintillation analyzer. The x-axis is a linear plot of the energy or light intensity of the beta particles, and the y-axis is the number of counts/unit time that a pulse of that intensity has been seen by the photomultiplier tube. If the area under the spectrum is integrated, then the total amount of radioactivity present in the sample, as measured by the counter (CPM) is obtained. The CPM value measures of how efficiently the energy transfers from the beta particle to the solvent, and how efficiently it transfers to the scintillator. It measures how efficiently the scintillator produces light, and how efficiently the instrument electronics convert the photons to voltage pulse. All of the steps together determine the sample CPM seen by the system. The CPM value is measured and compared to the actual DPM in the sample (obtained from the μCi in the sample). The efficiency of counting the sample is then calculated by the following equation:

$$\frac{\text{cpm}}{\text{dpm}} \times 100\% = \text{counting efficiency}$$

The difference between DPM and the CPM measured by the liquid scintillation analyzer, is a result of a process termed quenching. This quenching phenomenon is involved in the scintillation process at two points: energy transfer and light quantitation. The first process of quenching (the energy transfer) is called chem-

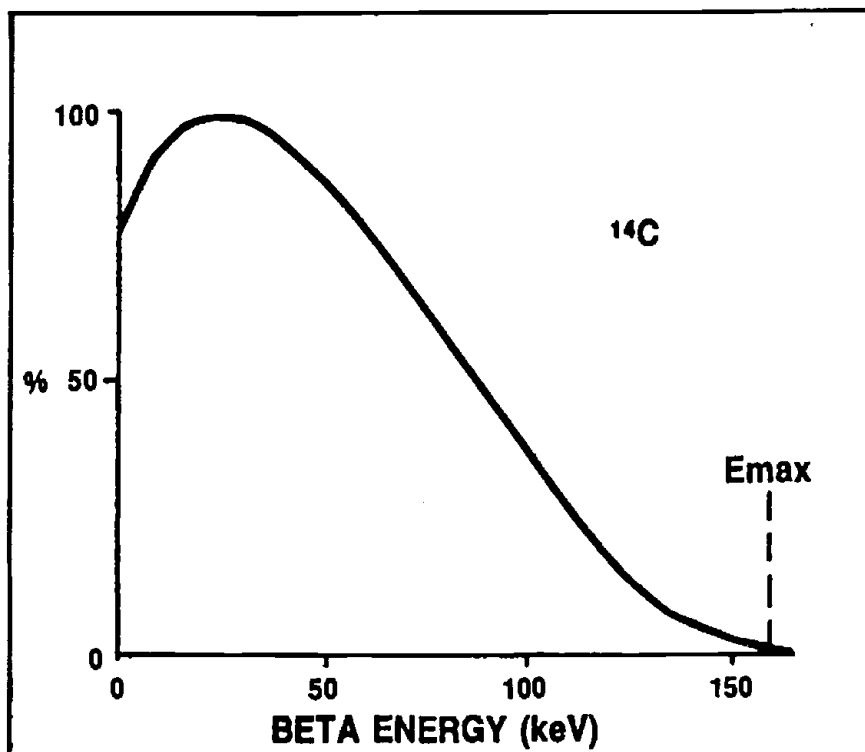


Figure 2. Energy spectrum of unquenched ^{14}C sample on Packard 2500TR liquid scintillation analyzer.

ical quenching. It involves the energy transfer of the beta particle to the solvent, or the transfer of energy from the solvent to the scintillator. Common chemical quenching agents (H_2O , nitromethane, CHCl_3 , CCl_4 , etc.) reduce the transfer of energy from the beta particle to the solvent. The quenching agent absorbs or reduces the energy of the beta particles transferred to the scintillator. The second process of quenching (photon reduction) is called color quenching. This phenomenon reduces the intensity of the scintillator produced photons seen by the photomultiplier. This phenomenon is similar to the use of a color filter on a photographic camera, which filters out certain colors of light on the photographs. The color in the scintillation process reduces the intensity of the photons seen by the photomultiplier. A typical spectrum for a ^{14}C sample, quenched by chemical and color quenching agents, is demonstrated in Figure 3.

The results of these quenching processes on the CPM can be extremely variable depending upon the quenching in the sample. For example, a sample prepared from a binding study may contain 2000 CPM, but a similar sample counted in solution might give 5000 CPM. In order to be able to compare these two samples, it is necessary to compare the DPM values (CPM compensates

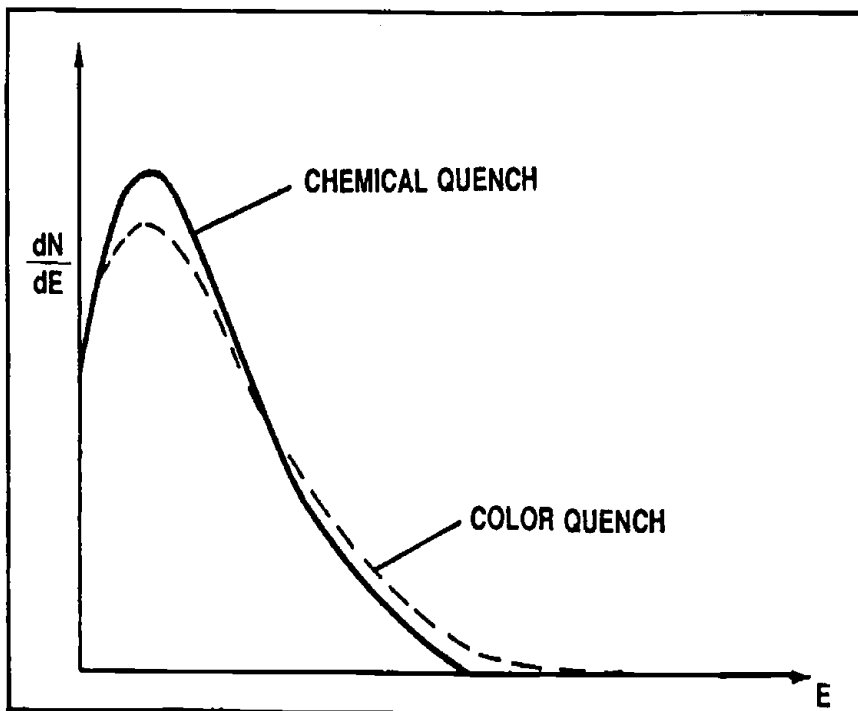


Figure 3. Chemical and color quenched spectrum.

for quenching). Three methods of quench correction can be used to obtain accurate DPM values: internal standard, sample spectrum, and external standard spectrum analysis.

Transformed External Standard Spectrum—tSIE

Most of methods for DPM determination involve the preparation of a quench curve (% efficiency vs quenching level) for a set of standard quenched vials. The most accurate method of determining DPM presence in the sample is by using the external standard spectrum technique. This technique involves exposing each sample to an external gamma source (^{133}Ba). The gamma radionuclide creates a Compton spectrum by way of the Compton scattering phenomenon. The gamma ray interacts with an electron to create a new gamma ray with less energy and a Compton electron. This Compton electron is similar to a beta particle and creates a Compton energy spectrum, Figure 4, in the LSA.

