LIQUID SCINTILLATION ANALYSIS
The Liquid Scintillation Analysis, Science and Technology reprint is used to describe the science of liquid scintillation and does not represent the latest instrument, reagents and cocktails. Customers should use this handbook for the basic principles of understanding Liquid Scintillation Analysis.
Acknowledgement

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Michael J. Kessler, Ph.D.
Editor
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THEORY OF LIQUID SCINTILLATION COUNTING
LIQUID SCINTILLATION ANALYSIS

Theory of Liquid Scintillation Counting

Introduction

Liquid Scintillation Counting (LSC) today is the most sensitive and widely used technique for the detection and quantification of radioactivity. This measurement technique is applicable to all forms of nuclear decay emissions (alpha and beta particle, electron capture, and gamma ray emitting radionuclides). Liquid scintillation counting is an analytical technique which measures activity of radionuclides from the rate of light photons emitted by a liquid sample.

The technique of liquid scintillation counting is based on a few well-known physical phenomena A brief explanation/review of these physical processes is given in this section in order to provide an understanding and appreciation of the power and limitations of the technique in the various studies involving the use of radioactive tracers.

![Decay of a Beta Radionuclide](Figure 1-1. Decay of a Beta Radionuclide.

The Beta Particle and Photon Production

Perhaps a brief explanation of the structure of matter and radioactivity will provide an understanding of the first of the physical phenomena referred to above. All matter is made up of a few building "blocks" of which the arrangement determines the element and its chemical behavior. In its simplest form an element consists of a nucleus, where the major mass resides, and an orbital electron cloud system. This is similar to the solar system where the planets gyrate around the sun, the sun in this analogy is the nucleus and the planets are the electron cloud. An element contains in its nucleus the basic building blocks of protons and neutrons, the orbital particles in the cloud are electrons. Protons are positively charged particles, neutrons have approximately the same mass but are electrically neutral and the electrons are negatively charged and have a mass approximately two thousandths that of the neutrons and protons. Generally, the elements at the lower end of the atomic table contain an equal number of protons and neutrons in the nucleus. As the atomic weight increases in the atomic table, the number of neutrons exceeds the number of protons. The number of electrons in the orbital system must equal the number of neutrons in the nucleus making the atom electrically neutral. The number of electrons also determine the chemical behavior of the element. When an imbalance exists between the number of protons and neutrons in the nucleus, the element becomes unstable and a rearrangement takes place. This represents a decay or a radioactive event and energy is released in the form of radiation (Figure 1-1.)
Nuclear decay is a highly complex process involving the laws of relativity, so this description is obviously an oversimplification of radioactivity. The object of this discussion is to provide a basis of understanding of the liquid scintillation process, the main aspect of interest is the radiation accompanying a decay event, which will now be discussed.

Consider the case of tritium. This is a radioactive isotope of hydrogen, chemically the same as hydrogen but with an excess of neutrons in its nucleus. The decay results in the emission of two particles, an electron, given the name beta particle because it originates in the nucleus, and a neutrino (Figure 1-2).

These two particles are released simultaneously and carry the decay energy from the nucleus. This energy is characteristic of the radionuclide, in the case of tritium the energy is 18.6 keV. Kiloelectron volt (keV) is the unit of energy used in nuclear physics. The neutrino is of no interest in liquid scintillation counting apart from the fact that it carries away some of the decay energy. The energy of 18.6 keV is shared randomly between the beta particle and the neutrino. Thus, theoretically, the β-particle can possess any energy between 0 and 18.6 keV (Figure 1-3).
The energy is kinetic, that is energy of motion. Like any other mass in motion, the beta particle dissipates its energy by collisions in the medium in which it is released. In a liquid which is relatively dense, the tritium beta particle will travel only short distances before all of its kinetic energy is dissipated. The energy is absorbed by the medium in three forms, heat, ionization, and excitation of the molecules of the solution. It is excitation of the solution molecules which is of interest in the liquid scintillation technique. To assure efficient transfer of energy between the beta particle and the solution, the solution is a solvent for the sample material. Excited solvent molecules are not readily recognized, therefore the scintillation solution (the cocktail) consists of a mixture of a solvent and a solute. The solute is a scintillator. The excited solvent molecules can transfer energy to one another and also to the solute. An excited solvent molecule which passes on its energy to a solute molecule, disturbs the orbital electron cloud of the solute, raising it to a state of excitation. As the excited orbital electrons of the solute molecule return to the ground state a radiation results, in this case the radiation is a photon of light. Thus, a single beta particle will manifest its presence by colliding with solvent molecules and the excitation of many scintillator molecules (Figure 1-4).

Figure 1-3. Energy Distribution (Spectrum) of ³H.

Figure 1-4. Illustration of the Collision Process.
The total number of photons from the excited scintillator molecules constitutes the scintillation. To a first approximation this is a linear conversion of energy into photons and, therefore, the intensity of light in the scintillation is proportional to the initial energy of the beta particle. Referring again to the case of tritium, the scintillation intensity will range from 0 to the maximum possible for a particle of 18.6 keV. Figure 1-5 shows the distribution of photons per energy level from a quantity of tritiated material. The scintillation solution has converted the kinetic energy of the beta particle into light energy.

![Figure 1-5. Photon Spectrum of Tritium.](image)

**Photon Detection**

Placing a glass or plastic vial containing the radiolabeled analyte with the scintillation solution into a dark enclosure allows the photon intensity to be observed. The relative photon intensity is dependent on many factors, including the type of liquid scintillation cocktail, radiolabeled analyte, other solutes dissolved along with the analyte, sample vial material, quantity of radiolabeled analyte, maximum energy of emission, and other chemically related phenomena affecting radiochemical distribution. The nature of the nuclear and chemical interactions produce emitted photons in the ultraviolet region of the electromagnetic energy spectrum, typically with low intensity.

In order to efficiently detect the emitted photon, a photosensitive device is required to amplify the light, and transduce the detected photons into an easily workable form. This device is known as a photomultiplier tube (PMT) or multiplier phototube (MPT) and was first developed by Gworykin et al in 1936. In today’s scintillation counters, two photomultiplier tubes collect the total light produced within the scintillation vial which either falls directly onto the two photocathodes, or is reflected onto each photocathode by a centrally mounted optimizing reflector mounted between the PMTs.
The inside face of the PMT is uniformly coated with a photosensitive material which has the property of converting the absorbed photon (light) energy into electrical energy by the release of photoelectrons which are negatively charged particles. The electrons are attracted to an electrode within the tube by the positive potential of the electrode and the result is the production of more electrons. These secondary electrons are attracted to the second electrode where a similar process takes place (Figure 1-6.)

![Figure 1-6. Principles of Operation of a Photomultiplier Tube.](image)

The tube consists of a number of electrodes (usually 11-13). The passage of the secondary electrons with an amplification at each electrode stage produces a cascade of electrons at the final electrode. This creates an electrical pulse, representative of the photons responsible for the pulse. The PMT is a linear device so the amplitude of the pulse is directly proportional to the number of photons detected by the photocathode. Hence, the scintillation is detected and converted into an electrical pulse. Registering each pulse during the time of the measurement provides an indication of the number of scintillation events occurring in that time (Figure 1-7.)

![Figure 1-7. Simplified Liquid Scintillation System.](image)
If all that is required of a liquid scintillation counter is the measurement of light produced by the interaction of the nuclear decay event with the scintillation solution, then additional instrument components would not be necessary. However, few scintillation counters have actually been developed based on this principle. Inherent to this measurement technique are some fundamental limitations which have been addressed over the years as liquid scintillation counting technology has evolved. A basic limitation of the technique is associated with photomultiplier tube technology in that a PMT will produce small pulses even when no light is present. The device is so sensitive that the application of voltage between the electrodes is sufficient to produce small "noise pulses" which would be responsible for a background to the observed sample measurements.

Thermal changes in the surroundings is another factor responsible in producing noise pulses with respect to PMT operation. A known limitation of photomultiplier tubes is the thermionic effect produced which is based simply on the fact that the migration of electrons is dependent on temperature. The result is that the noise pulses can often be extraneous and resemble electronic pulses resulting from photons representing nuclear decay events. The noise pulses must be recognized to obtain an accurate representation of nuclear emissions. The other limitation is the difference in amplitude of the PMT pulse which will depend on the location of the event within the vial. An event producing, say, 100 photons will produce a pulse amplitude inversely proportional to the distance between the event and the PMT. The amplitude of the pulse is important, particularly if more than one radionuclide is contained within the vial.

**LSC Instrument Technology**

Until the early 1950's, the radiolabeled sample was not in direct contact with the organic based scintillation cocktail. Aqueous accepting scintillation solutions had not yet been developed. The sample was placed external to the scintillation cocktail, and hence the term "external liquid scintillation counting" was used.

Liquid scintillation counting techniques as known today had its beginnings in 1953 when Hayes et al first introduced radiolabeled biologic material into the scintillation solution. This technique quickly became known as "internal liquid scintillation counting." The technique of internal liquid scintillation counting, known today as simply "liquid scintillation counting" or "LSC," offers many unique measurement advantages such as homogeneous sample geometry, no adsorption effects, and maximum radionuclide counting efficiency. These and other advanced concepts in scintillation technology are discussed in later sections of this manual.

During the same year, 1953, the first commercial liquid scintillation counter was manufactured by Packard Instrument Company. Scintillation counters until this time were "homemade" and often designed to satisfy a specific measurement task with little or no flexibility to accommodate changes in the desired measurement conditions. The performance of these early scintillation counters provided a firm foundation in the art of the technology, but suffered from some serious fundamental limitations.

In 1953 with the introduction of the first commercial liquid scintillation counter, the Packard Tri-Carb® Model 314 EX, these basic limitations were resolved through a novel instrument design. The first Tri-Carb counter was a revolutionary device which incorporated two unique features: coincidence pulse detection (Kallman and Accardo), and the use of two diametrically opposed photomultiplier tubes. Coincidence pulse detection (Figure 1-8) is an electronic logic circuit which compares the signal output from both photomultiplier tubes. So successful was this design that for many years it was the only liquid scintillation counter in widespread use.
Nuclear decay events produce approximately 10 photons per keV of energy. The energy is dissipated in a period of time of the order of $5 \times 10^{-9}$ second (5 nsec). Thus, a beta decay yielding a multiplicity of photons will stimulate both PMTs at the same instant in time. The signal from each PMT is fed into a circuit which produces an output only if the two signals occur together, that is within the resolving time of the circuit, approximately $20 \times 10^{-9}$ second (20 nsec). This is termed a coincidence circuit and the output is a coincidence pulse. The electrical noise from the PMTs is produced randomly in time, but occurs at a sufficiently low rate to be excluded by the coincidence circuit (Figure 1-8).

In 1963 Packard was the first to introduce a technique which addressed another limitation, that of differing effects due to the geometrical location of the emitted photon within the scintillation vial in reference to each PMT. A number of different counters were designed in which the pulses from both PMTs were summed. By summing the amplitude of the pulses from each PMT, an output is obtained which is proportional to the total intensity of the scintillation. As each PMT produces a signal from the same event, the geometrical location of the event becomes less important (Figure 1-9). Using the technique of pulse summation improved the instrument counting performance of these Packard scintillation counters. The tritium efficiency was improved from approximately 30% to 45% and instrumental background was reduced to approximately 30 cpm from 50 cpm in a tritium counting window.
Today, most modern commercial liquid scintillation counters employ the methods of pulse coincidence detection and summation counting. The method of detecting the nuclear event and photon detection have remained basically unchanged over the years. It is from this point forward that the various scintillation counting systems may differ.

In principle, the summation output, provided that the signal is accompanied by a signal from the coincidence circuit, can be input into either discrete scalers to simply record the events (Figure 1-10) or these pulses can be qualified for energy and pulse height and input into an energy spectrum analyzer (Figure 1-11). Using discrete scalers to store the observed scintillation events has been utilized since the introduction of the first commercial Tri-Carb counters. Since then there have been no fundamental changes incorporated into the basic counting design. A scintillation counter utilizing linear amplification with three different counting windows and employing discrete channels to record observed counts requires a separate amplifier, pulse height analyzer, and scaler for each channel. An alternate pulse processing technique was introduced first in 1964 by Ansitron and again in 1965 by Beckman Instruments which was based on logarithmic amplification methods. Using a logarithmic amplifier eliminated the need for individual channel amplifiers.

**Figure 1-10. Simplified Counting System.**

**Figure 1-11. Energy Spectrum Analyzer.**
One of the most significant advancements in the technique of liquid scintillation counting was the introduction of pulse height energy spectrum analysis first presented on a Packard Tri-Carb 460C series liquid scintillation spectrometer. This technique, pioneered by Packard in 1980, provided the capability of windowless counting. The basic design concept was based upon collecting a pulse height energy distribution spectrum at a fixed gain over the entire energy range from 0 keV to 2000 keV. This technique allowed the user to interrogate any portion of the energy spectrum concurrent with sample counting or until the beginning of the next sample count. Dynamic pulse pattern recognition techniques are used to build the energy distribution spectrum with 12 second update intervals. Spectrum analysis of the detected events from a sample is made possible by the development of microprocessor-based electronics. This provides a most sophisticated and precise method of analyzing the events occurring within the vial. The method extracts more information from these events than the earlier techniques of pulse height analysis. Spectral analysis forms the basis of design criteria for Packard’s current liquid scintillation spectrometers.

It was shown earlier that to a first approximation the process of converting the beta particle energy to photons is linear. A particle of 100 keV, in dissipating its energy, produces approximately 1000 photons (i.e. ~10 photons/keV). The detection of these photons by the two photomultipliers is linear, thus the output from the summation circuit produces a pulse the amplitude of which represents 100 keV of energy released in the scintillation solution. Since the process is linear, a beta particle with an energy of 150 keV will cause a summation circuit output 50% higher in amplitude than the 100 keV particle. Typically a beta particle of this energy will take a few nanoseconds to dissipate all of its energy. This results in an analog pulse rising to its maximum amplitude and falling to zero. The amplitude of the analog pulse is converted into a digital value, providing the coincidence requirement is satisfied. The conversion is achieved in a high speed analog to digital converter (ADC) (Figure 1-11), and the digital value, which represents the beta particle energy, is the address of a memory slot of a Spectralyzer™ spectrum analyzer. The Spectralyzer spectrum analyzer consists of many storage slots or channels covering an energy range from 0 keV to 2000 keV.

![Figure 1-12. Accumulated Sample Spectrum.](image-url)
The conversion by the ADC is linear; therefore, during the measurement of a sample, the Spectralyzer spectrum analyzer will accumulate counts representing the complete energy spectrum of the radionuclide. It will be appreciated that the longer the counting period, or the more active the sample, the greater the number of events stored and the more precise the stored spectrum. The Spectralyzer spectrum analyzer has channels, each representing 0.5 keV increments of energy. In the example of analyzing all the events from a tritium sample, counts will be stored in the lower 38 channels of the storage system, that is 38 channels representing 18.6 keV. Thus, a beta particle with an energy of 6.3 keV will be recorded in the 6.0 to 6.5 keV channel of the Spectralyzer spectrum analyzer. At the end of the measurement every detected event from the sample is stored between 0 and 2000 keV (Figure 1-12), including those events due to background and other phenomena which can affect the counting of the sample.

Because the pulse amplification of a Tri-Carb liquid scintillation system is linear with respect to energy, it becomes logical and easy to select the conditions for measuring any beta emitting radionuclide. The Spectralyzer spectrum analyzer is calibrated in approximate keV and the user can choose any region between 0 and 2000 keV in which to measure a sample. For example the Region of Interest for $^3$H is from 0 to 18.6 keV ($^3H\beta_{\text{MAX}} - 18.6 \text{ keV}$) and for $^{14}$C it is from 0 to 156 keV ($^{14}C\beta_{\text{MAX}} - 156 \text{ keV}$). Preset Region limits in the Spectralyzer spectrum analyzer are assigned values for the more commonly used radionuclides, although user-defined counting regions are easily selected.

**Counting Considerations**

There are two aspects of these detecting system designs which affect the sensitivity of detecting radioactive events.