The Compton spectral distribution of ^{133}Ba can be used to monitor quenching in the sample. The Compton spectrum is stored in the Spectralyzer so that various features of this spectrum can be monitored. After close evaluation of the Compton spectrum of various quenched samples, it was apparent that this spectrum could not be used directly as a measure of quench; volume, wall effect,

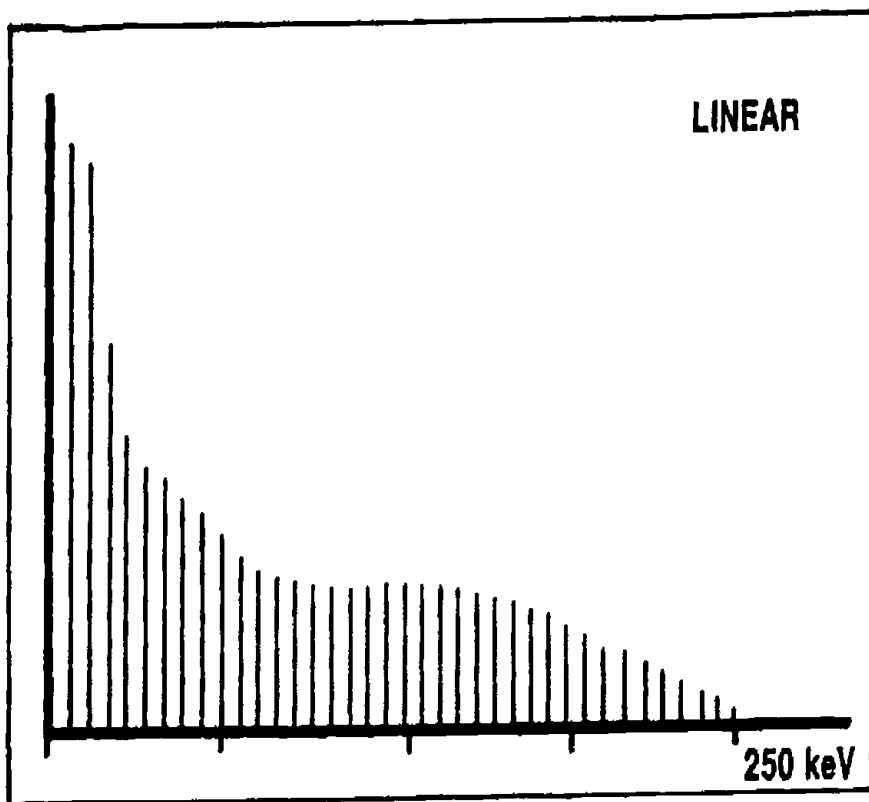


Figure 4. Compton spectrum of ^{133}Ba -linear.

and vial size changed the quenched indicating parameters in the Compton spectrum. After further investigation, it was found that if the integral spectrum of the ^{133}Ba external standard spectrum was computed, the end point of this transformed external standard spectrum (tSIE) could be used to measure sample quenching. The tSIE decreases as the quenching of the sample increases. This change of the tSIE with increasing quenching is illustrated clearly in Figure 5. This shows the Compton spectrum of two samples of different quench levels which have been transformed. The external standard end point of each sample is shown. This external standard end point is multiplied by a constant, such that the tSIE for an unquenched, argon purged ^{14}C sample is equal to 1000. The tSIE (quench indicating parameter—QIP) can be used as a method of relating the % efficiency of a standard quench set as a function of quenching the sample.

This plot of % efficiency can be used as an accurate method of obtaining DPM for samples with unknown DPM. This first requires the preparation of a standard quench curve (% efficiency vs tSIE) with a sealed set of standards containing the same radionuclide that is present in the unknown. Once each sample in the standard quench set has been counted, the % efficiency is deter-

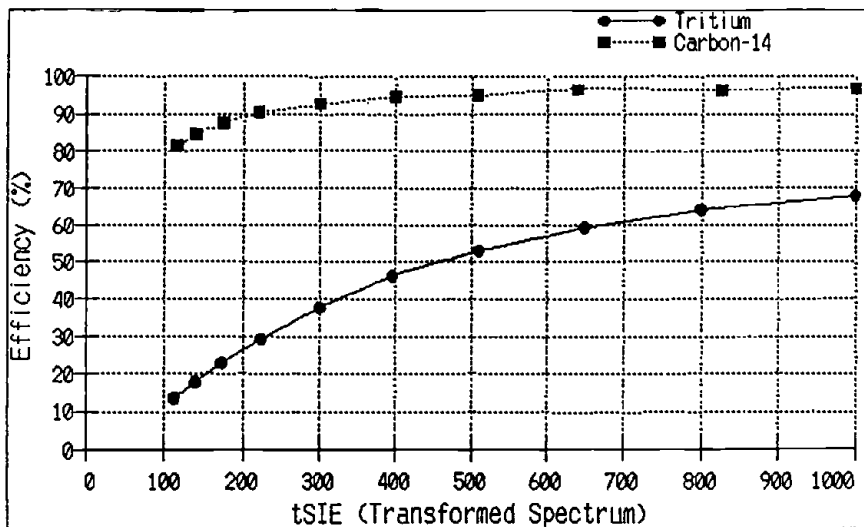


Figure 5. tSIE quench curves for tritium and ^{14}C .

mined. This is done by counting each sample and obtaining its CPM. Since the quenched set was made with a known amount (DPM) of radionuclides of interest, the % efficiency can be calculated by the following equation:

$$\frac{\text{cpm}}{\text{dpm}} \times 100\% = \% \text{ efficiency}$$

This is the y-axis number for each sample. The x-axis values are determined next. This is done by exposing each standard in the quenched set to the external standard (^{133}Ba). The Compton spectrum of each sample is then recorded in the Spectralyzer spectrum analyzer. This is converted to an integral spectrum. The tSIE value is determined from the extrapolated end point of this transformed spectrum. The tSIE value is then used as a measure of the quenching in the sample (QIP). Once the tSIE and % efficiency for each of the standard quench sets has been determined, it is plotted on a graph. The curve fit used to connect the points is a fixed point least square quadratic (FPLSQ). A typical quench curve for ^{14}C and ^3H is shown in Figure 5.

In summary, the following steps are used to prepare a quench curve for determining DPM:

1. prepare standards (two different methods can be used): (1) purchase sealed standards containing radioisotope of interest, (2) prepare and analyze set of standards of known DPM with different levels of quenching agent, and use a cocktail similar to that used in unknown sample
2. count standards in defined counting region determined by evaluation of sample spectrum of least quenched sample
3. count sample to determine CPM for each standard

4. compute % efficiency for each sample by using the following equation:

$$\% \text{ efficiency} = \frac{\text{cpm}}{\text{dpm}} \times 100$$

5. expose each sample to an external standard, ^{133}Ba , and obtain Compton spectrum. Transform spectrum to obtain the end point of this spectrum, and then determine tSIE value
6. plot % efficiency of each standard vs tSIE value
7. use fixed point least square quadratic curve fit (FPLSQ) to connect points and obtain quench curve for radionuclide of interest

Now that this quench curve has been obtained, how can it be used to obtain the DPM in an unknown sample? The first step in determining the DPM in the unknown sample, containing the same radionuclide as the nuclide used to prepare the standard curve, is to count the unknown sample under the same conditions used to create the standard curve. Then the CPM in the sample is determined. The sample is then exposed to the external standard, and the tSIE (quench indicating parameter) is determined. From the standard curve and the tSIE, the counting efficiency for the sample is extrapolated. The DPM for the unknown is then calculated from the equation:

$$\text{DPM} = \frac{\text{cpm}}{\% \text{ efficiency}}$$

In order to obtain accurate DPM values, it is necessary to have a quench indicating parameter (tSIE value) which has the following characteristics:

1. high dynamic range
2. reproducibly and accuracy at normal and high quench conditions
3. independence of sample volume
4. independence of "wall effect"
5. independence of vial type
6. independence of vial size
7. independence of cocktail density

The external standard used to determine the tSIE must have the following characteristics:

1. low radiation hazard not subject to stringent radiation safety regulation
2. the E_{max} low enough so as not to require excessive shielding which could increase background
3. maximum energy of Compton spectrum close to energy of ^3H and ^{14}C , most frequently used radionuclides in dual label counting
4. energy of external standard source sufficiently low to reduce or eliminate spectral distortions caused by gamma ray interaction with material in counting chamber environment
5. timely determination such that counting time of sample is not greatly increased in determining DPM

This chapter will evaluate each characteristic and present the data as to the accuracy of the DPM determination. This can be illustrated best by defining a term called % recovery. The % recovery of an experiment is the DPM determined by the liquid scintillation analyzer compared to the actual DPM in the sample times 100%.

$$\frac{\text{DPM counter}}{\text{DPM actual}} \times 100$$

For all characteristics of the tSIE, the % recovery is calculated and plotted.