1. The coincidence requirement that each PMT produce a response sets a limit of detection. The beta particle must have sufficient energy to produce at least two photons and one must interact with each PMT. Inevitably, because the photons are radiated in all directions, some will be lost despite the reflector. Thus, the probability of a photon entering each PMT decreases with decreasing beta particle energy. Below a few keV of energy the yield of photons, under ideal conditions, is 7-8 photons per keV.

2. The photocathode of a PMT is not 100% efficient. The conversion efficiency from a photon to a photoelectron (the quantum efficiency) is only about 30%. Hence, when dealing with $^3$H, where the average beta energy is less than 6 keV, this radionuclide cannot be detected at 100% efficiency as many events do not produce a sufficient number of photons.

The net effect of these two factors creates a threshold of detection below which an event is unlikely to be recorded. This level is referred to as the coincidence threshold, and in energy terms, occurs below 1 keV.
Another phenomenon which influences all counting is the inevitable background produced by environmental radiation. Several sources of radiation can affect the sensitive scintillation solution and the detection process.

(a) Cosmic radiation which is variable and influenced by sun spot activity and barometric pressure.
(b) Fallout from nuclear tests which might be contained in the air.
(c) The building materials of the laboratory which contain activity.
(d) Natural activity in the glass sample vial and the walls of the photomultiplier tubes.
(e) Stray radiation from sources of activity in the laboratory, or apparatus producing radiation such as x-ray sets and linear accelerators.

The sample in the counting chamber and the PMTs are surrounded by lead, typically about 5 cm in all directions. This lowers the background to reasonable proportions for all but very low activity measurements. Background counts exist in all measurements and cover the range of energies associated with the detection of the radionuclides in the life science applications.

**Recent technological advances to improve counting performance**

Although the basic theory of liquid scintillation counting has remained unchanged, new techniques are continually being developed for this technology. For example, the Packard Tri-Carb 2000CA liquid scintillation analyzer, introduced in 1985, contains many innovative features which take advantage of the computational power of the computer and advanced microelectronics. These new features include:

- **Three-Dimensional Spectrum Analysis** for the reduction of background. With this feature, carbon dating becomes possible with liquid scintillation counting (Chapter 2).
- **Vector Qualitative Analysis** for the detection of heterogeneous samples. Heterogeneous samples present serious problems in liquid scintillation counting (Chapter 2).
- **A new approach to counting efficiency determination** based on a transformed external standard spectrum, tSIE. This method overcomes the shortcomings of other external standard methods (Chapter 3).
- **Full Spectrum DPM calculations** have presented a new, unique, and windowless method of looking at dual label samples (Chapter 4).
- **The Efficiency Tracing Technique** has introduced a method of determining DPM without quench correction or efficiency curves (Chapter 3).

All of these features as well as the fundamentals of liquid scintillation counting are described in detail in the following chapters:
References

Applied Spectrum Analysis

Qualitative and quantitative analysis of the beta energy spectrum to enhance counting results.

Introduction

The objective of counting radioactivity, and beta radiation in particular, is measuring the amount of activity associated with individual radionuclides.

In liquid scintillation counting, the basis for either qualitative or quantitative analysis is the behavior of the appropriate beta spectrum under various counting conditions. The traditional method is based on pulse height analysis of the spectrum and the use of selected windows or counting channels. In more recent times, another approach based on full spectrum analysis has emerged. This newer method essentially involves windowless counting and has proven to be an improvement over the traditional method used in earlier Packard counters (as well as those of other manufacturers). The following section describes the basis for this newer approach and its advantages.

The term "qualification of radioactivity" as used in this chapter, is defined as the identification of a radionuclide based on pattern recognition of its beta energy spectrum.

The Beta Spectrum

Spectral Shape

The beta particles emitted by a nucleus produce a continuous energy spectrum from zero up to a maximum value. This maximum energy is typical for the measured radionuclide. The energy used for calculations of absorbed energy is the average energy which is a function of the maximum energy and the atomic number of the nucleus. Again, this is an energy characteristic of a particular radionuclide.

Mantel has calculated the theoretical beta energy spectra and average energy for all nuclides of interest in medicine and biology (1). Appendix A shows all these spectra (59 radionuclides) with a listing of the pertinent and average energies for each radio nuclide.

Typical theoretical spectra for some commonly used pure beta emitters are shown in Figure 2-1.
The pulse height distribution spectrum obtained with a liquid scintillation counter is shown in Figure 2-2. The pulse height is calibrated in energy units (keV).

![Pulse Height Distribution](image)

Figure 2-2. Pulse Height Distribution.

This pulse height distribution deviates from the theoretical beta spectrum due to:

1. For low energies, the pulse height distribution does not have its characteristic form due to the coincidence condition, that is, the total (theoretical) spectrum minus the single photon pulses. The average energy required to produce a photoelectron (to register a count) in the coincidence spectrum is 0.7 - 1 keV (2).

2. Due to the quantum threshold of the photocathode and pulse summation, the pulse height scale is not linearly proportional to the electron energy at the lower energy range:
   
   **Quantum Threshold** - Assuming a cathode quantum efficiency of 30%, the pulse height from a large 1000 photon scintillation is equal to 300 photoelectrons. However, the pulse height from a single photon, when detected, is equal to the pulse height of a single photoelectron. The probability that it will be detected is only 30%, but when detected it will be registered as one photoelectron.

   As a conclusion, with a relative photon input of 1000 to 1, the relative pulse height output is only 300/1. This nonlinearity in pulse height distribution is confined to scintillations up to 3 keV.

   **Pulse Summation** - The pulse height distribution for low energies is further altered by the summation concept (3). The ability to register a weak scintillation at one phototube depends on the detection probability and pulse height of the same event at the second phototube. This distorts the pulse height distribution linearity further (up to 3.5-4.0 keV).
Conclusion: The beta spectrum as registered by modern liquid scintillation counters looks nothing like the theoretical spectrum at the low-energy end. Moreover, the shape is instrument-dependent. Coincidence threshold, photomultiplier quantum efficiency, summation circuit, and detector chamber optics will affect the spectral shape (Figure 2-3).

![Linear Versus Logarithmic Energy Scale](image)

**Figure 2-3.** Theoretical vs. Actual Spectrum Shape.

**Linear Versus Logarithmic Energy Scale**

**Disadvantages of the logarithmic scale:**

1) The origin of the energy scale is not 0 keV. Most commercial instruments with logarithmic amplification must use channel numbers instead of keV units to mark the energy level.

2) More than half of the tritium spectrum is heavily distorted when logarithmic scaling is used. Emphasis is on the inaccurate and instrument dependent part of the spectrum rather than on the higher pulse height distribution point, which is theoretically and experimentally correct. On a linear scale, only one-sixth of the tritium spectrum is slightly distorted.

3) An accurate determination of the typical spectral energies is difficult as the scale becomes more and more compressed at higher energy levels.

4) In some commercial instruments, logarithmic response is obtained by logarithmic photomultiplier amplification. This results in a logarithmic response before summation. The summed pulse is: \( \log A + \log B = \log AB \). This means that the pulse output heights from both photomultipliers are multiplied rather than summed. While summation corrects for the unequal distribution of decay energy between the two photomultipliers, multiplication will cause a nonstatistical distribution of pulses along the energy scale which results in a higher contribution of \(^{14}\text{C}\) in the \(^{3}\text{H}\) channel. Peak resolution and bad isotope separation are characteristic.

5) Logarithmic gain amplifiers are difficult to stabilize (no linear feedback for gain stabilization is possible) and the window selection must be made based on channel numbers rather than on energy levels. This usually requires spectrum search abilities or live spectrum displays to correctly set the windows.

6) Calculating the average pulse height of a logarithmic spectrum gives a figure which is not related to the characteristic average energy of the radionuclide. It has no physical meaning.
Disadvantage of the linear scale:
1) An amplifier with a high dynamic range and a multichannel analyzer with a great number of channels is required to store the spectrum with sufficient high resolution. With modern electronics, this is more of a cost disadvantage than a technological disadvantage. It is certainly less expensive to store the complete energy range in 256 or 1024 channels with logarithmic amplification than in 4096 channels with linear amplification.

Spectrum Recognition
Recognition of a beta energy spectrum (Figure 2-4) is usually based on one of the following characteristics of the pulse height distribution:

- The maximum beta particle energy (E_{\text{max}})
- The pulse height at peak energy (5) (E_p)
- The inflection point of the upper range (4) (H-number concept)
- The average pulse height (1) or the spectral index (6) (E_{av}, SIS)

1) The maximum beta particle energy: E_{\text{max}}

The maximum energy is determined as the end point of the pulse height distribution. Measuring and calculation accuracy is not very precise because one is looking at the end of the spectrum where the number of counted particles is low per energy level.

Regression techniques can be applied to improve estimation of the end point. The end point is calculated as the intercept of the curve that describes the theoretical equation of the pulse height distribution (e.g. the Birks equation) (7,8). However, these equations are only valid when pure beta emitters are studied. They are not applicable to composite beta emitter spectra.
2) Pulse height at peak intensity: $E_p$

The energy at the maximum decay rate is another typical point of the beta ray spectrum. Again, precise determination is very difficult. The peaks are broad and detection must be based on calculating the maximum of a function obtained by peak regression (first derivative equal to zero). Although more counts are accumulated in this region of the spectrum, precise location is impossible due to the broad shape.

3) The inflection point of the upper edge: $H#$

The position of the inflection point can be used to characterize the radionuclide. This method also looks at a particular region of the spectrum and calculations are based on the second derivative of the curve fitted through the points obtained by counting in narrow channels located in this region.

The precision looks better when calculating the inflection point on a logarithmic pulse height distribution (refer to Figure 2.5). Calculations are certainly easier because of the decreased spectrum resolution on a logarithmic scale.

![Figure 2-5. Inflection Points.](image)

Inflection point calculations are usually very difficult and virtually unusable when noncharacteristic pulse height distributions or composite beta spectra are encountered (refer to Figure 2.6).

![Figure 2-6. Composite Beta Energy Spectra.](image)
See composite spectra for $^{47}\text{Ca}$ and $^{47}\text{Sc}$. Both have more than one beta group present.

4) The Average Energy ($E_{av}$) and the Spectral Index (SIS)

Mantel has calculated the average energy of 59 isotopes of interest in medicine and biology (1). Each radionuclide can be uniquely identified by its average energy. (Refer to Appendix A.)

The average kinetic energy $E_{av}$ is calculated from the relation:

$$E_{av} = \frac{\int_0^{E_{max}} E \cdot N(E) \, dE}{\int_0^{E_{max}} N(E) \, dE}$$

$E_{av}$ = average kinetic energy
$N(E)$ = number of particles of energy $E$
$E$ = energy of a particle

The calculated value is the first moment of the beta energy spectrum, also called the center of gravity.

With the application of a high resolution linear Spectralyzer spectrum analyzer, Packard is able to apply this formula to the calculation of SIS (Spectral Index of the Sample).

$$\text{SIS} = K \frac{\sum_{x=0}^{u} x \cdot n(x)}{\sum_{x=0}^{u} n(x)}$$

$n(x) =$ number of counts with pulse heights between $X$ and $X + \Delta X $
$x =$ pulse height (proportional to beta particle energy).
SIS = Spectral Index of the sample.
$u =$ Upper level of pulse height distribution (end point)

This is the normalized (normalization factor $K$) average energy calculation, based on the definite integral using discrete energy channels with width $\Delta X$.

Based on the unique relationship between SIS and $E_{av}$, one is able to qualify the isotope spectrum. This index is the most accurate of the four characteristic parameters of a spectrum, since it uses the entire spectrum and thus the maximum counting accuracy to calculate the index.

A great amount of technological instrument requirements must be fulfilled in order to perform this calculation:

1) Linear amplification with a wide dynamic range (the average energy of a logarithmic spectrum has never been published to qualify a radionuclide spectrum).

2) High resolution spectrum analysis-Based on Mantel’s equation, the average pulse height can be accurately calculated only when the energy resolution is very high (theoretically infinite). Therefore, Packard uses the highest resolution MCA that is available in commercial liquid scintillation counters (more than 4000 channels).
Composite Beta Spectra

Multiple Beta Group Decay

Many beta emitters have more than one beta group present. Of the 59 spectra, theoretically defined by Mantel, 45 are composite spectra. A typical multiple beta group spectrum is shown in Figure 2-7 (linear and logarithmic scale).

![Composite Beta Spectrum](image)

All of these composite beta ray spectra can be uniquely identified by their average energy and thus also by their SIS value, using a linear energy scale.

As the composite spectrum derives its characteristic form from the upper end of the spectrum, it is very difficult to distinguish most of these spectra from pure beta emitter spectra on a logarithmic scale (refer to Figure 2-7).

Dual Label Spectra

Since many experiments are carried out with dual-labeled samples, the qualification of a dual-labeled spectrum (Figure 2-8) is very important for proper nuclide separation. A typical dual-labeled spectrum (¹H and ¹⁴C) has the following characteristic shape:

![Dual-Labeled Spectrum](image)
The $^3$H spectrum is superimposed on the $^{14}$C spectrum. The resulting Spectral Index of the Sample (SIS) falls somewhere between the two single-labeled SIS values. In fact, the position relative to the single-labeled values is a direct measure of the proportion of each radionuclide in the mixture. If $N_H(x)$ and $N_L(x)$ are the distributions due to the high energy and low energy components of the mixture, then the total SIS($T$) according to equation 1 can be written as:

$$SIS_T = K \left[ \frac{\sum x * N_H(x) + \sum x * N_L(x)}{\sum N_H(x) + \sum N_L(x)} \right]$$

The sum in the denominator represents the counts due to the high and low energy components, respectively. This is equal to the total counts in the composite spectrum.

In this case the SIS cannot be used to identify the individual radionuclide, but its value is uniquely related to the count fraction of the low and the high energy isotope. A high ratio $^3$H/$^{14}$C will yield a low total SIS value, while a low ratio will result in a high total SIS value.

Again, the SIS value of the total spectrum is a unique parameter to qualify and quantify the two radionuclides of dual-labeled samples. No other characteristic parameter of the spectrum yields this information. This qualification of the two fractions is very important ensuring accurate dual label DPM results. (Refer to Chapter 4, THEORY OF DUAL LABEL MEASUREMENTS.)

**Alpha Particle Spectrum Analysis**

The use of liquid scintillation counting for alpha emitters is attractive because it offers counting efficiency of 100% and simplicity of sample preparation.

Alpha radionuclides emit high energy particles in the range of 4 MeV to 6 MeV. A characteristic property of alpha particle interaction with liquid scintillation media is a low scintillation or photon yield as compared to beta or even gamma emitters. (The light yield is approximately a factor of 10 lower.) Almost all of the kinetic energy associated with an alpha particle emission is given up to the scintillation media in a relatively short distance. The relative scintillation yield resulting from this interaction depends on the specific ionization. The higher the specific ionization energy, the lower the relative photon yield. This effect limits alpha radionuclide energy resolution. Though alpha particles are mono-energetic, the pulse height distribution peaks obtained with liquid scintillation counters are broad. A peak width of 1 MeV is typical for the resolution of an alpha ray spectrum. This does not allow effective separation of alpha emitters of different energies. (Refer to Figure 2-9.)

By optimizing experimental conditions [D.L. Horrocks (9)], improved resolution can be obtained. Further experimenting with single PMT counting and by decreasing the sample/scintillator cross-sectional area (e.g. by using 6mm diameter tubes), near baseline resolution can be obtained from these radionuclides with a separation of only 650 keV (refer to Figures 2-10 and 2-11).
It is obvious from these experiments that spectrum analysis is a valuable tool to count alpha radionuclides. Pictures were taken from the accumulated spectra (spectraview). Spectrum analysis allows the experimenter to quickly evaluate the procedure and to qualify the obtained data for activity determination. Optimal selection of the regions of interest are now possible using linear spectrum analysis.

**Gamma Ray Spectrum Analysis**

Gamma ray counting plays an important role in liquid scintillation counting for two distinct reasons:

1. The biomedical interest in radioimmunoassays and steroid receptor assays. In a few procedures, the substance of interest is labeled with $^3$H, but in most cases iodination with high-specific-activity $^{125}$I is carried out.