The first characteristic of the tSIE is its volume independence. This can be illustrated by preparing a series of samples:

1. standard 20 mL vial—19, 15, 12, 8 mL
2. miniature 7 mL vial—6, 4, 3, 2, 1, 0.5 mL
3. microfuge tube—0.4, 0.2, 0.1, 0.05, 0.025 mL

The samples were counted and a tSIE determined for each sample with a subsequent DPM printed out. The % recovery was then determined from the DPM of the counter, and the DPM actually present in the sample. A graph of the % recovery vs sample volume for ^3H is shown in Figure 6. For the 15 sample volumes, a straight line was obtained near 100% recovery. The exact statistical numbers of ^3H were a mean of 98.19%, a standard deviation of (SD) 1.58, and a % coefficient of variation of (%CV) 1.61. For ^{14}C , the numbers

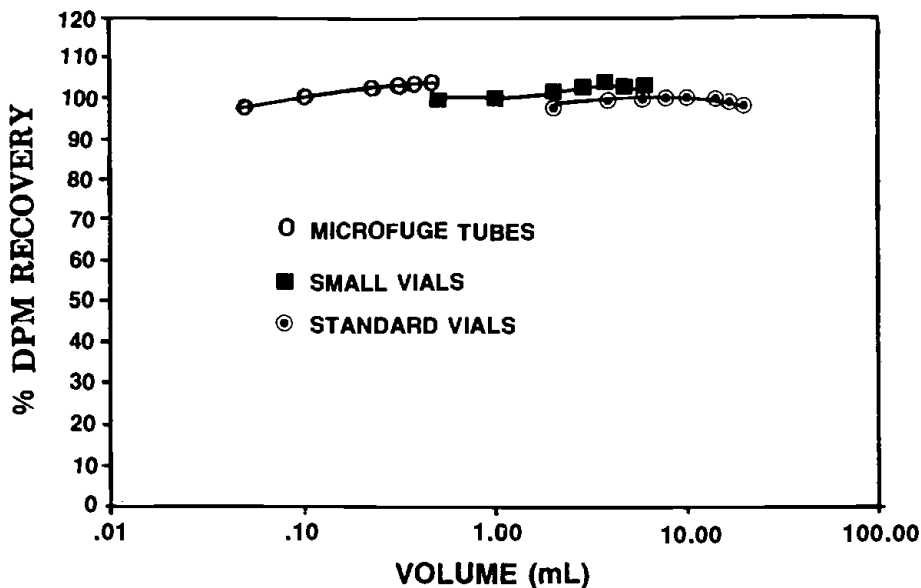


Figure 6. ^3H DPM % recovery as a function of sample volume.

Table 1. Quantitation of ^3H and ^{14}C and Dual Label Samples at Various Volumes Using tSIE as a Quench Monitor

Volume	Nuclide	% Recovery	tSIE
400 μL	^3H	98.9	663
200 μL	^3H	98.5	617
100 μL	^3H	97.6	555
50 μL	^3H	96.8	528
25 μL	^3H	96.4	453
400 μL	^{14}C	100.6	698
200 μL	^{14}C	101.5	630
100 μL	^{14}C	100.0	600
50 μL	^{14}C	98.3	538
25 μL	^{14}C	99.6	494
400 μL	$^3\text{H}/^{14}\text{C}$	93.3/99.6	677
200 μL	$^3\text{H}/^{14}\text{C}$	95.1/99.2	629
100 μL	$^3\text{H}/^{14}\text{C}$	95.3/104.5	610
50 μL	$^3\text{H}/^{14}\text{C}$	96.9/104.5	543
25 μL	$^3\text{H}/^{14}\text{C}$	99.2/98.5	475

were even closer to 100% with a mean of 99.51%, a SD of 0.917 and %CV of 0.922. This data clearly indicates that the DPM values obtained using the tSIE is volume independent. Because of the increasing use of microvolume counting, a special series of samples were prepared with volumes of 400, 200, 100, 50, and 25 μL containing ^3H , ^{14}C , or dual label $^3\text{H}/^{14}\text{C}$. These volumes were chosen because they are commonly used in the microvolume counting procedure. The dual label samples were incorporated to determine if at low volumes, the DPM of a dual label sample could be determined accurately. Both single label ^3H and ^{14}C DPM % recoveries (Table 1) show excellent recoveries. The % recoveries are 97.6% (^3H) and 100% (^{14}C) with a small SD of 1.1 to 1.2% for these isotopes. For the $^3\text{H}/^{14}\text{C}$ dual label samples, the % recoveries are 96.56% for ^3H , and 101.2% for ^{14}C . The standard deviation for ^3H was 1.70, and for ^{14}C it was 2.94. The data in Table 1 clearly indicate that the tSIE is decreasing as the sample volume decreases. If the tSIE and % efficiency decreases, then the CPM/% efficiency increases, and the corrected DPM is determined accurately. As mentioned in the requirements for a DPM (quenching method), the counting time should not be substantially increased. Despite these low sample volumes, the time required to calculate the tSIE (external standard quench indicating parameter) is short (0.5 to 1.0 minutes).

The optimal geometry of the external source positioned directly below the sample and the ease of measurement of the Compton spectrum from the ^{133}Ba gamma source requires only a short time period for external source quench correction. The sample throughput, therefore, is not substantially affected by counting samples containing small volumes. These results are illustrated in Table 2.

As can be clearly seen, the tSIE is very reproducible over the large quench range, with the % efficiency for ^3H decreasing from 58.47 to 3.32%. The data shows that the % recovery is very close to 100%, with a maximum of %CV of 0.640 for the sample, with a tSIE = 83.59. The tSIE value is also very repro-

Table 2. Reproducibility of % Efficiency, % Recovery and tSIE for ^3H Quenched Samples, Counting Each Sample Ten Times

Sample	% EFF	% Rec.	% CV	tSIE	% CV/tSIE
1	58.47	99.94	0.362	815.2	0.412
2	55.78	99.77	0.200	725.9	0.334
3	44.49	100.23	0.224	472.2	0.240
4	24.77	99.82	0.304	231.1	0.246
5	18.07	99.42	0.303	177.1	0.179
6	10.81	99.6	0.318	119.8	0.352
7	6.15	99.29	0.640	83.59	0.480
8	3.32	99.00	0.381	58.75	0.230

ducible even for the most heavily quenched sample of the sample set $t\text{SIE} = 58.75$ ($\%CV = 0.230$). The maximum $\%CV$ for any $t\text{SIE}$ is 0.480.

Now that the $t\text{SIE}$ has been shown to be reproducible at samples containing normal quenching values, what about samples that are severely quenched? Does the $t\text{SIE}$ value become less reproducible as the quench becomes more severe? In order to assess this, a special set of ten samples was prepared. The $t\text{SIE}$ values varied from 39 to 10, and the $\%$ efficiency varied from 1.388 to 0.011%. Each of these samples was counted 10 times with the statistical data presented in Table 3.

The samples (^3H -labeled) are severely quenched with efficiency decreasing to 0.011%. Even at this level, the $\%$ efficiency has a $\%CV$ of 3.31 which is extremely good for these heavily quenched samples. The $t\text{SIE}$ values decrease to 9.956 with a $\%CV$ of 0.296. The table also shows the three $t\text{SIE}$ values; the $t\text{SIE}$ was identical each of the 10 times that the $t\text{SIE}$ was determined. This data clearly indicates that the $t\text{SIE}$ method of determining quench is very reproducible and has a large dynamic range. These experiments were also conducted with ^{14}C at similar quench level. Almost identical results for $t\text{SIE}$ reproducibility were obtained. A graphic summary of the type of quench curve which could be obtained with these 18 standard quenched samples is shown in Figure 7. The major portion of the quench curve ($\%$ efficiency vs $t\text{SIE}$) is shown in the graph. The heavily quenched sample region of the curve has been exploded in order to assess the nature of these curves at the extremely heavily quenched levels.

Table 3. Reproducibility of % Efficiency, % Recovery and tSIE For Extended ^3H Quench Samples, Counting Each Sample Ten Times

Sample	% EFF	% CV/EFF	tSIE	% CV/tSIE
1	1.388	0.922	39.00	0.209
2	0.617	0.868	26.99	0.324
3	0.311	0.476	21.08	0.300
4	0.166	1.072	18.15	0.290
5	0.096	2.876	15.41	0.205
6	0.060	1.587	14.88	0.283
7	0.038	3.990	12.70	0.000
8	0.023	2.410	12.30	0.000
9	0.016	2.740	10.20	0.000
10	0.011	3.310	9.956	0.296

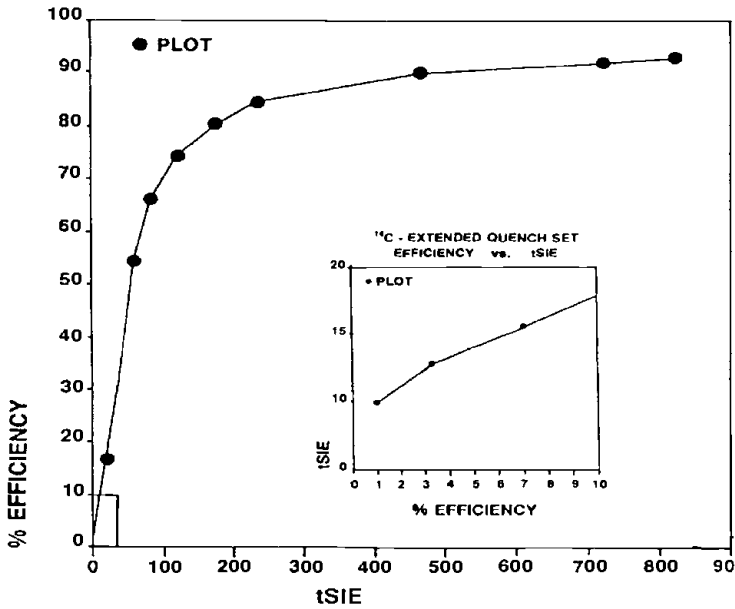
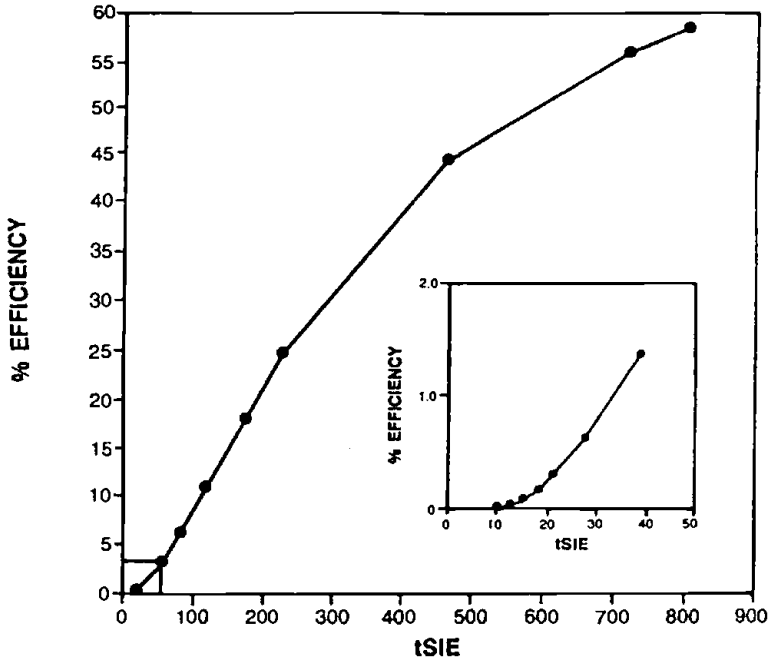


Figure 7. Quench curve for ^3H tSIE = 815-9.956 (top). Quench curve for ^{14}C for tSIE = 815-9.956.

The dynamic range for the tSIE method of determining efficiency can be defined by the following equation:

$$\text{Dynamic Range}^* = \frac{\% \text{ eff. of unquenched sample}}{\% \text{ eff. of most highly quenched}}$$

Using this definition, the dynamic ranges are as follows:

$${}^3\text{H} = \frac{63.57 \text{ (tSIE} = 1000\text{)}}{0.011 \text{ (tSIE} = 9.956\text{)}} = 5779$$

$${}^{14}\text{C} = \frac{96.54 \text{ (tSIE} = 1000\text{)}}{1.13 \text{ (tSIE} = 9.956\text{)}} = 85.43$$

These results clearly indicate that the tSIE method of determining quenching in a sample have a very broad dynamic range with very reproducible values even at a heavily quenched sample.

The third characteristic of the tSIE is its ability to eliminate the "wall effect" problem, which may be present in samples prepared in liquid scintillation vials. The common organic solvent present in liquid scintillation solutions (toluene, xylene, pseudocumene) can readily penetrate the walls of plastic liquid scintillation vials. Two problems can be observed when using plastic liquid scintillation vials. First, the solvent can permeate the plastic wall of the scintillation vial. This gives in inside of the liquid scintillation counter a strong organic odor. The penetration of the solvent into and through the scintillation vials results in actual swelling of the vials; this makes it impossible to store and recount samples prepared in plastic vials. Second, not only the solvent but the scintillator can penetrate into the plastic wall. This can result in the plastic vial wall acting as a plastic scintillator. This "plastic scintillator wall" has a lower efficiency than the sample in solution. Thus, if the plastic vial with plastic scintillator wall is exposed to the external gamma source, extra low energy photons will be emitted which could affect the low energy external standard spectrum. This could thus affect the external standard quench indicating parameter and result in incorrect DPM values. The wall effect on the % recovery of a sample can easily be assessed by counting a sample initially at 12 hour periods. If the wall effect is affecting the DPM, then the % recovery would change as a function of time. This experiment was conducted for both the plastic standard (15 mL) and miniature vials (5 mL) over an 84 hour period.

The results in Table 4 clearly show that the % recovery of neither the standard vial nor the miniature vials change as a function of time. The %CV for this entire time period is 0.71 and 0.36 for the 5 and 15 mL samples respectively. This indicates that the wall effect commonly seen in some liquid scintillation counters has been completely eliminated by the Packard quench indicating parameter (tSIE).

The fourth characteristic of tSIE is its ability to give accurate DPM values

*% recovery of all samples must be $100 \pm 5\%$.

Table 4. Effect of Possible "Wall Effect" on DPM Recovery in Plastic Vials Using ³H-Alanine in Instagel

Time (hr)	Volume (mL)	% DPM recovery
0	5	100.2
0	15	100.2
12	5	101.1
12	15	100.9
24	5	100.3
24	15	100.1
48	5	100.4
48	15	99.9
60	5	100.2
60	15	99.9
72	5	100.1
72	15	100.5
84	5	98.8
84	15	100.0

for different scintillation vial types (glass or plastic), different sample sizes (standard, miniature, and microfuge tubes), different scintillation solution types, and different quenching agents types (chemical and color). Let us divide these into two sections. The first will address the vial size and the vial type question. The second will cover the different types of scintillation solution, as well as various types of quenching agents. The results for different vial size and vial type is shown in Table 5.