2. Gamma ray sources are commonly used in commercial scintillation counters as external standardization sources (used in quench correction techniques).
Because of their low density (1g/cm$^3$) and low atomic number of their constituent elements (H = 1, C = 6, N = 7, O = 8) organic scintillators have much lower gamma ray absorption coefficients than inorganic scintillators, such as NaI (Tl). The photoelectric absorption is small when the energy is greater than 30 keV and the Compton scattering becomes the main absorption process up to E = 2 MeV. Above 2 MeV, pair production becomes appreciable.

In function of their energy, the following phenomena contribute to the observed pulse height spectrum in LSC:

- $E \leq 30$ keV: conversion electrons (e.g. $^{60}$Co, $^{61}$Cr) and Auger electrons (e.g. $^{125}$I).

- $30$ keV $\leq E \leq 2$ MeV: Compton scattering produces electrons with energies from zero to some maximum energy, given by the equation:

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

- $E > 2$ MeV: pair production

A typical gamma emitting radionuclide that is often used in LSC for RIA and steroid receptor determinations is $^{125}$I. The counting efficiency can be as high as 76% in a typical emulsifier type liquid scintillation cocktail. The composite spectrum of the 27.5 keV x-rays and the 35.5 keV y-rays is shown in Figure 2-12.

Spectrum analysis can be successfully applied to qualify the isotope (using SIS) as well as the counting efficiency. The two peaks are produced by conversion electrons and Auger electrons. Their ratio, and thus the SIS value, is a measure for the counting efficiency (with increasing quench level, the spectrum will not only shift to the left but the two energy peaks will compress and appear as a single peak).
Compton Spectrum Analysis

For quench compensation, the popular technique of external standardization uses a gamma emitting standard source to irradiate the scintillation solution. Usually, gamma emitters with energies between 300 keV and 2 MeV are used for this purpose. Due to these high energies, photoelectric absorption is negligible and Compton scattering becomes the major gamma absorption process. The Compton electrons cover a continuous spectrum from 0 keV up to $E_{\text{max}}$ equal to:

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

The following external standard sources are commonly used: (Figures 2-13 to 2-15):

- $^{133}\text{Ba}$ ($E\gamma = 356 \text{ keV}, E_{\text{max}} = 207 \text{ keV}$): Kontron, TM-Analytic (ex-Nuclear Chicago), Packard
- $^{187}\text{Cs}$ ($E\gamma = 662 \text{ keV}, E_{\text{max}} = 478 \text{ keV}$): Beckman
- $^{152}\text{Eu}$ ($E\gamma = -1.408 \text{ MeV}, E_{\text{max}} = 1.192 \text{ MeV}$): Philips
- $^{226}\text{Ra}$ ($E\gamma = 2.43 \text{ MeV}, E_{\text{max}} = 2.20 \text{ MeV}$): LKB, Philips

![Figure 2-13. $^{133}\text{Ba}$ Compton Spectrum.](image)
Figure 2-14. $^{137}$Cs Compton Spectrum.

Figure 2-15. $^{226}$Ra Compton Spectrum.
Characteristics of the Compton Spectrum

The Compton spectrum generated by the external standard (a gamma source) can be qualified as any other beta spectrum, as previously described, by one of the following methods:

1) The maximum endpoint energy of the spectrum
2) The pulse height at peak energy
3) The inflection point of the upper edge (Compton edge) or "H-number"
4) The average pulse height or the spectral index

In addition

5) The transformed spectral index, a Packard innovation, can be used. This technique is described in detail in the next chapter, "Quenching and Efficiency."

The following brief remarks can be made with respect to the utility of the various qualification methods applied to the Compton spectrum.

(1) **The maximum endpoint energy:** looking at the linear spectra, it is obvious that it is difficult to accurately determine the end point. There is no sharp intercept of the Compton spectrum with the x-axis, so that an accurate and reproducible determination is questionable. A logarithmic transform creates the visual illusion of a well-defined end point because of the compressed scale on the high end, i.e. on a scale of 1 to 10, values between 5 and 10 are plotted on the upper 30% of the x-axis, whereas values between 1 and 5 are plotted on 70% of the x-axis.

(2) **Pulse height at peak energy:** no well-defined peaks can be identified on the linear spectra. Again, logarithmic transformation compresses the higher energy portion of the spectra. Attempting to define the position of these peaks may result in the same errors as determining the peak on a linear scale.

(3) **H-number or inflection point:** all three spectra show inflection points at the upper end. The barium and the cesium ends of the spectrum have a steeper slope than the radium spectrum. Looking at the linear $^{137}$Cs spectrum, which best lends itself to this type of spectrum characterization, it is not clear where this inflection point is actually located. It appears to be between 880 and 930 which represents on a maximum scale of 1000, an error of 5%.

More accumulated counts may make this portion of the spectrum better defined. Again, by compressing the upper end of the energy scale, the logarithmic transform creates the visual illusion of a sharp, well-defined Compton edge. This technique is used by Beckman to identify the position of the spectrum.
(4) Spectral Index or Average Energy: the calculations as used by Mantel to uniquely identify many beta spectra can also be applied on Compton spectra. The precision of this calculation is directly related to the integrated counts of the spectrum. From the three spectra, accumulated a similar length of time and with sources of the same activity, it is obvious that $^{226}$Ra with its broad energy distribution, will yield the best accuracy when calculating the average energy.

$$E_{av} = \frac{\int_{E_{max}}^{E_{min}} E \cdot N(E) \, dE}{\int_{E_{min}}^{E_{max}} N(E) \, dE}$$

This method to identify the Compton spectrum is used by TM Analytic and Packard.

- Analytic uses the barium spectrum and calculates the ESP (External Standard Pulse)

$$ESP = \frac{k}{E_{av}}$$

- In older systems Packard used the radium spectrum and calculated the SIE (Spectral Index External Standard) (10).

$$SIE = K \cdot \frac{\sum_{x=L}^{u} x \cdot N(x)}{\sum_{x=L}^{u} N(x)}$$

$L$ = lower level used to cut-off lower energy portion of spectrum that is subject to counting interference.

(5) The transformed Spectral Index of External Standard ($tSIE$): In Packard's most recent systems, a novel technique is used to evaluate quenching by means of an external standard source.

Barium is used to activate the scintillator and a spectral transform technique is applied to the produced Compton spectrum. This patented Reverse Spectrum Transform eliminates spectral distortions due to counting artifacts (wall effect, volume variations, color effects, vial wall thickness differences, etc.). A regression technique is used to fit a curve to the transformed spectrum. The $tSIE$ is calculated from the regression coefficients ($tSIE = transformed \ Spectral \ Index \ of \ External \ Standard$).
Counting Interference and Spectrum Analysis

By virtue of using spectrum analysis, the modern liquid scintillation counter is now able to correct for a number of counting interferences that have plagued LSC users since the introduction of this counting technique. The following interferences are inherent to the liquid scintillation process:

1) Quenching
2) Chemiluminescence
3) Static electricity
4) Wall effect
5) Scintillation volume variations
6) Heterogeneous samples
7) Random noise
8) Background

Quenching

The counting efficiency of the solvent-solute system can be affected by many different factors which may reduce detection efficiency. These may briefly be described as:

(1) chemical quenching (sometimes called impurity quenching)-causes energy losses in the transfer from solvent to solute;

(2) color quenching-which is the attenuation of light photons in the solution.

The total effect is collectively referred to as "quenching" and results in the reduction of the number of emitted photons from the sample. As a result, the energy spectrum detected appears to shift toward lower energies (Figure 2-16).

Figure 2-16. Example of Quenching Phenomenon.
From this change in energy distribution, it appears that the counting efficiency is dependent on the degree of quenching and thus on the nature of the sample, the scintillator used, and the preparation method.

It is, therefore, essential to monitor the counting efficiency in each sample for comparisons with standards or other samples to be meaningful. In modern automatic scintillation counters, the counting efficiency is determined for each sample and the detected counts are converted to disintegrations to correct for quenching effects.

As this is the most prevalent interference in the counting process, several methods have been developed to correct for quenching and will be described later. Spectrum analysis is a very attractive means to correct for this quenching phenomenon. As the pulse distribution shifts to lower energies, any change in position of characteristic points of the spectrum will reveal quench information.

Chemical versus Color Quench

As mentioned above, there are in essence two different types of quenching: chemical quenching and color quenching.

Though these two types of quenching result in a decrease of counting efficiency, there is a fundamental difference between them. Chemical quenching absorbs beta energy before it is converted to photons while color quenching results from the passage of the photons through the medium. The wavelength of the produced light is altered if the solution is colored to a value where the PMT response is reduced. This results in different pulse height distributions for color and chemical quenching (refer to Figure 2-17).

In a chemically quenched sample, all energy radiations are equally affected whereas, for a colored sample, events that take place at the edge of the vial close to one phototube will yield a large pulse on one phototube and a small one in the other. By summation, these pulses are added together. The resulting pulse heights may well be as large as those obtained from a virtually unquenched sample. Only the number of events will be significantly reduced. Therefore, at equal quench levels (identical counting efficiencies), the pulse heights for a colored sample are spread over a wider range than for chemical quenched samples.

With the aid of spectrum analysis, these two types of quenching can be easily identified. For equal counting efficiencies, the SIS of the color quenched sample will be higher than for the chemical quenched sample.
Chemiluminescence/Photoluminescence Detection and Correction

Chemiluminescence is the production of light as a result of a chemical reaction. This most typically occurs in samples of alkaline pH and/or those containing peroxides, when mixed with emulsifier-type scintillation cocktails.

Photoluminescence results in the activation of the cocktail and/or vial by ultraviolet light. This can occur by exposure to sunlight or UV lights used in the laboratory.

There is a difference between chemiluminescence and photoluminescence in that chemiluminescence has a fairly slow decay rate (from 0.5 hr to more than a day dependent on temperature) while photoluminescence generally decays within a few minutes.

With regard to spectrum, there is no apparent difference between chemiluminescence and photoluminescence. Luminescence is a single photon event and is registered as a count due to the probability of having coincident events at high luminescent activity. The pulse height distribution depends only slightly on the intensity of the reaction (refer to Figure 2-18 which shows the spectrum analyzed at different time intervals). The spectrum has a pulse height distribution overlapping the $^3$H spectrum.

The maximum pulse height corresponds to approximately 6 keV and the spectrum is chemical quench independent (as the events are not generated by converting beta particles to photons but by the reaction itself). These characteristic properties of the luminescence spectrum make it easy to distinguish from a beta energy spectrum, using spectrum analysis. The SIS of the composite spectrum will decrease with increasing luminescent activity. The typical spectral shape can be stored in the Spectralyzer spectrum analyzer so that the count results can be corrected for the interfering events based on spectral shape and fraction subtraction. The lower the SIS, the higher the luminescence contribution to the total uncorrected spectrum.

![Chemiluminescent Spectrum at Different Time Intervals.](image)

*Figure 2-18. Chemiluminescent Spectrum at Different Time Intervals.*
Origin of Chemiluminescence

Many photon-producing interferences in liquid scintillation counting result primarily from different sample preparation methods used for different sample types. Chemiluminescence is the light produced from chemical reactions between components of the scintillation sample. Usually the analyte or solutes in the analyte can react with one another or the scintillation cocktail, resulting in light emitted due to chemical excitations. Similar to the energy conversion process described in previous sections initiated by radioactive decay, chemiluminescence results from the chemical excitation energy which can be converted into electronic excitation energy and emission of light. Chemiluminescence typically results when alkaline tissue solubilizers are added to emulsifier type scintillation cocktails. Another common source of chemiluminescence is the presence of oxidizing agents in the sample. The reactions are usually exothermic and result in the production of a large number of single photons. Chemiluminescence is a single photon event. For a liquid scintillation counter to observe these luminescent events, the photons must be produced at a rate fast enough to give multiple photons within the coincidence gating time of the liquid scintillation counter. It has been demonstrated that the pulse height energy distribution spectrum of luminescence largely overlaps the tritium distribution spectrum. Luminescence events cannot be excluded by channels ratio method or adjusting the threshold discriminator. The measurement of samples exhibiting chemiluminescence interference invalidates the assay.

Chemiluminescence Control

There are two basic manners in which to control luminescence to background levels; 1) using electronic logic on board scintillation counters or 2) through additional sample preparation methods. In 1979, Packard introduced the first luminescence detection and correction circuit on board modern liquid scintillation counters. Each Packard scintillation spectrometer equipped with the luminescence detection and correction feature has the ability to monitor the accumulated luminescence events and correct for the interference to within statistical limits of the actual amount of radioactivity.

Counting Interference

One of the serious interfering processes which affects the counting results of a liquid scintillation counter is due to the sample material or the method of sample preparation. Interactions take place within the scintillation solution which excite molecules. These chemically induced excitations produce photons. A single excited molecule will produce a single photon. Although the coincidence requirement normally prevents these from affecting the counts, if the rate of production of single photons is sufficiently high, each PMT could be stimulated within the resolving time of the coincidence circuit indicating this as a legitimate event. Counts will be accumulated which are unassociated with radioactivity. This phenomenon is called chemiluminescence.

Luminescence Correction

The Tri-Carb analyzer:

- Corrects for chemiluminescence
- Corrects for bioluminescence
- Corrects for photoluminescence (due to ultraviolet light)
There are two main types of events which can take place within the counting vial, one due to the beta particle and the other due to chemically induced events not associated with the radioactivity being measured.

The coincidence requirement, precludes the acceptance of events from one PMT. Single events due to electronic noise or single photons are generally noncoincident from each PMT. With these restrictions it is legitimate to ask why there is so much concern about chemiluminescence. The problem arises when the production of single photons occurs at a rate sufficiently great that separate luminescence events stimulate each PMT within the resolving time of the coincidence and summation circuits.

This can be shown diagrammatically. The following illustration (Figure 2-19) shows the output pulses from each PMT under conditions where beta events ($\beta^-$) and occasional single photons or noise events are accepted or rejected by the coincidence circuit. Each time interval is $20 \times 10^{-9}$ seconds, the approximate resolving time of the coincidence circuit. When single events occur at this rate, the coincidence circuit successfully rejects them and accepts only the beta events.

![Figure 2-19. 2$\beta^-$ COINCIDENCES—0 CHANCE COINCIDENCES.](image)

If the single events occur more frequently, this arrangement cannot distinguish between beta events and the chance coincidences of single events from each PMT. The diagram (Figure 2-20) shows this situation.

![Figure 2-20. 2$\beta^-$ COINCIDENCES—3 CHANCE COINCIDENCES.](image)

How can a system distinguish between these coincidence signals? If a second circuit is used which recognizes the beta events, then the problem is solved. The second circuit delays the signal from one of the PMTs by a period of time at least equal to the resolving time. Below is illustrated such a scheme delaying the PMT 1 signal and establishing coincidence only between the delayed PMT 1 signal and the PMT 2 signal (Figure 2-21). It will be seen that the beta events are not accepted in this circuit, but the chance coincident rate is changed.

![Figure 2-21. 2$\beta^-$ COINCIDENCES—3 CHANCE COINCIDENCES.](image)
In the same period of time the number of single events accepted by this circuit will be the same, but no beta events are accepted unless these coincide with luminescence events. If chance coincidence events from this circuit are accumulated and recorded during a sample measurement, these can be subtracted from the data accumulated in the Spectralyzer spectrum analyzer. It will be realized that during the sample measurement the Spectralyzer spectrum analyzer will have accumulated the counts of both the beta events and the chance coincidence due to luminescence. To appreciate how this subtraction is achieved in the correct channels of the Spectralyzer spectrum analyzer, the following explanation is offered.

Due to the coincidence requirement, luminescence results in a count only when both phototubes register a photoelectron within the 20 nsec. resolving time \( t \). This will result in a summation pulse equivalent to a beta particle with an energy less than 0.5 keV. The spurious counts due to luminescence can be calculated according to:

\[
\text{Luminescence} = C_1 \times C_2 \times 2t
\]

where,  
\( C_1 = \text{count rate in Phototube 1} \)
\( C_2 = \text{count rate in Phototube 2} \)
\( t = \text{coincidence resolving time} \)

Consider luminescence producing single photon events in each phototube at a rate of 100,000 cpm. With a 20 nsec. resolving time, the rate of spurious counts due to luminescence would be:

\[
10^5 \times 10^5 \times 2 \times 20/60 \times 10^{-9} = 6.67 \text{ cpm}
\]

Therefore, it is clear that if the single photon rate is less than 100,000 cpm for each phototube, the signal due to luminescence will be virtually eliminated by the coincidence circuit. However, as the luminescence count rate in each phototube increases, the effect of luminescence becomes more severe, and will contribute more spurious counts to the actual sample count rate. Also, extremely high count rates may cause the phototubes to become totally saturated such that single events cannot be detected. For example, let the count rate in each phototube equal 1,000,000 cpm. Then:

\[
10^6 \times 10^6 \times 2 \times 20/60 \times 10^{-9} = 666.67 \text{ cpm}
\]

Furthermore, saturation from extremely high single photoelectron count rates will increase the dead time to such a degree that normal operation is impaired.