The results in the table indicate the % recovery using glass or plastic, and any vial size is 100% for all of the samples assayed. This shows that the DPM using tSIE is independent of the type or size of vial used to hold the sample.

The second aspect to be investigated was the effect of various scintillation solutions and various quenching agents (color or chemical). The results are shown in Table 6.

The first four samples indicate the result of the various quenching agents (color and chemical) present in the sample. The results in Table 6 indicate that the three chemical quenching agents (CHCl₃ and CCl₃, and CH₃NO₂) do not affect the % recovery of the ³H-toluene in the sample. The addition of a red colored organic material, eosin, does not affect the % recovery with a 100.2% value obtained. This is further shown for various quench levels for ³H and ¹⁴C in Figure 8. The next three samples show that the sample density in g/cc does not

Table 5. Assessment of DPM/% Recovery-Variou Vial Sizes and Types Using ³H-Toluene

Sample	Vial Type	% Recovery
1	20 mL glass	99.9
2	7 mL glass	99.6
3	6 x 50 mm Sealed	98.9
4	20 mL plastic	100.2
5	7 mL plastic	100.2
6	1.5 mL microfuge tube	99.8
7	0.5 mL microfuge tube	98.9

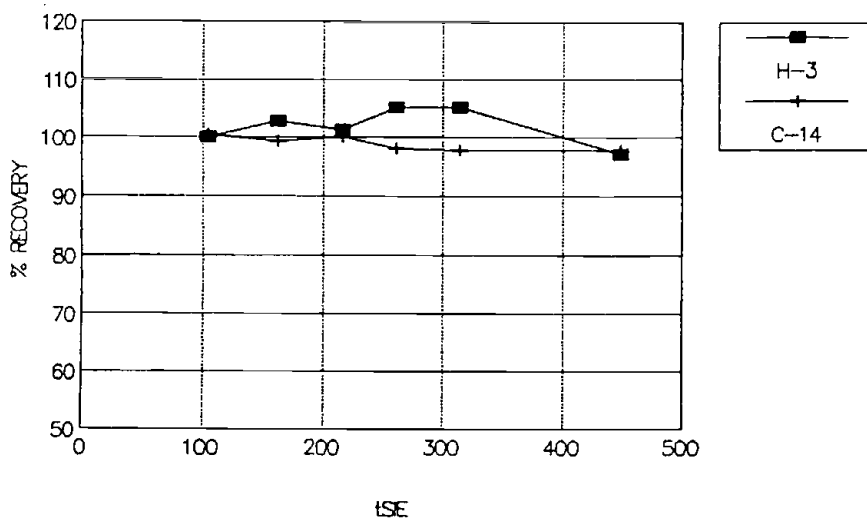
Table 6. Effect of Type of Quenching Agent on DPM Recovery for ^3H

Treatment	Density (g/cc)	$\times 10^{23}/\text{mL}$ Elect Density	% Recovery
CHCl_3 (10 μL)	—	—	99.2
CCl_4 (10 μL)	—	—	98.5
CH_3NO_3 (10 μL)	—	—	99.6
Eosin Red (10 μL)	—	—	100.2
Toluene + PPO	0.863	2.82	99.9
Xylene + PPO	0.877	2.89	100.2
Pseudocume/PPO	0.860	2.84	100.8

affect the % recovery of the sample. In addition, the cocktail electron density of the samples was calculated. The cocktail electron density is the sum of the weight of each component, times the electrons per molecule, times 6.0238×10^{23} molecules/mol. The first units for the electron density is electrons/mL. The effect of this electron density on the tSIE/DPM and % recovery was evaluated and found not to affect the % recovery for the samples tested.

Now, the tSIE method of evaluating sample quenching has been shown to be independent of most of the factors which affect DPM determination in simple single radiolabeled analysis. What about its accuracy in counting the more complicated dual label and the most complicated triple label samples? First, let us evaluate the method used for quantitating dual labeled samples. The problem is how to obtain the true DPM for each of the two single labels in a dual label sample. This problem is shown in Figure 9 for dual labeled $^3\text{H}/^{14}\text{C}$ samples.

In order to obtain accurate DPM values for both radionuclides, the following steps should be taken:

**Figure 8.** ^3H and ^{14}C recovery for colored samples at various tSIEs.

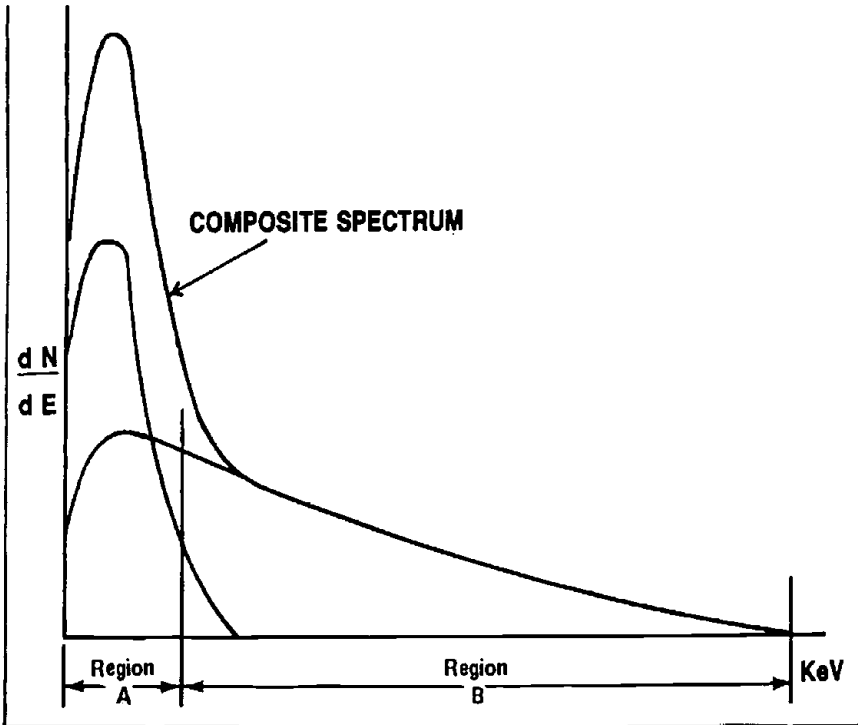


Figure 9. Dual label $^3\text{H}/^{14}\text{C}$ region settings for dual label counting region A = 0-12 keV, region B = 12-156 keV.

1. Prepare a set of ^3H samples, each containing a constant number of DPMs, with zero to a maximum amount of quenching agent. A commercial sealed set can also be obtained for each radionuclide.
2. Prepare a set of ^{14}C samples similar to the ^3H samples with a constant DPM in each and different quench levels.
3. Next, count each sample in the ^3H quench set to obtain DPM.
4. For each sample, calculate the % efficiency = $\frac{\text{cpm}}{\text{cpm}}$ equation is for both regions A and B.
5. Expose each sample to the built-in external gamma source, ^{133}Ba , with the tSIE being determined for each sample. This tSIE is independent of the radionuclide present or the region settings. The tSIE is an indicator of the quench level in the sample.
6. Obtain two plots of obtained—(Figure 10) of % efficiency, ^3H in region A and ^3H in region B, as a function of tSIE. From the plots, the % efficiency in both the A and B regions can be obtained.
7. Assay the ^{14}C standard in the same manner as the ^3H standards, with two additional plots being obtained (Figure 11). The plots are of % efficiency of ^{14}C in region A and ^{14}C in region B. From these plots, the % efficiency_{CA} and % efficiency_{CB} can be obtained using fixed point, at least square quadratic curve fit.