The spectrum of luminescence has a distribution which is confined to the equivalent of a few keV of beta particle energy. The maximum number of events will occur between 0 and 2 keV and remain there independent of quenching. Quenching reduces the number of luminescence events recorded but does not alter the distribution. The Tri-Carb liquid scintillation analyzer therefore records the luminescent distribution in memory for subsequent correction. At the end of the counting period the subtraction is made in the appropriate energy channels of the accumulated counts in the Spectralyzer spectrum analyzer to eliminate the luminescence contribution. The Spectralyzer spectrum analyzer now stores the corrected beta spectrum. The region settings will determine the limits for readout of the sample cpm. If the user selects a region which excludes the first few channels, no corrections will apply to the sample cpm. (However, the % contribution will be shown in the printed data since a user should be aware of luminescence.)
Reference Distribution of Luminescence Events (% Luminescence Calculation with Chance Coincidence)

Because the performance of each pair of PMTs can be different and can change with time (albeit over a long period), the Tri-Carb liquid scintillation analyzer offers the facility of entering a distribution pattern based on the performance of the individual system to maintain the precision obtained during manufacture.

The original distribution entered during installation of the Tri-Carb liquid scintillation analyzer is stored in the system. If for any reason, the system memory is lost, it is a simple procedure to re-enter the distribution.

The storage of the luminescence distribution pattern is very important to assure a correctly functioning luminescence correction system. Counting a luminescent sample in absence of radioactivity allows compensation for the inherent differences between the responses of the delayed coincidence and the actual coincidence circuits. Some manufacturers offer luminescence correction based on the same correction scheme, but are not compensating for response differences. These nonequalized count rates of delayed coincidence and normal coincidence circuits, result in suboptimal luminescence corrections.

Result Reporting (Statistics of Counting)

In addition to correcting the cpm, the % of luminescence contribution is printed on the Tri-Carb analyzer's printout.

This is a statistically reliable method, as the delay circuit measures the same events as accepted by the coincidence and summation circuits.

As luminescence is a randomly occurring phenomenon, it is subject to the normal statistical distribution. Two examples will illustrate this statistical uncertainty. A luminescence measurement of 1000 events per minute will have a 2σ uncertainty of ±63 counts. If the sample has a radioactive count rate of 8000, the luminescence correction contributes an uncertainty of less than 1%. If during a measurement of a sample, the detector of luminescence records 5 x 10^4 chance coincidence event per minute, the 2σ statistical uncertainty is equal to 2 \sqrt{5 \times 10^4} = 447 counts. Although 447 events per minute is relatively insignificant in 5 x 10^4 (50,000), if 50,000 counts per minute are subtracted from a sample with a true count of 1000 cpm, the statistical variation of the luminescence correction is ± 447 and the corrected count is 1000 ± 447, i.e. an approximate error of 50%. This assumes the sample count of 1000 is not subject to a statistical uncertainty.

Correction Performance

The exceptional performance of the luminescence detector is shown in the following graphs (Figure 2-22). The sample is a tritium-labeled compound in a scintillation cocktail containing a chemiluminescent material. With the luminescence detector on, the correct cpm was obtained as shown with the solid line. Counting without the luminescence detector would have produced the cpm results indicated by the dotted line.

The curves produced are with Insta-Gel® scintillation cocktail and Soluene® -350 tissue solubilizer which produce luminescence with a shorter life than some competitive products. Even if a competitive product is being used, the luminescence detector in the Tri-Carb analyzer will make the correction.

Many users are unaware of these interfering phenomena. Now they can be assured the printed data is correct.
Luminescence and Sample Preparation

Another method by which luminescence can be controlled is through additional sample preparation steps or alternate preparation protocols. The luminescence rate is affected by temperature. Heating the luminescent scintillation sample accelerates the reaction rate and is usually recommended when the scintillation counter does not possess the luminescence detection correction capability. Cooling the luminescent scintillation samples reduces the photon intensity to low levels but the interference may still be present and thus provides a false indication of luminescence control. Chemiluminescence is produced mainly during sample preparation methods which add peroxides or basic sample digests.

When peroxides are used in sample preparation procedures for bleaching, the chemiluminescence should be allowed to decay by incubating at elevated temperatures. Chemiluminescence produced from oxidation-reduction reactions can be forced to an end point by adding a reducing reagent to the sample mixture. In acid base chemiluminescence, neutralizing the sample environment with an acid will also drive the reaction to the endpoint. Neutralization should be approached cautiously, as many labeled proteins may precipitate if the sample becomes too acidic. Additions to the scintillation cocktail may change the sample holding capacity which can affect counting results.

Recent developments in liquid scintillation cocktail design have produced a cocktail which inhibits and reduces luminescence while accommodating the aqueous radio labeled material Hionic-Fluor™ was designed as a unique aqueous-accepting scintillation cocktail designed to control luminescence to nominal background levels.
Photoluminescence
Some materials can be excited by ultraviolet light; they give up the excitation energy as light, normally in the visible region. This process is called photoluminescence. Scintillation cocktails have photoluminescent properties. Materials used to make the scintillation vial and the vial cap may be photoluminescent. Photoluminescence normally decays rapidly; dark adaption for one-half to one hour in the counter is normally adequate for photoluminescence to decay to an insignificant level. For experiments in which measurements near background are important, dark adaption for as long as 24 hours may be necessary.

Electrostatic Discharge
Electrostatic discharge is a photon-producing interference in liquid scintillation counting. Liquid scintillation counting is an analytical method for quantification of radioactivity by carefully measuring emitted light. Photons produced in the detection chamber by causes other than nuclear decay will interfere with the measurement. Photomultiplier tubes are designed to collect emitted light within the effective area and convert these photons into electronic signals, regardless of their origin. The basic design nature of the electronic logic in today’s liquid scintillation counters excludes PMT noise using coincidence and threshold conditions. However, many pulses from interferences such as static discharge closely resemble electronic pulses representing light produced from nuclear decay events. Static electricity is an interference which has recently gained renewed attention by scintillation counter manufacturers.

Causes and Effects of Electrostatic Discharge
The separation of two nonconductive materials can generate significant amounts of static electricity. Static may be produced by the friction or the pressure between two materials. When separated, one of the materials can develop a positive charge and the other material a negative charge. Static electricity consists of charged ions, positive or negative, which are atoms that are electrically out of balance due to the removal or addition of one or more electrons. Like magnets, ions of like charge repel and opposite charge attract. The intensity of static electricity can be measured in terms of voltage. In many cases the positive or negative voltage on the surface of a material can be many tens-of-thousands of volts which carry very small electric current.

Materials which are common to the construction of commercial liquid scintillation counters and other material which we contact in our daily routines are in themselves generators of static electricity. A triboelectric chart represents the typical static charge intensity and polarity of many common substances (Figure 2-23).
From the chart it is apparent that air and Teflon® or human skin and polyethylene can represent two of the more intense static generating systems. Many of the materials listed are inherent to the design of modern scintillation counters as well as counting vials and other supply accessory items. In general the scintillation counter environment is quite conducive to the development of static charges. Because the surface of the human body is covered at all times by a microthin moisture layer, which promotes the generation of static electricity, plastic material can acquire an intense level of static electricity. Static charges can build up on the surface of glass or plastic scintillation vials, serpentine sample changer belts, sample holding cassettes, and other moving parts in a scintillation counter.

Static charges can develop in the scintillation vial or in the scintillation cocktail; the resulting discharges will produce light which will be detected by a liquid scintillation counter. Scintillation cocktails which contain emulsifiers are subject to a build-up of static charges if they are pumped through plastic tubing. Static charges on the vial surface can be produced during shipping and as a result of movement in the sample changer of the liquid scintillation counter. Glass vials have less problems with static than do plastic vials. Small vials in adapters are particularly prone to static charge build-ups.

_Teflon is a registered trademark of E.I. duPont de Nemours and Co._
Effect of Electrostatic Discharge on Count Results

Electrostatic discharge can occur on the surface of the counting vial in the detection chamber during a sample measurement. Stability is a common characteristic of static electric charges and does not easily leak off of the material’s surface. Electrostatic discharge can be thought of as a localized lighting storm on the surface of the counting vial and is the photon producing effect. Electrostatic discharges occur randomly with time during the sample count and contrary to belief are not always associated with the beginning of a sample count. A time-based frequency distribution of static discharges is demonstrated in (Figure 2-24).

Implementing a Static Control Program

The major goal of static control criteria is to effectively neutralize the charged ions on the surface of interest. Since static electricity is related to surface associated positive or negative charged ions, then by presenting the charged surface with an atmosphere of counter charged ions, electrostatic discharge results. The counter ions annihilate the surface static charge, resulting in a neutral surface effect. There are two basic methods of producing a counter ionized atmosphere 1) ionizing radiation and 2) electricity.

In the early liquid scintillation counters the technique of using radioactivity to generate an atmosphere of counter ions was accomplished with an alpha particle emitting source. The alpha source was chosen primarily for its long half-life, short range in air (local effect), high energy, and relatively limited exposure. $^{210}$Po was the common alpha particle emitter used for the radioactive method. The radioactive method of ionizing the local atmosphere is proven to be effective only when there is a sufficient time period in the presence of the counter ion field. Typically a minimum exposure time of ten minutes is required to achieve nominal effectiveness.

Using electrical current as opposed to radioactivity is another technique used to generate an ionized atmosphere of mixed counterions. In 1983, Packard first introduced an electronic static discharge device know as the Electrostatic Controller. The Electrostatic Controller reduces surface static charge on the vial being counted in essentially the time the scintillation counter takes to load the sample into the counting chamber, approximately two seconds. The theory of operation is based on charging electrodes which are arranged in a geometric configuration to an LSC vial with a high voltage at very low current. The high voltage causes a localized electrical disturbance in the atmosphere around each electrode, resulting in the production of counterions. When the counting vial is passed through the Electrostatic Controller device the controller sweeps the entire vial to be counted with a 360° field of electrically produced counterions, neutralizing charged regions on the vial surface. There are several advantages associated with using the electronic method of producing counterions.
The major advantage of the Packard Electrostatic Controller is that it provides a safe, clean, nonradioactive approach to static dissipation. The Packard Electrostatic Controller is only activated when the instrument loads a sample to the counting chamber. Since the counter ions are electrically produced, as opposed to alpha radioactivity, there is no need for periodic disassembly and replacement. The operable lifetime is approximately six months for a $^{210}$Po source and must be replaced along with associated wipe tests' to confirm source integrity. The Electrostatic Controller never needs replacement during the life of the instrument.

The Electrostatic Controller ensures immediate static dissipation in Packard’s liquid scintillation analyzers. (Figure 2-25).

The eight electrodes produce a 360° field of electrically produced ions thus neutralizing all static electricity on the vial surface.

![Figure 2-25. Packard Electrostatic Controller.](image)

**Wall Effect**

The common organic solvents contained in the scintillation cocktail can penetrate the walls of plastic vials which then indirectly affect the counting accuracy of the sample.

The sample energy spectrum is not distorted by this phenomenon but the Compton spectrum, induced by the external standard and used to calculate an external standard quench indicating parameter, can become heavily distorted.

The diffusion of the cocktail in the wall converts the wall to a plastic scintillator. When this plastic scintillator (together with the scintillator solution) is irradiated by the external standard source, it will act as an extra scintillator medium, and due to Compton scattering, low energetic photons are emitted. The energy distribution is dependent on the energy of the external standard source (refer to Figure 2-26 for external standard spectra of $^{226}$Ra and $^{137}$Cs).

---

1. *Title 10 Code of Federal Regulations, Part 31, Section 31.5(c) (2) NRC guidelines state in 10CFR 31.3(a) that static elimination devices which use not more than 500 uCi of polonium-210 are subject to a general license.*
From Figure 2-26(a) which is a logarithmic presentation of the $^{137}$Cs and the $^{226}$Ra Compton spectra, it appears that the wall effect heavily distorts the Compton spectrum. However, such a logarithmic presentation gives a completely distorted picture of the real spectrum. It is suggested that the spectrum is not affected at low energy levels. This is of course not true. As a matter of fact, the plastic scintillation of the vial wall induces a low energetic pulse distribution spectrum that extends to about 40 keV for $^{226}$Ra (Figure 2-26b). This parasite spectrum is not affected by quenching and thus becomes relatively more important as the quenching increases.

Quench parameters based on the spectral index of the external standard are relatively unaffected by this wall effect when they are calculated from a lower threshold:

$$\text{SIE} = \kappa \left( \sum_{x=L}^{u} x \cdot N(x) \right) \left( \sum_{x=0}^{u} N(x) \right)$$

$L =$ wall effect cut off level

The Packard SIE is calculated according to this formula and is thus independent of wall effect.

Figure 2-26(a) also shows the nonrealistic presentation of radionuclide spectra on logarithmic energy scales. The emphasis is on the lower end of the spectrum which is undefined and not sensitive to any interference due to limited quantum efficiency, coincidence counting, and summation effect (refer to a typical shape of a beta spectrum).

Packard's tSIE calculated from the Transformed Barium Spectrum is insensitive to wall effect because the Reversed Spectrum Transform filters out the parasite spectrum.
**Scintillation Volume Variations**

The LSC counting problems related to volume are twofold:

a) As the volume decreases, the light output falls on the less efficient areas of the PMT. Therefore, photons with the same energy will produce less counts on lower points of the PMT face than they would if they fell nearer the center.

Figure 2.27 shows typical relative quantum efficiencies of the PMT for photons striking in the areas:

![Plot of equal response areas of a typical photocathode. The most sensitive area is given a value of 100.](image1)

As a result, the energy detection is less efficient at small volumes. This has the same effect as quenching, and the SIS value will decrease as a function of decreasing efficiency.

b) The gamma rays used to produce the external standard Compton spectrum, require a certain mass of solvent to be present to interact and produce Compton electrons. With decreasing volume, the mass decreases and the number of Compton electrons will also be reduced. For composite gamma sources, the lower energy gamma rays will proportionally produce more Compton electrons than the high energy gamma rays because they are more likely absorbed by the lower volume. Figure 2.28 shows the influence of volume on the Compton spectra from $^{137}$Cs and $^{226}$Ra.

![External Standard Spectrum of $^{228}$Ra and $^{137}$Cs as a Function of Volume.](image2)
The logarithmic transformation distorts the real energy distribution. It is suggested that the Compton edge of the $^{137}$Cs spectrum is volume independent (doesn’t change position), while the average pulse height energy or SIE is volume dependent due to the spectral shape changes. Since the SIE calculation is independent of the accumulated counts (the integral of the counts is in the numerator and denominator of the equation) and only for small volumes, and energy distortion is observed for the lower energies (but well below the cutoff energy for the SIE calculations), the SIE is only slightly volume dependent. As both CPM results and SIE decrease slightly with decreasing volume, the final DPM results are virtually volume independent. Volume dependence has effectively been eliminated by using the recently developed transformed spectral index system (tSIE), explained in detail in the next section.

The Transformed Spectral Index of the External Standard (tSIE) exhibits volume dependency similar to the volume dependency of the counting efficiency. This allows accurate determination of single and dual label DPM of samples ranging in volume from 25 µL to 20 mL.

**Heterogeneous Samples**

In liquid scintillation counting, it is always assumed that the sample is in solution and that when any quenching effects are observed, they are due to either color or some chemical component. When samples are homogeneous, any valid quench correction method such as SIS, SIE or tSIE can be used to determine the correct counting efficiency of the sample. Unfortunately, there are situations encountered routinely where insoluble radiolabeled samples must be counted. These samples include compounds isolated on solid supports such as on paper chromatograms, filter paper and in polyacrylamide gels. When the solid support is placed into a counting solution, some of the deposited radiolabeled material will dissolve and some will remain on the support. The labeled material which dissolves will count with solution efficiency and that which remains on the support will count with less than solution efficiency because some of the beta energy will be lost by self-absorption within the support medium. The observed count rate for a heterogeneous sample will depend on the distribution of the radiolabeled sample between the solid support and the counting solution. This is what makes the determination of counting efficiency of heterogeneous samples tenuous. If possible, heterogeneous samples should be avoided in liquid scintillation counting.

To warn the user against heterogeneous counting, a patented Vector Qualitative Analysis is used (Figure 2-29) (11). The relation between the external standard quench parameter (tSIE) and the sample spectrum endpoint (SEP) can be expressed by vector mathematics. By comparing the vector of an unknown sample against the vector of a reference, homogeneous sample, the quality of the sample can be assessed. At the same external standard quench level, the endpoint of the spectrum of an heterogeneous sample is different from the spectral endpoint of an homogeneous sample.

![Figure 2-29. Vector Qualitative Analysis for Heterogeneous Sample Determination.](image)
Random Noise

Noise introduced in the electronic circuits by line interference (high voltage transients and line transmitted switching noises) and radio frequency noise (switches, motors, relays and fluorescent lights) are known to contribute to sporadic background pulses.

Especially for low level counting, this is a major concern. Spectrum analysis helps to eliminate these pulses by spectrum smoothing. These noise pulses usually show up as single energy peaks superimposed on the spectrum (Figure 2.30).

By smoothing the spectrum using digital filters, these interferences can be eliminated.

The Background Spectrum

To analyze the shape of a background spectrum (Figure 2.31), we first categorize the contributions to overall background in a typical LSC system:

1) Instrument 10%
2) Crosstalk 22%
3) Vial glass & PMT face 37%
4) Scintillator (14 mL) 31%

100%

1. Instrument background: This contribution results from the noise (dark noise, after pulse noise) of the PMTs. In a well-designed counter with a fast-resolving coincidence circuit and low dark noise from the photomultiplier, the background represents only 10% of the total observed background. This background is low energetic.

2. Crosstalk: A scintillation event (e.g. spontaneous release of photoelectrons from the cathode), initiating photons within one PMT (face of envelope body) will be seen by the other PMT. These events are usually within the coincidence resolving time and by summation they may appear as low energy background pulses. PMT masking or electronic crosstalk correction will reduce these background pulses.
3. Vial glass and PMT face: Background scintillation in the vial wall and the PMT face are generated by:
effect by cosmic or environmental radiation of glass walls and PMT face. (Radioactive 40K radiation is
present as a residue in the glass wall and PMT face.) The distribution of these background pulses is flat
over a broad energy range.
4. Scintillator background pulses are also caused by cosmic and environmental radiation, and they too cover
a broad energy spectrum.

By virtue of the Spectralyzer spectrum analyzer, this background spectrum can be stored as a reference
spectrum for subsequent background correction. Again, the SIS can be used to qualify the background
radiation. A decrease in SIS alerts the user that excessive instrument and crosstalk background is observed,
while an increasing SIS indicates an increase in cosmic, environmental, or vial radiation.
3-D Spectrum Analysis

Traditionally, the experimental spectrum of a radionuclide is represented in a two dimensional plane, using the x-axis as the pulse height (energy) dimension and the y-axis as the count rate dimension. This representation is obtained by analyzing the pulse height of the scintillation event (proportional to the energy of the decay event) and storing each count as a function of the pulse height.

However, when analyzing the shape of a scintillation pulse, the following observations are made:

1. A scintillation pulse originated from a beta decay event consists of (refer to Figure 2-32)
   - A fast or prompt pulse component
   - A slow or delayed pulse component.

   ![Figure 2-32. Typical Beta Scintillation Pulse.](image)

   As the energy of the beta decay event is proportional to the pulse area rather than the pulse height, it is a misconception to consider the energy as proportional to the pulse height. Consequently, storing pulses in function of pulse height yields an inaccurate representation of the energy spectrum. For "real world" samples, this pulse spectrum distortion is minimal. The dissolved oxygen in scintillation solutions, induced by preparing LS samples under normal laboratory conditions (exposed to air), acts as a quenching agent and eliminates most of the fluorescence that causes the delayed pulse.

2. A close examination of the nonquenchable background pulses reveals that the characteristics are substantially different from the characteristics of beta scintillation pulses (nonquenchable background is the pulses that are a result of the interaction of background radiation with the material in the environs of the counting chamber):

   ![Figure 2-33. Nonquenchable Background Pulse.](image)
The pulse actually consists of a pulse train with a major fast component, resembling a beta scintillation pulse, followed by a burst of low intensity pulses. The number of "after-pulses" is different from one background pulse to the other (Cerenkov radiations in the glass envelope of the PMT and surrounding material). The total number of "after-pulses" is called the Pulse Index (12).

**Three-Dimensional Spectrum**

In order to quantify the energy of a decay event, the total area of the detected pulse must be taken into account. This can be done by distributing the prompt pulses over the pulse height scale as a function of the pulse index. This yields a three-dimensional (3-D) representation of the pulse energy spectrum (Figures 2-34a, b and c).

The pulse index of the tritium pulses is defined as the number of sampling slices required to integrate the delayed pulse component. The effect of oxygen quenching on this slow pulse component is demonstrated in Figure 2-34b and 2-34c.
Analysis of the 3-D Spectrum

The 3-D distribution of e.g. $^3$H shows a limited number of spectral planes. The background distribution, however, covers up to 15 spectral planes (pulse index for some background pulses is equal to 15).

Based on these characteristic differences between the real beta decay distribution and the background radiation spectrum, most background pulses can be discriminated from beta decay events.

Using the pulse index as a discriminator, background pulses can be excluded from the $^3$H or $^{14}$C spectrum. $E^2/B$ optimization can be obtained by selecting the optimal pulse index discriminator setting. Typical improvement in $E^2/B$ achieved for both tritium and $^{14}$C are shown in Table 2-1 A & B counting the samples for 600 minutes or 0.5% 2s whichever occurs first.

Table 2-1. Effect of Pulse Index Discrimination on Background, Efficiency, $E^2/B$.

<table>
<thead>
<tr>
<th>AMOUNT OF PULSE-INDEX DISCRIMINATION</th>
<th>$^3$H EFFICIENCY (%)</th>
<th>BACKGROUND (CPM)</th>
<th>$E^2/B$</th>
</tr>
</thead>
<tbody>
<tr>
<td>NONE</td>
<td>26.50</td>
<td>18.45</td>
<td>38.06</td>
</tr>
<tr>
<td>NORMAL</td>
<td>26.24</td>
<td>12.75</td>
<td>54.08</td>
</tr>
<tr>
<td>HIGH SENSITIVITY</td>
<td>24.68</td>
<td>9.25</td>
<td>65.85</td>
</tr>
<tr>
<td>LOW LEVEL</td>
<td>22.59</td>
<td>3.33</td>
<td>153.25</td>
</tr>
</tbody>
</table>

A 402.6% increase in $E^2/B$

<table>
<thead>
<tr>
<th>AMOUNT OF PULSE-INDEX DISCRIMINATION</th>
<th>$^{14}$C EFFICIENCY (%)</th>
<th>BACKGROUND (CPM)</th>
<th>$E^2/B$</th>
</tr>
</thead>
<tbody>
<tr>
<td>NONE</td>
<td>83.45</td>
<td>9.67</td>
<td>720.15</td>
</tr>
<tr>
<td>NORMAL</td>
<td>81.87</td>
<td>7.07</td>
<td>948.05</td>
</tr>
<tr>
<td>HIGH SENSITIVITY</td>
<td>78.50</td>
<td>4.74</td>
<td>1300.05</td>
</tr>
<tr>
<td>LOW LEVEL</td>
<td>70.10</td>
<td>1.38</td>
<td>3560.00</td>
</tr>
</tbody>
</table>

A 495.5% increase in $E^2/B$

The data in Table 2-1 clearly indicate how much this unique patented technique of Three Dimensional Spectrum Analysis improves counting sensitivity. Counting sensitivities achieved by this technique rival those only previously possible by the use of very large amounts of passive shielding and/or the use of active (anticoincidence) shielding.

Quantification of Radioactivity

In the first chapter, the unique ability of the Spectralyzer spectrum analyzer to qualify sample radiations were discussed. By virtue of spectrum analysis, one is able to eliminate a number of counting interferences to obtain a clean beta ray spectrum. Spectrum analysis also allows identification of the obtained spectrum in terms of maximum and average energies. This information is of major importance to quantify the sample radiation.
Establishing Spectral Limits

Due to the capability of the Spectralizer spectrum analyzer to store a broad energy range of pulses (0-2000 keV), optimal channel settings can be made after the spectrum has been accumulated. No preconditions are set for pulse heights and no radiations are excluded from the measurement by pulse height discrimination. Optimal settings are obtained after analyzing the complete spectrum.

For single labeled samples, optimal settings are usually from 0 keV to $E_{\text{max}}$. This optimizes the efficiency and excludes all radiations falling outside the region of interest.

The observed $E_{\text{max}}$ energy is not necessarily the maximum energy of that particular radionuclide. As a function of quenching, the end point of the pulse height distribution shifts along the energy axis and it is important that the region of interest is adjusted accordingly (Figure 2.35). This excludes all unwanted background pulses while maintaining optimum efficiency. This automatic window tracking may be performed automatically in the Packard Tri-Carb systems.

![Figure 2-35. Automatic Region Tracking.](image)

Optimizing Counting Sensitivity

The lowest detection limit of a measurement is determined by the signal to noise ratio. In liquid scintillation counting, this signal to noise ratio is expressed in function of efficiency and background. This is termed the Figure of Merit (FOM) and is derived from statistical considerations.

$$\text{FOM} = \frac{S^2}{S + 2B}$$

- $S$: net sample count
- $B$: background count

As the signal to noise ratio is most important in low level measurements, $2B$ is greater than $S$ and the equation approximates to:

$$\text{FOM} = \frac{S^2}{2B}$$

As $S$ is proportional to $E$, this equation has been modified to:

$$\text{FOM} = \frac{E^3}{B}$$

This is currently the most widely used parameter to assess counter sensitivity and performance.
Optimizing counting sensitivity (E²/B) with spectrum analysis is easily achieved. The procedure is as follows:

1) Count and store background spectrum
2) Count and store sample spectrum
3) Increase lower level until maximum E²/B is obtained
4) Decrease upper level until maximum E²/B is obtained

The obtained LL and UL set the region of interest to maximum E²/B. This requires only the counting of two samples. Without a Spectralyzer analyzer which uses discrete channel settings, this procedure requires a very long time because both sample counts have to be repeated with each new region setting. The Packard Tri-Carb 2000CA analyzer performs this E²/B optimization automatically.

**Dual Label Region Settings (conventional)**

Consider the typical composite spectrum of a dual-labeled ³H-¹⁴C sample. It is apparent that no pulse height discrimination can be established that isolates both radionuclide spectra from the composite spectrum (Figure 2-36).

By raising the lower level for the ¹⁴C window to the end point of the ³H region (LL₉₂), all ³H counts can be excluded from the ¹⁴C region. This is at the expense of ¹⁴C efficiency.

However, for ³H, there is no means to eliminate by region selection the ¹⁴C counts that fall together with the ³H counts. For statistical reasons, the UL for ³H is usually set to a lower value than the end point of the ³H spectrum. With the upper level at UL₉, the contribution of ¹⁴C in the region can be restricted to 7-14% of the total ¹⁴C counts. It is obvious that the counts obtained in the region LL₉-UL₉ (³H region) must be corrected for this *spilldown* of ¹⁴C counts.

With modern LSC counters, the region setting for ¹⁴C allows also for a spillup of ³H in ¹⁴C (LL₉¹). This spillup factor is usually kept low and is selected in function of maximum sensitivity of the ¹⁴C window. Modern scintillation counters incorporate powerful calculation facilities in order to correct the measured counts in the two regions for spillup and spilldown.

Typical settings for an unquenched dual label sample containing ³H and ¹⁴C are:

³H: \( LL₉ = 0 \) keV  
\( UL₉ = 12 \) keV  
¹⁴C: \( LL₁₄ = 12 \) keV  
\( UL₁₄ = 156 \) keV
These settings are typical for a nonquenched sample. For quenched samples, both spectra will shift at a different rate. This complicates the region setting method for dual label samples even further. Automatic window tracking for both radionuclides is a prerequisite to obtain optimal isotope separation. In the Packard Tri-Carb systems, this accomplished with AEC (Automatic Efficiency Control).

![Composite Spectrum of Dual Label $^3$H/$^{14}$C Sample.](image)
References


Quenching And Efficiency

Introduction

In the foregoing section on the Spectralyzer spectrum analyzer, quenching was recognized as the most important interference occurring in liquid scintillation counting. Quenching affects the efficiency of the conversion process: beta particle energy to photoelectron. Therefore, the measured counts are only representative for the radioactivity of the sample if they are corrected for this quenching effect.

In this section, the effect of quenching on efficiency is further analyzed and common quench compensation methods and quench parameters are evaluated.

The Effects Of Quenching

To give a practical example, assume the sample material is readily soluble in toluene. The counting vial will now contain the solvent, the solute, and the molecules of the sample. A radioactive decay will result in the emission of a beta particle which will dissipate its kinetic energy by collision. The molecules of the sample will interplay with the solvent molecules to capture the beta particle energy. The energy given to the sample molecules is lost, incapable of energy transfer to the solute, and therefore, incapable of producing photons. Even excited solvent molecules can transfer their energy to the sample molecules. The higher the concentration of sample molecules, the lower the probability of light-producing collisions and, thus, fewer photons per keV of decay energy. This phenomenon is chemical quenching. All samples are quenched to some extent, in fact, it is impossible to produce an unquenched sample. Figure 3-1 illustrates the collision process (energy conversion) where "c" represents the molecules of the sample material. The diagram shows a beta collision with a solvent molecule (s) which transfers its energy to the solute (ø) with the production of a photon. The second collision is with a sample molecule where the energy is lost as heat(*). One other collision is shown where energy is transferred to the solvent and then to the sample molecule again resulting in heat and no photon.

Figure 3-1. Illustration of the Energy Conversion Process, with Quenching.
Another form of quenching exists with many samples. This form is unassociated with the energy exchange and occurs after the collision process. In their passage through the medium, the photons may be absorbed or scattered. The wavelength of light can be altered if the solution is colored, altered to a value where the PMT response is reduced. These forms of quenching of the emitted light are referred to as optical quenching. The most common cause is due to color from the sample or an ill-chosen scintillation cocktail.

To a first approximation, all forms of quenching have the effect of reducing the number of photons per keV of beta particle energy which reach the PMTs. Because photons interact with the PMTs, the pulse amplitude is reduced for the same energy particle. The result is a shift to lower amplitudes of the pulse height spectrum (Figure 3-2). For the radionuclides with high energy beta particles, this shift may have little or no effect on the counting efficiency, but with lower energy radionuclides the effect can be considerable. Tritium particularly is affected by quenching because the beta particle energy is so low that relatively few photons are produced even with no quenching. With no quenching, a 1 keV particle will produce fewer than 10 photons that exceed the coincidence threshold. With quenching a particle of higher energy will be required to produce the same number of photons. Events below the coincidence threshold are lost and there is no method of recovery without using a different sample preparation technique.