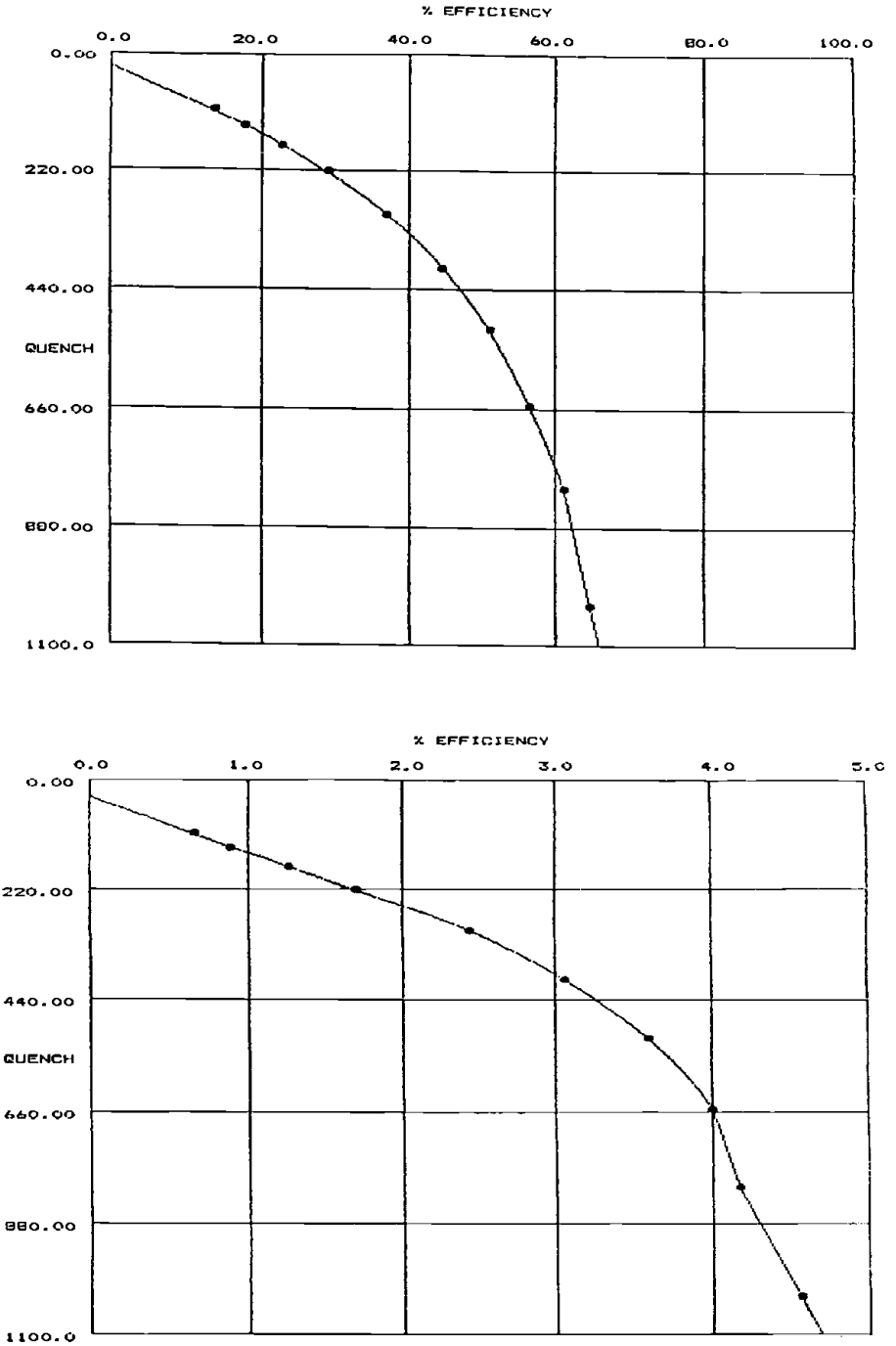


Figure 10. Dual label $^3\text{H}/^{14}\text{C}$ quench curves for ^3H using tSIE for region A (top). Dual label quench curve for ^3H using tSIE for region B.

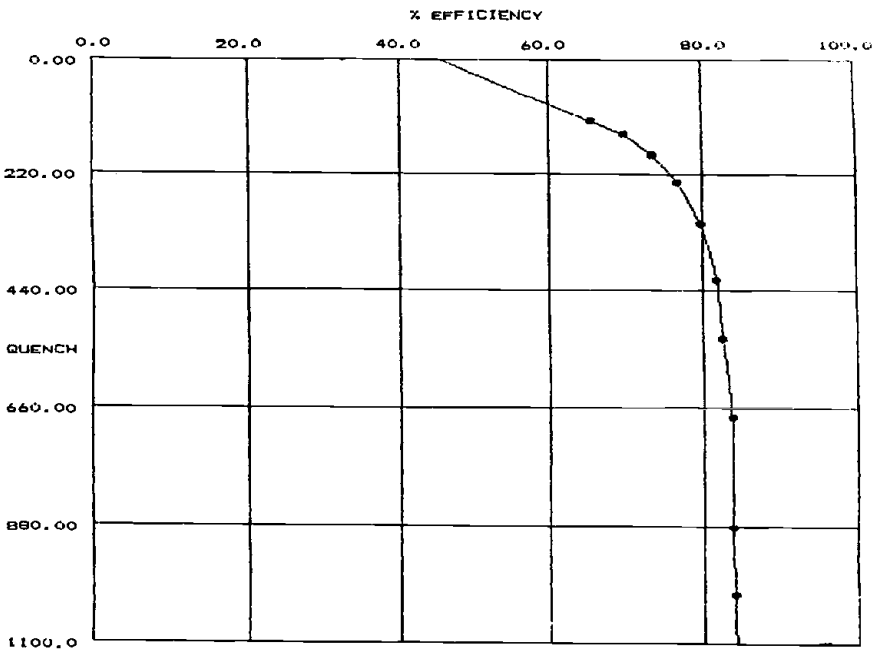
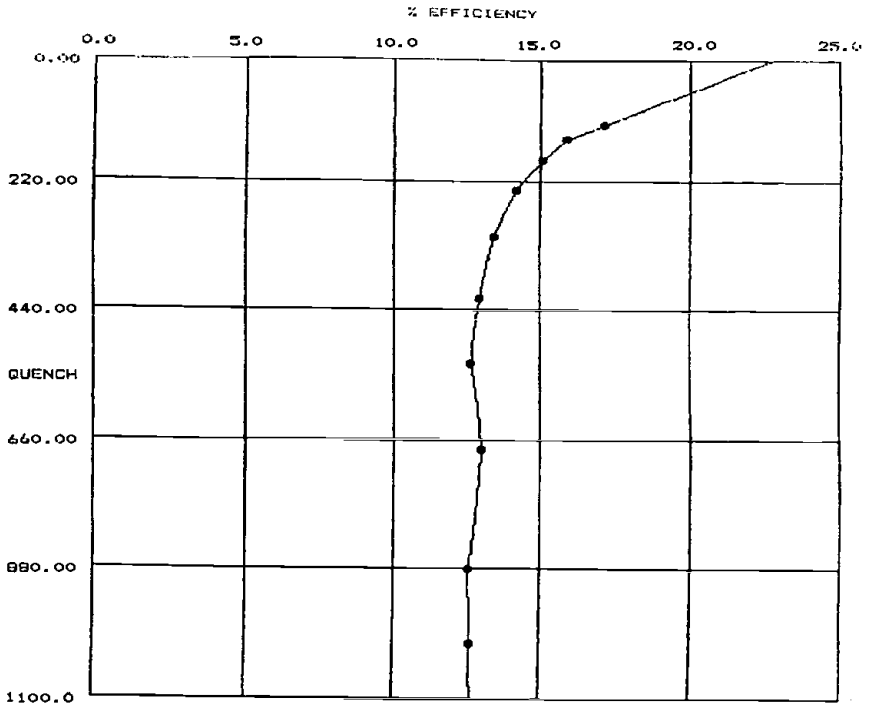


Figure 11. Dual label $^3\text{H}/^{14}\text{C}$ quench curves for ^{14}C using tSIE for region A (top). Dual label quench curve for ^{14}C using tSIE for region B.

8. Now the system is ready to determine DPM values for unknown samples. First the tSIE value is determined, and by using this value on the four plots above, four % efficiencies can be obtained (% efficiency_{HA}, % efficiency_{HB}, % efficiency_{CA}, and % efficiency_{CB}). The sample is then counted in each of the two regions with the CPM_A and CPM_B obtained. Then the following equation can be used:

$$\text{CPM}_A = (\% \text{ Eff.} \times \text{DPM}) + (\% \text{ Eff.}_{CA} \times \text{DPM}_C)$$

$$\text{CPM}_B = (\% \text{ Eff.}_{AB} \times \text{DPM}_H) + (\% \text{ Eff.}_{CB} \times \text{DPM}_C)$$

By rearranging and substituting into these equations, the DPM_H and DPM_C can be obtained.

$$\text{DPM} = \frac{(\text{CPM}_H \times \% \text{ Eff.}_{CB}) - (\text{CPM}_C \times \% \text{ Eff.}_{CA})}{(\% \text{ Eff.}_{HA} \times \% \text{ Eff.}_{CB}) - (\text{CPM}_C \times \% \text{ Eff.}_{CA})}$$

$$\text{DPM} = \frac{(\text{CPM}_C \times \% \text{ Eff.}_{HA}) - (\text{CPM}_H \times \% \text{ Eff.}_{HB})}{(\% \text{ Eff.}_{HA} \times \% \text{ Eff.}_{CB}) - (\% \text{ Eff.}_{HB} \times \% \text{ Eff.}_{CA})}$$

As can be seen from the equations and plots, two of the most important factors are the % efficiency and carbon in region A. The % efficiency_{CA} (crossover of ¹⁴C into ³H) increases drastically when the sample becomes more quenched. This could cause considerable problems when a large ratio of ¹⁴C to ³H exists in the sample. Therefore, a special feature termed AEC (Automatic Efficiency Control) was implemented in the system to help overcome this problem. This feature automatically tracks the theoretical end point of both the ³H and ¹⁴C spectrum in the sample. By doing this, the % efficiency of ¹⁴C in the A region is kept relatively constant, as shown in Figure 12. This enables accurate DPM determinations for ³H/¹⁴C dual labeled samples at various quench levels and at various radionuclide ratios. This technique can be used for any dual labeled sample separated by at least 175 keV of energy.

In order to assess the performance of the tSIE as a quench indicating parameter, a DPM for dual labeled samples and a series of samples were prepared. These samples contained ³H/¹⁴C ratios of 1:1, 1:5, 1:10, 1:20, and 1:50. Each of these samples were prepared at various quenched levels. The DPM for each of the dual labeled samples was determined using the previously described procedure with AEC activated (Figure 13). The results, shown in Table 7, indicate that for the 14 samples evaluated, the ³H recovery was 100.31 and the ¹⁴C was 100.85%. These % recoveries are extremely stable, even for samples at high ¹⁴C/³H ratios of 20:1 and 50:1. It is also clear from the table that a quench range from very small to moderate to severe, that the % recovery of both radionuclides is close to 100%. The results are also very reproducible with a %CV of 0.887 for ³H, and 0.886 for ¹⁴C.

In order to evaluate the DPM determination for a dual labeled sample of ³H/¹⁴C samples of a 1:20 ratio were prepared and quantitated. A plot of %

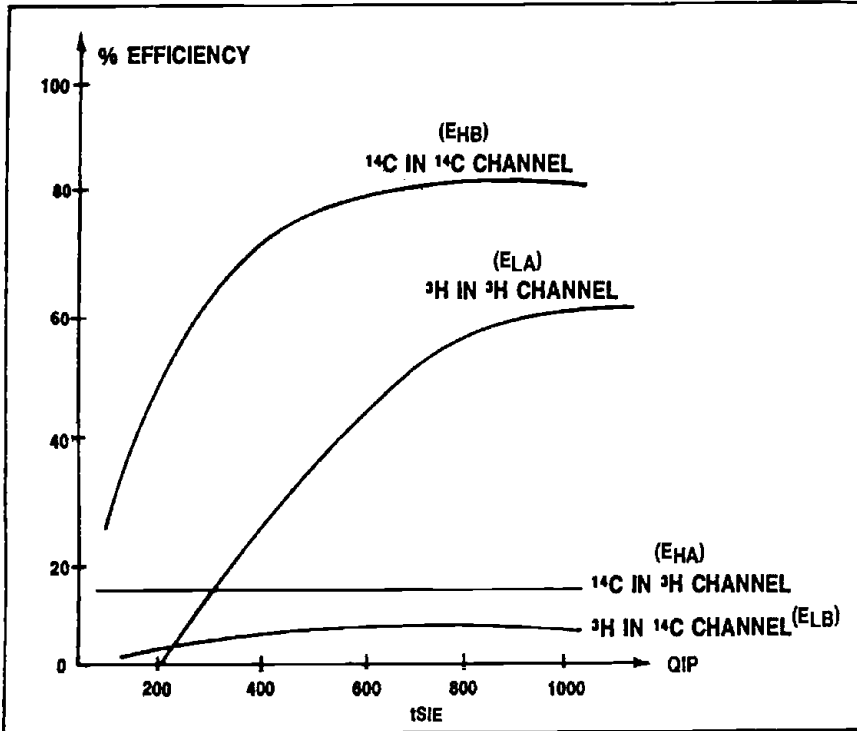


Figure 12. Dual label with AEC ^3H and ^{14}C in regions A and B.

recovery of both ^3H and ^{14}C is shown in Figure 10. The quenched levels range from a tSIE of 800 to 83.9% to 62.1% for ^{14}C . The results in the graph indicate a stable % recovery of ^3H and ^{14}C , even at a high ratio of 20:1.

Now that the dual label samples have been shown to use tSIE to determine accurate DPM values, what about the ultimate analysis of a triple label sample containing $^3\text{H}/^{14}\text{C}/^{32}\text{P}$? This triple label sample analysis is very similar to the dual labeled analysis except that instead of % efficiency vs tSIE curves, 9 are required. Three for each radionuclide in each of the three regions of interest. From these 9 % efficiencies, the three CPM values for regions A, B, and C, and the DPM of each of the three radionuclides can be quantitated by an inverse matrix analysis procedure. In order to completely evaluate the triple label DPM procedure, four different ratios of $^3\text{H}/^{14}\text{C}/^{32}\text{P}$ were used at various quenched levels. The DPM was determined for each radionuclide. The statistics, mean, SD and %CV were also calculated.