![Figure 3-2. Effect of Quenching On An Energy Spectrum.](image)

Independent of the degree of quenching each channel in the Spectralyzer spectrum analyzer will accumulate sample events during the counting period. Because the Spectralyzer spectrum analyzer records all events which satisfy the coincidence requirements and stores decay events according to energy, a measurement results in a complete sample spectrum. Illustrated in Figure 3-2 is an unquenched and a quenched spectrum. The distribution of events in the channels can be mathematically analyzed and compared with the distribution of an unquenched sample to provide an index of the degree of quenching (Figure 3-3). At the end of a measurement when the precision is at maximum, the first moment of the distribution can be calculated as the index. Because the information is derived from the sample contribution, the index is referred to as the Spectral Index of the Sample (SIS), or mathematically, the first moment of the pulse height distribution (also Mean Pulse Height or Center of Gravity).
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(1st moment of distribution is proportional to the average energy of the beta spectrum):

\[
\text{SIS} = 1\text{st moment of the distribution} \times K.
\]

where \( K = \text{a fixed factor} \)

or

\[
\text{SIS} = K \frac{\sum_{x=0}^{u} X \times n(x)}{\sum_{x=0}^{u} n(x)}
\]

In a single measurement the sample has provided both counts, which divided by time gives the sample cpm, and a unique quench indicating parameter, the SIS. This information is sufficient to determine the sample activity irrespective of the levels of quenching. Activity is the number of decays of the radioactive sample, such that:

Activity DPM is proportional to CPM and the reciprocal of the counting efficiency as determined by the quench factor:

\[
\text{DPM} = \frac{\text{CPM}}{(E) \text{ EFFICIENCY}} \text{ or as a percentage}
\]

\[
\text{DPM} = \frac{\text{CPM}}{E \%} \times 100
\]

By measuring a series of quenched standards, all with the same activity but with differing levels of quenching, a relationship between the SIS and measuring efficiency can be determined. This relationship provides an efficiency correlation for these standards to the calculated SIS of each standard, Figure 3-4 is a plot of a \(^{14}\text{C} \) efficiency correlation with the SIS. Once this relationship is established, the measurement of a sample with unknown quenching and activity provides an SIS value. Using the graph, the efficiency of the measurement can be determined. Any region of interest can be chosen for the measurement, providing it covers a sufficient amount of the spectrum to give statistically reliable results in a reasonable measuring time. Samples must be measured in the same region as the standards.
Figure 3-3. Effect of Quenching on the Accumulated Spectrum.

Figure 3-4. Efficiency Correlation Based on SIS.
Automatic Window Tracking/Gain Restoration

In order to maintain optimal counting conditions, it is necessary to keep the spectrum or spectra of interest in the optimal regions of interest (refer Spectrum Analysis chapter: quantification of radioactivity).

For single-labeled counting, the $E^2/B$ is always maintained at an optimum level while for dual label counting, optimum spectrum separation can be maintained at different quench levels.

Two approaches have been used to maintain these optimum counting conditions:

Automatic Window Tracking

Automatic window tracking adjusts the discriminator levels of the counting regions as a function of the spectrum shift.

This method was first used by Packard in 1967 with the introduction of the Absolute Activity Analyzer. This feature has been renamed AEC (Automatic Efficiency Control) by Packard and is now also used by Beckman (AQC: Automatic Quench Compensation), Kontron (SSC: Spectral Shift Compensation) and LKB (AWS: Automatic Window Setting).

Automatic Gain Restoration

Instead of a decrease in the windows with the spectrum, the windows can be kept constant and the decrease in pulse height can be compensated electronically by increasing the gain. Increasing the gain will shift the spectrum to the right so that the end point can be located at its unquenched position. Though the visual impression is a correction for quenching, this gain restoration cannot compensate for the efficiency loss. By quenching, radiation of low energy is attenuated so that it no longer exceeds the threshold of detection sensitivity.

This gain restoration method was introduced by Beckman in 1967. At that time, this method was attractive for logarithmic amplifiers. Due to the single amplifier approach, gain restoration could be achieved in all two or three counting channels by merely adjusting the gain of the amplifier. The drawback was that the spectra in the three channels were compensated to the same extent. This is not the case with automatic window tracking. Each channel can be altered to optimize individual counting conditions.

Quench Indicating Parameters (QIP)

Several quench indicating parameters have been introduced throughout the history of liquid scintillation counting. They can be classified into four main categories:

1. Sample Spectrum QIPs:
   - SIS (Spectral Index Sample)
   - SCR (Sample Channels Ratio)
   - SQP(I)
2. External Standard Spectrum QIPs
   - ESC (External Standard Counts)
   - ESR (External Standard Ratio)
   - H# (Inflection Point Compton Edge)
   - ESP (External Standard Pulse)
   - SIE (Spectral Index External Standard)
   - SQP(E) (Spectral Quench Parameter of the External Standard)
   - tSIE (Transformed Spectral Index of the External Standard)

3. Internal Standardization

4. Efficiency Tracing DPM
   - DPM without quench curves

**Sample Spectrum QIPs**

* (Spectral Index of the Sample) SIS *

The spectral index of the sample (derived from the average energy) is the most sensitive quench indicating parameter. Its calculations and applications have been discussed extensively in the chapter on Spectrum Analysis.

Maximum accuracy is obtained from a sample spectrum as the integrated counts of the entire spectrum are used to calculate the index:

\[
SIS = K \frac{\sum_{x=0}^{u} X \cdot n(x)}{\sum_{x=0}^{u} n(x)}
\]

An attractive feature is that this index is count rate independent! It does not shift with increasing or decreasing activity.
Advantages:
- DPM results independent of sample volume (Figure 3-5)
- DPM results independent of wall effect.
- Independent of cocktail density (Figure 3-6)

Proportional to average energy of beta energy spectrum. This characteristic energy can be used to qualify the radionuclide (refer to Appendix A).

- High dynamic range.
- Unique quench monitor for Čerenkov counting.
- Insensitive to structure in the spectrum. Allows correction of structured spectra (e.g. \(^{125}\text{I}\)) over a wider range of quench than is possible using more conventional methods (e.g. channels ratio).
- Efficient: requires no external standard time and is two times faster than SCR.

Disadvantages:
The statistical accuracy is limited by the statistical uncertainty of the total accumulated counts. However, accurate DPM calculations can still be obtained at activity levels of 1000 counts/min with reasonable counting times.
Sample Channels Ratio (SCR)

This technique calculates the ratio of counts between two regions of the energy spectrum to detect spectral shift and thus quenching.

In the case of $^3$H, the regions are typically set from 0 to 19 keV in region A and 2 to 19 keV in region B. The ratio is calculated as either A/B or B/A. Figure 3-7 illustrates the principle. The SCR value changes depending on the level of quenching.

![Figure 3-7. Sample Channels Ratio A/B or B/A as a QIP Method.](image)

Advantage of SCR versus SIS:
- SCR calculation requires only two counting channels versus a high resolution multichannel analyzer for SIS.

Disadvantages SCR versus SIS:
- Limited quenching range for high quenched samples because the energy spectrum shifts to the lower energy region
- Precision is dependent on instrument region settings.
- Less accurate than SIS because only part of the energy spectrum is counted.
- The relationship between quench parameter and efficiency is dependent on region settings. Optimizing regions can be very laborious.
- Longer counting times required to obtain similar precision as compared to SIS.

Spectral Quench Parameter of Isotope (SQP(I))

SQP(I) is used by LKB as Sample Spectrum quench parameter. This quench parameter is analogous to Packard’s SIS.

Instead of calculating the center of gravity or first moment of the linear spectrum, LKB uses the logarithmic spectrum to derive SQP(I).

SQP(I) indicates the channel number which is in the center of gravity of the radionuclide spectral distribution. This channel represents the mean pulse height of the logarithmic spectrum of the radionuclide.
External Standard QIPs

External Standard Count: ESC

As the energy spectrum originated from Compton-scattering events shifts with quenching, the accumulated counts in a higher energy region will decrease with increasing quenching (Figure 3-8). This decrease in count rate can be used to construct a quench correction curve.

![Figure 3-8. Compton Spectrum of External Standard-Counts in External Standard Region](image)

This method is volume dependent. The count rate is directly proportional to the volume of the sample. Due to this severe limitation, it is no longer employed as a quench monitor.

External Standard Ratio: ESR

To solve the volume dependency problem with the ESC factor, the External Standard Ratio was introduced (early 1960’s).

This method is based on the channels ratio technique as used for SCR. Two channels on the counter are usually reserved for counting external standard events (refer to Figure 3-9).

![Figure 3-9. External Standard Regions for ESR Calculations. (137Cs Compton Spectrum)](image)
The ESR calculation is similar in concept to the SCR:

\[
ESR = k \left( \frac{CNTS_b}{CNTS_a} \right)
\]

\( k \): scaling factor  
\( CNTS \): accumulated counts in external standard regions

As the external standard energy spectrum is always produced by the same source, as opposed to the sample spectrum, two fixed discriminators can be used to calculate the ESR (factory optimized windows). This makes the measurement and determination completely automatic and eliminates the laborious work involved in finding the optimal settings.

This is done in an attempt to minimize some severe drawbacks of this technique:

1. **Limited dynamic range:** with increasing quenching level the energy spectrum shifts out of the upper window. This reduces the statistical precision of the ESR calculation at moderate and high quench levels.

2. **Wall effect:** The LL of the lower channel has to be set to a low energy value in order to maintain statistical precision. However, wall effect introduces a parasite spectrum in the low energy region of the Compton spectrum. This results in more counts in channel A and thus a decrease in ESR. As the CPM remain constant, wall effect will introduce severe errors in the DPM calculations.

3. **Volume dependency:** at low volumes, the spectral shape changes in the lower energy region. This effect on the spectral shape is analogous to the wall effect. As a result, DPM results are volume dependent.

These three major deficiencies are accentuated when low energetic external standard sources are used. For that reason, manufacturers using low energetic sources were forced to use other quench indicating parameters:

- **Nuclear Chicago (T.M. Analytic)** uses a \(^{133}\text{Ba}\) source. It switched to the ESP calculation (inversely proportional to the average energy of the spectrum with calculations based on the entire spectrum).

- **Beckman** uses a \(^{137}\text{Cs}\) source and was confronted with severe problems when the plastic vials were introduced. It started using the H\# or Compton edge location.

- **Packard, LKB and Philips** are using \(^{226}\text{Ra}\) as external standard source. Due to the high energy of the source the lower level of the lower counting channel could be set to sufficient high energy level to eliminate wall effect without compromising the dynamic range. With the introduction of lower cost plastic vials (higher penetration rate of solvent) and the advent of mini-vials, the wall effect became more and more important. Packard realized that one had to give up the superior dynamic range of the \(^{226}\text{Ra}\) when one wanted to eliminate the wall effect. For this reason, Packard started using in 1979 the SIE (Spectral Index External Standard) as the QIP factor.
Today only LKB, Kontron and Philips still use the external standard ratio as the quench parameter on current production models. Only with the latest version, the Spectral 1219, LKB uses SQP(E) similar to SIE—as the external standard quench indicator. Their less expensive 1211/12 and 1217/18 models use the ESR.

With the introduction of the Tri-Carb 2000CA liquid scintillation analyzer (1985), Packard has taken a completely new approach to quench monitoring using external standard sources. $^{133}$Ba is used as a low energy gamma source to activate the scintillator and a spectral transform is applied to the Compton spectrum to filter out all counting interferences. The spectral index is now calculated from the transformed spectrum. This yields a high quality quench parameter ($tSIE$: Transformed Spectral Index External standard), independent of counting interference. This approach offers the additional practical advantages of using a low energetic external standard source.

**H# or Compton Edge**

The Compton Edge determination has been introduced by Beckman and is named H-number after the inventor, Dr. Horrocks.

Originally the H# was defined as the projection on the log energy scale of the point located on the Compton Edge at half peak height. This determination was not very accurate as it was relying on the count rates accumulated in narrow channels near the Compton Edge. The precision was highly dependent on accumulated counts and energy width of the channels. Long counting times were required to obtain reasonably precise answers.

Later (1980), the technique was modified in an attempt to improve accuracy and reproducibility. Instead of determining the position at half pulse height, the inflection point is used. Again, the method relies on counts accumulated in narrow channels near the end point and the accuracy of the counts in these channels. To increase the counting precision, a higher activity $^{137}$Cs source is used. This allowed Beckman to bring down the counting time to 6 seconds per determination. However, more cycles are allowed to improve the accuracy. This reproducibility problem of the H# can be explained when looking at the Compton edge on a linear scale (Figure 3-10).

![Figure 3-10. External Standard Spectrum of $^{137}$Cs (a) Linear or (b) Logarithmic Scale—Compton Edge.](image)
On a logarithmic scale, the spectrum shows a distinct, sharp edge. However, this is only visual illusion. The precise location of a point in geometry can only be judged when linear scales are used. In the linear representation of the spectrum, it is less obvious where the inflection point of the Compton edge is situated. It can be anywhere between channel 900 and 920. On a scale of 1000, this represents an error of 2%.

With decreasing volume, the total number of external standard counts decreases. This means that the spectrum will look flatter at the Compton edge and an accurate determination becomes more difficult. The H# determination has to be repeated a number of times to come up with a reproducible result.

Another problem associated with H# accuracy is chemical versus color quenching. As discussed in Chapter 2, color quenching tends to flatten-out the spectrum. Fewer pulses are spread over a broader pulse distribution. This flattened Compton spectrum increases the inaccuracy of the H# determination and a significant error will result if the DPM of color-quenched samples are calculated using chemical-quenched standards.

Summary:
- Questionable reproducibility at low volumes. Repeated external standard cycles are required to improve precision.
- High activity source required.
- Poor precision with color-quenched samples.

**Mean Pulse Height Calculations (SIE)**

In Chapter 2, Spectrum Analysis, it was demonstrated that a factor, based on the calculation of the average energy of a radionuclide spectrum, is a very accurate factor to describe the pulse height distribution and the spectral shape.

This factor is very accurate as it is calculated from the total accumulated counts:

\[
SIE = k \left( \frac{\sum_{x=L}^{u} X \cdot N(x)}{\sum_{x=L}^{u} N(x)} \right)
\]

The lower level (L) is introduced to eliminate the effect of solvent diffusion in plastic walls on the Compton spectrum shape. SIE is thus virtually independent of wall effect.

A similar technique is used by T.M. Analytic (formerly Nuclear Chicago). However, due to the use of a low energy gamma emitter as the external standard source and the method used to calculate the quench indicating parameter, the wall effect cannot be eliminated. Only a spectrum transform technique can eliminate this effect with low energy sources (refer to page 3-19: tSIE calculations). Using a lower level to cut out the distorted portion of the spectrum would significantly reduce the dynamic (quench) range of the ESP technique (ESP -External Standard Pulse -is inversely proportional to the average energy of the spectrum).
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SQP(E) or Spectral End Point Calculations

On their most recent counters, LKB uses the end point of the external standard spectrum as a quench indicating parameter.

SQP(E) or the Spectral Quench Parameter of the External standard is the estimated end point of the external standard spectrum. The spectrum is stored in 1024 logarithmic channels of an MCA and SQP(E) indicates the channel number were 99.5% of the total counts of the external standard spectrum are accumulated (Figure 3-11).

![Figure 3-11. SQP(E) Determination Using $^{226}$Ra External Standard Spectrum.](image)

The formula for SQP(E) is:

$$\sum_{j=i}^{n} R_j \geq (1 - r) \sum_{j=400}^{n} R_j \Rightarrow \sum_{j=i+1}^{n} R_j$$

- $R_j$ = external standard count in channel $j$.
- $r = 0.995$, $n = 1024$.
- $\sum_{j=400}^{n} R_j$ = total external standard counts above channel 400.
- $i = SQP(E)$

This formula indicates that the first 400 channels of a total of 1024 are excluded from the calculations. This demonstrates again the dubious validity of using a logarithmic pulse height distribution. 400 channels corresponds approximately to 20 keV. On a logarithmic scale, this inaccurate representation covers about half of the energy scale. On a linear 2000 keV scale, it would only distort 1% of the displayed spectrum.

This lower portion is eliminated to reduce the effect of solvent diffusion (wall effect) on the quench parameter. However, eliminating 400 channels is certainly not enough to make SQP(E) independent of wall effect. This is illustrated in Figure 3-12.

As discussed in Chapter 2, volume variations distort the lower portion of the spectrum. As a result, SQP(E) is volume dependent.
Because color quenching tends to flatten the spectrum spreading it out over a broader energy range, the end point location is very sensitive to color quenching. To reduce the difference between color and chemical quenching, LKB had to introduce a mathematical correction of the SQP(E) parameter. Because the SQP(E) of a sample with a given counting efficiency is dependent on chemical or color quenching and on glass or plastic vial, the user has to instruct the system what type of samples and what type of vials are being used.

Due to the use of a logarithmic scale, the SQP(E) quench parameter is not very sensitive to quench changes. SIE is a three times more sensitive quench monitor than SQP(E).

Summary:
- SQP(E) shows wall effect and volume dependency.
- User has to specify sample type and vial type to correct for severe color dependency.
- Not a very sensitive quench monitor.