The results clearly indicate that the % recoveries are very close to 100% (99.65, 101.46, and 100.15), and all samples have a %CV of less than 1.36 over the entire quenched range. This would be appropriate if all experiments were done at a ratio of 1:1:1, but most scientists use various ratios of the three radionuclides present. How do the tSIE perform under conditions when one of

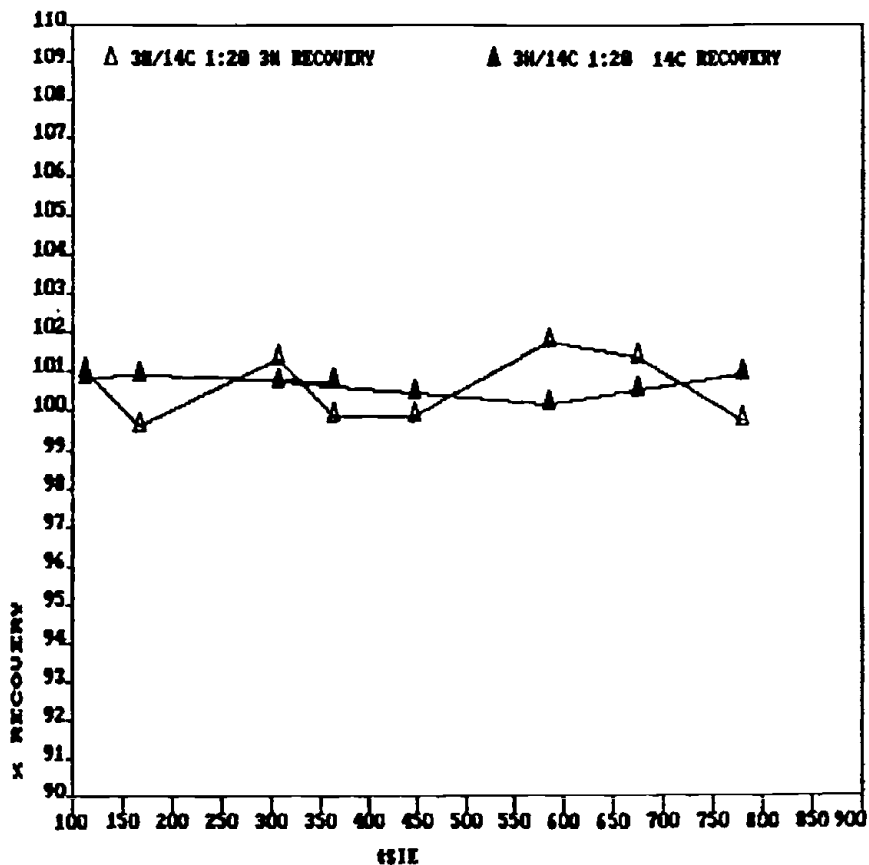


Figure 13. % recovery of ^3H and ^{14}C for dual label with AEC $^3\text{H}/^{14}\text{C}$ ratio 1:20.

Table 7. % Recovery of Dual Label $^3\text{H}/^{14}\text{C}$ at Various Quench Levels and Isotope Ratios

Ratio $^3\text{H}/^{14}\text{C}$	^3H % REC	^{14}C % REC	tSIE
1:1	100.6	101.0	808
1:1	100.6	101.3	473
1:1	100.2	98.3	161
1:5	99.9	101.8	828
1:5	102.3	100.5	463
1:5	100.5	102.0	174
1:10	100.1	101.3	786
1:10	99.7	101.1	464
1:10	100.8	101.5	173
1:20	99.7	100.9	780
1:20	99.8	100.5	448
1:20	99.5	100.9	166
1:50	100.9	100.6	786
1:50	101.5	100.2	681

Table 8. % Recovery of $^3\text{H}/^{14}\text{C}/^{32}\text{P}$ at Ratios of 1:1:1 at Various Quench Levels

	^3H	^{14}C	^{32}P	tSIE
	98.90	101.89	100.04	811
	98.12	100.78	99.16	724
	98.79	100.57	100.01	622
	98.98	101.78	100.51	499
	99.23	101.14	100.35	385
	99.80	101.04	101.13	329
	101.9	101.94	100.10	185
	101.50	101.78	99.87	117
Mean	99.65	101.46	101.15	
SD	1.35	0.56	0.62	
% CV	1.36	0.55	0.62	

Table 9. % Recovery of $^3\text{H}/^{14}\text{C}/^{32}\text{P}$ at Ratios of 10:1:1 at Various Quench Levels

	^3H	^{14}C	^{32}P	tSIE
	100.5	99.05	97.50	817
	100.1	94.71	98.90	753
	100.2	95.54	100.9	639
	100.1	98.23	98.96	572
	101.1	98.54	100.62	498
	100.5	97.80	99.2	420
	102.3	96.50	99.33	262
	102.5	97.40	102.50	178
Mean	100.91	97.24	99.68	
SD	0.97	1.52	1.41	
% CV	0.96	1.57	1.41	

the radioisotopes is in a tenfold excess over the other two radioisotopes present in the sample. This can be demonstrated by analyzing the data in Tables 9 to 11. Table 9 shows the results with an excess (10×) of the lower energy ^3H in the sample.

The results show that the recoveries are very close to 100%. The mean % recoveries are 100.91 (^3H), 97.24 (^{14}C), and 99.68 (^{32}P). All show a standard deviation of less than 1.52. Table 10 displays the results for a sample contain-

Table 10. % Recovery of $^3\text{H}/^{14}\text{C}/^{32}\text{P}$ at Ratios of 1:10:1 at Various Quench Levels

	^3H	^{14}C	^{32}P	tSIE
	100.24	99.88	99.70	813
	103.70	99.54	100.02	738
	98.71	101.56	101.11	646
	105.17	100.14	100.18	572
	99.81	100.28	101.43	496
	103.40	100.48	100.73	435
	104.03	100.45	99.98	361
	103.21	100.78	104.67	265
	97.89	100.75	99.53	179
Mean	102.44	100.42	100.81	
SD	2.56	0.59	1.58	
% CV	2.51	0.59	1.57	

Table 11. % Recovery of $^3\text{H}/^{14}\text{C}/^{32}\text{P}$ at Ratios of 1:1:10 at Various Quench Levels

	^3H	^{14}C	^{32}P	tSIE
	96.72	99.89	100.49	817
	98.51	108.28	99.67	751
	102.19	95.53	101.38	655
	98.40	99.99	102.04	605
	95.92	106.95	99.97	504
	96.66	102.09	101.36	443
	103.73	91.66	101.24	269
	103.55	107.47	100.29	183
Mean	99.45	101.45	100.83	
SD	3.20	5.90	0.80	
% CV	3.22	5.82	0.80	

ing a $^3\text{H}/^{14}\text{C}/^{32}\text{P}$ ratio of 1:10:1. The results show a mean of 102.44 (^3H), 100.42 (^{14}C), and 100.81 (^{32}P). These results are over the entire quench range analyzed by the liquid scintillation analyzer. The %CV are all less than 2.51.

Finally, the most difficult triple label sample ratio to analyze is at a $^3\text{H}/^{14}\text{C}/^{32}\text{P}$ ratio of 1:1:10. The reason that this is the most difficult is that both ^{14}C and large amounts of ^{32}P must be compensated for within the ^3H region. The results for this ratio are in Table 11. The statistical analysis of all of the quenched samples indicates extremely good % recoveries (99.45 [^3H], 101.45 [^{14}C], and 100.83 [^{32}P]). Because of the high ratio of ^{32}P in the sample, and consequently the high crossover of ^{32}P into both ^3H and ^{14}C , the %CV for these isotopes increased to 3.22 for ^3H , and 5.82 for ^{14}C . These numbers are extremely accurate considering that % efficiencies and three CPM values are used to calculate the DPM of each of the three radionuclides. Thus, the tSIE is an accurate method of determining the DPM in a triple label sample at various quench levels and with various isotope ratios.

SUMMARY

This paper definitely demonstrates that the use of the quenched indicating parameter, tSIE, is a rapid and accurate method of determining DPM of single, dual, and triple labeled samples. The DPM obtained with this technique is independent of sample volume, extent of quenching, wall effect, vial size, vial type, cocktail density, radionuclides number being analyzed (maximum = 3), and quenching agent type.

Accurate DPM can be obtained for dual label samples at 100:1 to 1:50 ratios and at various quenched levels. The DPM for triple label samples can be determined accurately, and with a small % coefficient of variation for various ratios of radionuclides. In conclusion, the use of tSIE as a quench indicating parameter results in accurate DPM values independent of most interferences found in liquid scintillation counting.