**tSIE or Transformed Spectral Index Calculations**

With the recent introduction of the Tri-Carb 2000CA liquid scintillation analyzer, Packard has taken a completely new approach to quench monitoring with an external standard source.

Instead of using the plain Compton spectrum, generated in the sample by the external gamma source, the transformed spectrum is used. A mathematical technique is applied to the energy distribution to correct for spectral distortions. Spectral distortions can be introduced by LSC artifacts such as wall effect (distortion in lower energy portion of spectrum due to diffusion of solvent in wall of plastic vials), volume variation (at lower volumes, more Compton electrons are generated in the low energy region), and color quenching (spectrum flattens and extends over broader energy range).

In conventional techniques of external standard quench monitoring, the effect of these spectral distortions on the quench parameter can be reduced by:

1) Eliminating the distorted part of the spectrum.

2) Looking at a particular point on the spectrum rather than using all of the spectral information from the regression coefficients (tSIE = transformed Spectral Index of External Standard).

The first approach reduces the dynamic range of the quench monitor as well as the statistical accuracy.
The second technique suffers from low statistical precision. Moreover, both the lower energy region and the higher energy portion of the spectrum can become distorted so that monitoring a particular point of the spectrum will not eliminate all counting artifacts.

Packard has taken the approach of conserving spectrum integrity by filtering-out spectral distortions. The applied technique is the proprietary Reversed Spectrum Transform method, RST, (patent applied for). The objective of the RST technique was to develop an ideal quench indicating parameter (QIP). The following criteria were used:

1) QIP must be independent of counting artifacts such as volume variations, color quenching, cocktail density, wall effect, vial type, vial material, etc.

2) The QIP must have a high dynamic range.

3) The energy of the external standard source must be sufficiently low to reduce or even eliminate the spectral distortion caused by the interaction of the gamma rays with material in the environs of the counting chamber (Cerenkov radiation in glass envelope of PMTs, vial walls, counting chamber material, etc.).

4) The maximum Compton energy must approximate as closely as possible the energy of $^3$H and $^{14}$C, the most frequently used radionuclides in dual label counting. This criterion assures a linear relationship between the sample spectra end points and the end point of the external standard spectrum as a function of quenching. This optimizes automatic region tracking and assures optimal dual label performance at all quench levels.

5) A low $E_{\text{max}}$ reduces the amount of shielding required to eliminate influence of background level when the external standard is stored.

6) An external standard source should be used with low radiation hazard which is not subject to stringent radiation safety regulations.

$^{133}$Ba was selected as the external gamma source because its low energy satisfies criteria 3, 4, 5, and 6. Besides, $^{133}$Ba has been used for many years with more conventional quench correction parameters (ESR, ESP).

The major disadvantage of using $^{133}$Ba with conventional QIP calculations was the inability to satisfy all conditions of criteria 1 and 2. In order to eliminate wall effect and volume dependency, the lower energy portion of the spectrum could not be used, thus limiting the dynamic range. Manufacturers traditionally using barium (T.M. Analytic and Kontron), usually opted for a high dynamic range. As a result, the performance of these systems is rather poor when counting plastic vials (refer to Figures 3-13a and 3-13b).
The Reverse Spectrum Transform eliminates spectral distortion by a mathematical spectral transform technique and conserves as such spectral integrity with maximum dynamic range. The technique is illustrated in Figures 3-14 a and b.

![Figure 3-14a](image1)

**Figure 3-14a**  $^{133}$Ba External Standard Spectrum.

![Figure 3-14b](image2)

**Figure 3-14b**  $^{133}$Ba Transformed External Standard Spectrum.

The simplified mathematical expression of the Reversed Spectral Transform (RST) is (refer to Figure 3-14b):

$$
\int_{E_{\text{max}}}^{E} N(E) \, dE = \left( \int_{E_{\text{max}}}^{E} N(E) \, dE - \int_{E_{\text{max}}}^{E_{1}} N(E) \, dE \right) \frac{E - t\text{SIE}}{E_{1} - E_{2}},
$$

where $E$ : transformed energy.

$t\text{SIE}$ is calculated as one of the parameters of this RST function:

$$
t\text{SIE} = E - (E_{2} - E_{1}) \frac{\int_{E_{\text{max}}}^{E} N(E) \, dE}{\int_{E_{\text{max}}}^{E_{1}} N(E) \, dE - \int_{E_{\text{max}}}^{E_{2}} N(E) \, dE}
$$

$t\text{SIE}$ is expressed for unquenched as 1000 and is independent of count rate or accumulated counts ($\int N(E) \, dE$ appears in numerator and denominator).
The major advantages of the RST technique for calculating tSIE are:

1) DPM results are independent of wall effect (Figure 3-15).

![Figure 3-15. Wall Effect as a Function of Time on DPM Recovery of Tritium Using tSIE.](image)

2) DPM results are volume independent from 25µL to 20mL (Figure 3-16).

![Figure 3-16. Effect of Volume on DPM Recovery Using tSIE as QIP.](image)

3) Allows optimal geometrical source positioning. The $^{133}$Ba source is positioned below the sample. This optimal geometrical placement collimates the radioactivity through the length of the vial and provides a longer gamma radiation path for maximum Compton electron formation.

Absorption of radiation in the bottom material of the vial (varying thickness), in sedimentation or filter paper will not affect the quench factor. tSIE depends only on the spectral distribution and is not dependent on accumulated counts activity. Absorption will only slightly reduce the number of Compton electrons but will not alter the spectral distribution.
4) Provides maximum statistical precision, even at high quench levels or extreme low volumes. All counts in the spectrum are used in the calculations.

5) Very efficient quench monitoring technique. Minimum extra counting time required to count even the most demanding samples.

6) Capable of using only one quench curve per radionuclide regardless of LS cocktail, quenching agent, sample type or volume, vial type and vial size.

The excellent performance of a quench parameter derived from the Compton spectrum from a low energetic external standard source would have been impossible with conventional equipment.

The storage of the complete Compton spectrum in a high resolution linear Spectralyzer spectrum analyzer allows full exploitation of the capabilities of applied spectrum analysis. This results in a performance which would be inconceivable using previous techniques.

**Internal Standardization**

The counting efficiency of a sample can be determined by adding to the activity already in the vial, a known amount of activity of the same radionuclide and computing the increase of the sample cpm. The internal standard technique for computing counting efficiency involves a three-step procedure.

1) The sample is counted and its cpm determined.
2) A known amount of activity from a standard source is added to the sample.
3) The sample plus the added activity is counted and the new cpm is determined.

The following formula is then used to compute the counting efficiency:

\[
E = \frac{C^2 - C^1}{D}
\]

where, 
- \(C^1\) is the net cpm of the sample without the internal standard.
- \(C^2\) is the net cpm of the sample with the internal standard.
- \(D\) is the dpm of the internal standard.

**NOTE:**
*Net cpm is the gross count rate per minute minus the background count rate.*

There are a number of considerations in performing this efficiency determination technique. The internal standard source must satisfy the following requirements for an accurate efficiency determination.

1) The material should be the same material as the sample.
2) The radioactive label should be the same as that in the sample.
3) Activity added should be greater than or equal to the sample activity.
4) The internal standard activity must be accurately determined.
5) The activity added must be accurately known, i.e., the error in introducing the internal standard must be small.
Counting the sample plus internal standard should be performed as soon as possible after the addition of the standard. Counting conditions should be the same in the two measurements.

**Efficiency Tracing**

The liquid scintillation efficiency tracing technique is a new and powerful method of quantitating radionuclides being analyzed in a liquid scintillation analyzer.\(^1\)\(^-\)\(^7\) This technique has several advantages over conventional DPM analysis. First, no quench curve (quenched standard set) is required for each nuclide being analyzed. Second, the technique can be used effectively for almost all pure beta and beta-gamma emitters. Third, only a single unquenched \(^{14}\text{C}\) sample (same as that used to normalize the liquid scintillation analyzer) is required to calculate DPM results. Fourth, the efficiency tracing technique provides a simple method for quantitation of DPM in the sample. Fifth, relatively small errors (1-5%) in the calculation of DPM, can be achieved using this technique. Sixth, different radionuclides can be intermixed in the sample batch.

This efficiency tracing technique is based on a patent-pending procedure which requires a liquid scintillation analyzer to contain a multichannel analyzer, a sophisticated data reduction system, and a standard radionuclide (sealed calibration standard) of known absolute activity. The efficiency tracing procedure is accomplished in the following manner. First, the system is standardized (normalized) with an unquenched \(^{14}\text{C}\) standard. The reference spectrum of this standard is analyzed, and the counting efficiency is determined in six separate regions simultaneously. Second, the % efficiency in each of the six regions is calculated and plotted against the actual number of counts in each region. Figure 3-17 shows a typical % efficiency versus CPM plot for the \(^{14}\text{C}\) standard.

Third, an extrapolated CPM value for the 100% efficiency is determined based on the plot of the resultant line analyzed by the method of least squares. Fourth, when an unknown sample is analyzed, its spectrum is analyzed in the same six regions and the results are plotted using the same x-axis (% efficiency) generated by the \(^{14}\text{C}\) standard.
A line is then drawn through the resultant points. A curve is fitted through these six points and extrapolated to 100% efficiency. The extrapolated CPM value at this point is equal to the number of DPM in the sample. Over a dozen radionuclides (\(^{14}\text{C}\), \(^{32}\text{P}\), \(^{36}\text{Cl}\), \(^{46}\text{Sc}\), \(^{59}\text{Fe}\), \(^{60}\text{Co}\), \(^{63}\text{Ni}\), \(^{86}\text{Rb}\), \(^{90}\text{Sr-Y}\), \(^{131}\text{I}\), \(^{134}\text{Cs}\), \(^{147}\text{Pm}\)) have been assayed\(^6,^7\) using this technique with excellent statistical results (SD = 1.4\%) between the DPM calculated by the efficiency tracing and the absolute activity (DPM) of the sample. Specific examples of the DPM results achieved using the efficiency tracing technique on the LSA Model 2000CA for radionuclides \(^{36}\text{Cl}\), \(^{59}\text{Fe}\), \(^{63}\text{Ni}\), and \(^{14}\text{C}\) are shown in Table 3-1.

**Table 3-1. Efficiency Tracing Results of Various Nuclides at Various Quench Levels.**

<table>
<thead>
<tr>
<th>RADIONUCLIDE</th>
<th>SIS</th>
<th>ISIE</th>
<th>DPM (ET)</th>
<th>DPM (ACTUAL)</th>
<th>% REC.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(^{36}\text{Cl})</td>
<td>973</td>
<td>919</td>
<td>117.381</td>
<td>118.414</td>
<td>99.1</td>
</tr>
<tr>
<td>(^{36}\text{Cl})</td>
<td>580</td>
<td>537</td>
<td>117.607</td>
<td>118.414</td>
<td>99.3</td>
</tr>
<tr>
<td>(^{36}\text{Cl})</td>
<td>188</td>
<td>174</td>
<td>116.606</td>
<td>118.414</td>
<td>98.5</td>
</tr>
<tr>
<td>(^{36}\text{Cl})</td>
<td>117</td>
<td>109</td>
<td>117.154</td>
<td>118.414</td>
<td>99.0</td>
</tr>
<tr>
<td>(^{59}\text{Fe})</td>
<td>241</td>
<td>460</td>
<td>2.231.719</td>
<td>2.310.000</td>
<td>97.0</td>
</tr>
<tr>
<td>(^{59}\text{Fe})</td>
<td>223</td>
<td>428</td>
<td>2.204.944</td>
<td>2.310.000</td>
<td>95.4</td>
</tr>
<tr>
<td>(^{59}\text{Fe})</td>
<td>151</td>
<td>315</td>
<td>2.283.158</td>
<td>2.310.000</td>
<td>98.8</td>
</tr>
<tr>
<td>(^{59}\text{Fe})</td>
<td>115</td>
<td>305</td>
<td>2.306.399</td>
<td>2.310.000</td>
<td>99.9</td>
</tr>
<tr>
<td>(^{63}\text{Ni})</td>
<td>30</td>
<td>583</td>
<td>200.026</td>
<td>200.000</td>
<td>100.4</td>
</tr>
<tr>
<td>(^{63}\text{Ni})</td>
<td>29</td>
<td>535</td>
<td>197.908</td>
<td>200.000</td>
<td>99.0</td>
</tr>
<tr>
<td>(^{63}\text{Ni})</td>
<td>16</td>
<td>235</td>
<td>195.066</td>
<td>200.000</td>
<td>97.0</td>
</tr>
<tr>
<td>(^{63}\text{Ni})</td>
<td>15</td>
<td>206</td>
<td>185.546</td>
<td>200.000</td>
<td>93.0</td>
</tr>
<tr>
<td>(^{14}\text{C})</td>
<td>173</td>
<td>1.000</td>
<td>111.280</td>
<td>111.700</td>
<td>99.6</td>
</tr>
<tr>
<td>(^{14}\text{C})</td>
<td>86</td>
<td>505</td>
<td>112.603</td>
<td>111.700</td>
<td>100.8</td>
</tr>
<tr>
<td>(^{14}\text{C})</td>
<td>39</td>
<td>209</td>
<td>116.373</td>
<td>111.700</td>
<td>104.1</td>
</tr>
<tr>
<td>(^{14}\text{C})</td>
<td>24</td>
<td>120</td>
<td>125.131</td>
<td>111.700</td>
<td>112.0</td>
</tr>
</tbody>
</table>

Specific examples of the actual efficiency tracing plots of the data shows excellent statistical results, % recovery, 100% ± 2% of counts versus % efficiency for \(^{36}\text{Cl}\), \(^{59}\text{Fe}\) and \(^{63}\text{Ni}\) are shown in Figures 3-18 through 3-20.

![Figure 3-18. Efficiency Tracing Curve for \(^{59}\text{Fe}\) at tSIE=437 Quench Level.](image-url)
As can be seen from the previous table and results, excellent DPM values can be obtained for radionuclides ranging from $^{63}\text{Ni}$ to $^{32}\text{P}$ over the energy range of 60-1700 keV.

In order to further assess the reliability and accuracy for determining DPM values, a series of samples with various types of counting vials, scintillation cocktails, sample volume, microvolume samples, and degree of color or chemical quenching, were quantitated using the efficiency tracing technique (Table 3-2).

**Table 3-2. Efficiency Tracing Results Study for $^{14}\text{C}$.

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>tSIE</th>
<th>DPM (ET)</th>
<th>DPM (ACTUAL)</th>
<th>% RECOVERY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Std. vial</td>
<td>625</td>
<td>143.457</td>
<td>144,000</td>
<td>99.6</td>
</tr>
<tr>
<td>Color quenched</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Mini vial</td>
<td>644</td>
<td>144.020</td>
<td>144,000</td>
<td>100.0</td>
</tr>
<tr>
<td>Color quenched</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Std. vial (2.0 ml)</td>
<td>576</td>
<td>128.636</td>
<td>129,500</td>
<td>99.3</td>
</tr>
<tr>
<td>4. Std. vial (8.0 ml)</td>
<td>967</td>
<td>127.808</td>
<td>129,500</td>
<td>98.7</td>
</tr>
<tr>
<td>5. Std. vial (15.0 ml)</td>
<td>959</td>
<td>128.432</td>
<td>129,500</td>
<td>99.2</td>
</tr>
<tr>
<td>6. Mini vial (0.5 ml)</td>
<td>608</td>
<td>128.608</td>
<td>129,500</td>
<td>99.3</td>
</tr>
<tr>
<td>7. Mini vial (2.0 ml)</td>
<td>776</td>
<td>128.099</td>
<td>129,500</td>
<td>98.9</td>
</tr>
<tr>
<td>8. Mini vial (5.0 ml)</td>
<td>862</td>
<td>128.768</td>
<td>129,500</td>
<td>99.4</td>
</tr>
<tr>
<td>9. Std. vial</td>
<td>690</td>
<td>142.734</td>
<td>144,000</td>
<td>99.1</td>
</tr>
<tr>
<td>Chemical quench</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10. Mini vial</td>
<td>653</td>
<td>143.072</td>
<td>144,000</td>
<td>99.4</td>
</tr>
<tr>
<td>Chemical quench</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11. Std. vial</td>
<td>207</td>
<td>119.173</td>
<td>120,800</td>
<td>98.7</td>
</tr>
<tr>
<td>Bray’s cocktail</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12. Micro volume 400 ul</td>
<td>739</td>
<td>10.992</td>
<td>10,760</td>
<td>102.1</td>
</tr>
<tr>
<td>13. Micro volume 200 ul</td>
<td>693</td>
<td>11.209</td>
<td>11,080</td>
<td>101.1</td>
</tr>
<tr>
<td>15. Micro volume 50 ul</td>
<td>590</td>
<td>10.747</td>
<td>10,650</td>
<td>100.9</td>
</tr>
<tr>
<td>16. Micro volume 25 ul</td>
<td>548</td>
<td>10.715</td>
<td>10,460</td>
<td>102.4</td>
</tr>
<tr>
<td>17. Std. Vial - No</td>
<td>989</td>
<td>110.129</td>
<td>111,700</td>
<td>98.6</td>
</tr>
<tr>
<td>Chemical quench</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18. Std. vial</td>
<td>785</td>
<td>111.312</td>
<td>111,700</td>
<td>99.7</td>
</tr>
<tr>
<td>Chemical quench</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19. Std. vial</td>
<td>281</td>
<td>112.279</td>
<td>111,700</td>
<td>100.5</td>
</tr>
<tr>
<td>Chemical quench</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20. Mini Vial - No</td>
<td>862</td>
<td>132.904</td>
<td>133,500</td>
<td>99.6</td>
</tr>
<tr>
<td>Chemical quench</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The mean% recovery is 99.91% with a very small standard deviation of only 1.15. These results conclusively demonstrate that using the efficiency tracing technique produces DPM results which are independent of:

1) Cocktail density variation
2) Different vial sizes
3) Varying sample volume
4) Color quenching
5) Chemical quenching
6) Vial composition

The independence of the DPM values on the chemical quench level of the sample can be further demonstrated using $^{63}$Ni at various quench levels (629, 501, 381, 221) plotted in Figure 3-21. The plot indicates that each efficiency tracing plot (different tSIE values) has a different slope but all intersect the 100% activity line at approximately 197,000 DPM. This clearly indicates that the final DPM results are independent of the color or chemical quench level of the sample.

![Figure 3-21. Efficiency Tracing Curves for $^{63}$Ni at Four Quench Levels.](image-url)
In addition, if efficiency tracing is used for low level DPM samples, results similar to those shown in Figure 3-22 can be expected. A set of samples containing 100 DPM was analyzed by this technique, and 98.813 DPM with a coefficiency of variation of 0.714 was obtained. Similarly from Dr. Ishikawa in Japan, DPM as low as 22.53 ± 1.11 were obtained for a set of low level samples. These results indicate that the efficiency tracing method can be used accurately to determine DPM in low level samples.

![Figure 3-22. Efficiency Tracing of ¹⁴C Sample Containing 100 DPM and Statistics of Four Samples.](image)

This technique can be used for most pure beta and beta-gamma emitters. The one exception is tritium whose efficiency tracing plot can result in large errors (up to 25%) for highly quenched sample. In addition, the efficiency tracing technique is not applicable to radionuclides which decay by isomeric transitions and electron capture (EC). The reason for this is that radionuclides that decay by electron capture are followed by the emission of an x-ray or Auger electron. This makes it difficult to find the true absolute activity of these radionuclides using this technique. ⁶

In summary, the efficiency tracing technique is an accurate and reliable method of calculating DPM (absolute activity) for most pure beta and pure beta-gamma emitters (minimum energy=60 keV). This method requires only counting an unquenched ¹⁴C standard of known activity and does not require the preparation of a quenched curve set for each individual radionuclide. The final DPM for each sample is calculated from the spectrum of each sample compared to that of the standard unquenched sample using six separate spectral regions. The data (DPM) are calculated using a special curve fitting routine and extrapolation technique to determine the absolute activity (DPM) of the sample.

**Efficiency Tracing References**

Cerenkov Counting

Cerenkov radiation is used for quantitating certain radionuclides in a liquid scintillation analyzer (LSA) in the absence of a scintillation cocktail.

When high energy beta particles pass through a medium, they can exchange their energy from the electron ($\beta$-particle) to the molecules of the medium (1,2). The exchanged energy produces localized electronic polarizations along the charged particle's path. As these polarized molecules return to their ground state they release photons in the energy range of 350-600 nm when the velocity of the $\beta$-particle exceeds that of light in the medium.

A certain velocity threshold exists for the production of the Cerenkov radiation. This threshold energy can be calculated from the following equation:

$$E_{\text{min}} = 0.511 \left[ \frac{1}{n^2} - \frac{1}{2} \right] \text{MeV}$$

In this equation 0.511 is the rest mass of the electron in MeV and $n$ is the refractive index of the medium. Using a medium of water and the radionuclides $^{36}\text{Cl}$, $^{32}\text{P}$, and $^{90}\text{Sr}/^{90}\text{Y}$, the $E_{\text{min}} = 263$ KeV. For $^{36}\text{Cl}$ ($E_{\text{max}} = 0.714$), 46% of the particles have an energy higher than the $E_{\text{min}}$ for $^{32}\text{P}$ ($E_{\text{max}} = 1.71$) 86% are above the $E_{\text{min}}$, and for $^{90}\text{Sr}/^{90}\text{Y}$ ($E_{\text{max}} = 0.545$) 61% are above $E_{\text{min}}$. The efficiency in water for these radionuclides is $^{36}\text{Cl}$ (7%), $^{90}\text{Sr}/^{90}\text{Y}$ (25%), and $^{32}\text{P}$ (53%). The highest energy spectrum of the three is for $^{90}\text{Sr}/^{90}\text{Y}$ (SIS = 20.74) and is illustrated in Figure 3-23.

![Figure 3-23. Energy Spectrum for $^{90}\text{Sr}/^{90}\text{Y}$ in 15 mL of H$_2$O.](image-url)
The next highest energy spectrum is that of $^{32}$P, which is used in biotechnology and other research (Figure 3-24). In the biotechnology research area, the use of microfuge tubes (0.5 and 1.5 mL) are routinely employed. With the use of a special Pico vial holder and adaptor ring, these samples can be easily quantitated accurately and the sample can be recovered for further analysis.

The spectrum $^{36}$Cl (SIS = 5.99) is shown in Figure 3-25. This radionuclide is used for the study of ion transport across semipermeable membranes.

The Cerenkov radiation produced is affected by a number of parameters associated with the samples including: (1) volume, (2) chemical quenching agents, (3) color quenching agents, and (4) wavelength shifters. All of these factors were investigated with respect to three radionuclides in H$_2$O, $^{36}$Cl, $^{90}$Sr/$^{90}$Y, and $^{32}$P. The first factor to be investigated is that of volume (Figure 3-26). As can be seen, the best efficiency is found at a volume of greater than 10 mL of sample. As the volume decreases to 1 mL, the recovery of the various radionuclides is reduced by 17% for $^{32}$P, 10% for $^{90}$Sr/$^{90}$Y, and 22% for $^{36}$Cl.
The volume effect is more dramatic for the lower energy radionuclides as shown by the 22% change in recovery for $^{36}\text{Cl}$ (Figure 3-26).

The second factor to be investigated was the effect of a chemical quenching agent, nitromethane, on the % efficiency for the three radionuclides counted in water. Nitromethane, a severe quenching agent in liquid scintillation, shows only a slight effect on the % efficiency for $^{32}\text{P}$, $^{90}\text{Sr}/^{90}\text{Y}$, $^{36}\text{Cl}$ (Figure 3-27). The higher energy radionuclide $^{32}\text{P}$ shows only a 5% loss in efficiency whereas $^{36}\text{Cl}$ shows over a 40% decrease in efficiency. The reduction in the efficiency could be a direct result of the presence of the nitromethane, which could change the refractive index of the medium, and thus increase the $E_{\text{min}}$ and reduce the photon intensity of the Cerenkov radiation. The major reason for the lack of the change of efficiency is that the light is produced directly by the medium and no solvent or fluor interaction is required to produce the photons. The effect of other additives, such as NaCl, HN03, NaOH, acetic acid, and Na2SO4, give less than a 5% deviation from theoretical recovery.

Figure 3-26. The Effect of Volume (1-15 mL) on % Efficiency for $^{32}\text{P}$, $^{36}\text{Cl}$, and $^{90}\text{Sr}/^{90}\text{Y}$.

Figure 3-27. The Effect of Chemical Quenching on % Efficiency for $^{32}\text{P}$, $^{36}\text{Cl}$, and $^{90}\text{Sr}/^{90}\text{Y}$.
The third factor to be investigated is that of the effect of color on Cerenkov counting. A red dye (Ab = 0.090 at 400 nm) was added to the samples in water, and the results are shown in Figure 3-28. This figure indicates that the color red, which absorbs light in the ultraviolet or near ultraviolet region, reduces the number of photons seen by the photomultiplier. This results in a lower % efficiency. The decrease of the % efficiency is from 7.5% to 1.0% for $^{36}$Cl, 49.8% to 13.1% for $^{32}$P, and 22% to 11% for $^{90}$Sr/$^{90}$Y.

Color quenching is similar to that found in liquid scintillation counting. Color quenching can be compensated for by using one or a combination of the following techniques. First, the sample can be decolorized (bleached) to remove all of the color. This is a rather time consuming process. Second, an internal standard of known amount and efficiency can be added. The sample is recounted and the accurate DPM are determined. The major problem is that the sample has been destroyed with the addition of the internal standard radioactivity. Third, a channels ratio can be determined. Two different channels are chosen and a plot of channels ratio vs. % efficiency is constructed. Probably the best and easiest method of obtaining true DPM present in the sample is to use the Quench Indicating Parameter (SIS) vs. efficiency. The accuracy of the SIS scheme to correct for color quenching in the sample is illustrated in Table 3-3.

The fourth factor is the use of a wavelength shifter to shift the energy of the photons from a region of low photomultiplier tube sensitivity to a region of high photomultiplier tube sensitivity. This helps to eliminate any directional properties of Cerenkov radiation and produce an isotropic photon emission characteristic of the radionuclide. The wavelength shifters include: acridine, coumarin, B-naphthol, 4-methylumbelliferone, and 7-amino-1, 3-naphthalenedisulfonic acid. These wavelength shifters show the greatest effects on low energy beta emitters by increasing the efficiency fourfold for $^{36}$Cl and 1.6 fold for $^{32}$P. These wavelength shifters must be added to the sample, thus eliminating the possibility of recovering the sample.
Cerenkov Counting References

THEORY OF DUAL LABEL DPM MEASUREMENTS
Theory Of Dual Label DPM Measurements

Introduction

Measuring a sample containing two radionuclides requires different considerations than those used for single label measurements. The two radionuclides each contribute to the scintillations that generate pulses. All beta-emitting radionuclides produce a continuum as a spectrum, and the spectrum extends from zero to the beta-maximum energy. If two radionuclides are contained in the same sample, the composite spectra will extend from zero to the beta-max of the higher energy radionuclide. A region from zero to the beta-maximum of the lower energy radionuclide will inevitably record events from both radionuclides. These are indistinguishable in any liquid scintillation system. The illustration in Figure 4-1 is a graphical representation of a composite dual label spectrum. To separate the events and estimate the contribution of each radionuclide using conventional methods of activity analysis, the information stored in the Spectralyzer™ spectrum analyzer requires two discrete energy regions for analysis of the data or Packard’s patented single energy region Spectrum Unfolding technique.

![Figure 4-1. Dual Label Composite Spectrum.](image1)

![Figure 4-2. Dual Label Region Settings.](image2)

Conventional Dual Label DPM

Figure 4-2 shows how two regions, Region A and Region B, can be set to analyze the contribution of each radionuclide. This diagram illustrates the method. (It is not drawn to scale). Because the area under each radionuclide profile is different, the accumulated counts from each radionuclide are not the same. Mathematically, the count contribution of each radionuclide to each region can be calculated from the following expressions in the computation of dpm(D).

Let

\[ A = \text{total cpm in Region A, and} \]
\[ B = \text{total cpm in Region B.} \]
\[ A = \text{cpm from the lower energy radionuclide +} \]
\[ \text{cpm from the higher energy radionuclide.} \]

But

\[ \text{Eff} = \frac{\text{cpm}}{\text{dpm(D)}} \]
\[ \text{Therefore cpm}=E \ast \text{dpm(D)} \]
Substituting in \( A = (E_{LA} \cdot D_L) + (E_{HA} \cdot D_H) \)

where the suffix LA refers to the lower energy radionuclide in Region A.

The suffix HA refers to the higher energy radionuclide in Region A.

\( D_L \) is the dpm of the lower energy radionuclide

\( D_H \) is the dpm of the higher energy radionuclide.

Substituting in \( B = (E_{LB} \cdot D_L) + (E_{HB} \cdot D_H) \)

where the suffix LB refers to the lower energy radionuclide in Region B.

the suffix HB refers to the higher energy radionuclide in Region B.

therefore

\[
D_L = \frac{A \cdot E_{HB} - B \cdot E_{HA}}{E_{LA} \cdot E_{HB} - E_{LB} \cdot E_{HA}} \quad \text{(equation 1)}
\]

and

\[
D_H = \frac{B \cdot E_{LA} - A \cdot E_{LB}}{E_{LA} \cdot E_{HB} - E_{LB} \cdot E_{HA}} \quad \text{(equation 2)}
\]

In the general case, where each radionuclide produces counts in both channels, the theoretical determination of optimum counting conditions has been derived. The reader is referred to P. D. Klein and W. J. Eisler, Anal. Chern. 38, 1453 (1966) for further details.

The above refers to the general case where each region receives counts from both radionuclides. It is possible to simplify these expressions in the special case where the lower limit to Region B is set to exclude events due to the lower energy radionuclide. will now be discussed.

In this case \( ELB = 0 \)

thus, reducing equation 1 to

\[ D_L = \frac{A \cdot E_{HB} - B \cdot E_{HA}}{E_{LA} \cdot E_{HB}} \quad \text{or} \quad \frac{A - (D_H \cdot E_{HA})}{E_{LA}} \]

and equation 2 to \( D_H = \frac{B}{E_{HB}} \)

Measuring samples of low activity where the background count rate in each region is significant requires modification to the formula A and B become the net cpm after subtraction of the background count rate.

An acceptable separation in the measurement of two radionuclides in one sample is obtained when the \( \beta \)-maximum energy ratio of the radionuclides is in excess of 2-3 to 1. Consider the case of dual labeled \(^3\)H and \(^14\)C with \( \beta \)-maximum energies of 18.6 keV and 156 keV respectively, the energy ratio is approximately 8.4 to one, which provides excellent separation. The Tri-Carb analyzer sets the region limits as 0-12 keV for Region A and 12-156 keV for Region B.
The determination of the activity of the lower energy radionuclide is calculated after subtracting the counts from the higher energy radionuclide from the lower energy region \((D_{HA} E_{HA})\). This cpm value must be subtracted from the gross cpm in Region A. If the number of counts of the higher energy radionuclide in the lower energy region is large compared with the counts due to the lower energy radionuclide, this subtraction can result in a small number. The accuracy of the result is decreased since small variations in this subtracted figure can produce large errors. Therefore, it is an advantage to maintain the counting efficiency of the higher energy radionuclide Region A as low as is practical. This can be accomplished by lowering the upper limit in Region A.

Inevitably this results in a decrease in the measuring efficiency of the lower energy radionuclide in Region A thus a compromise between radionuclide separation and counting efficiency is necessary.

The compromise will be influenced by the relative count rates of the two radionuclides. The user must determine region limits according to the experiment. As a guide to selecting the upper limit of Region A, the following procedure is suggested for the measurement of \(^3\)H and \(^14\)C.

Load an unquenched \(^3\)H standard and enter into conversation with the system. Determine the count rate of this standard with Region A set between 0 and 19 keV, record this value. Change the upper limit to 18 keV and record the cpm, repeat this procedure with the upper limit at 17, 16, 15 keV, etc. down to 4 keV. Calculate the counting efficiency of the \(^3\)H standard at each region setting. Unload the \(^3\)H standard and load an unquenched \(^14\)C standard. Repeat the above procedure using the same region upper limits for measuring \(^14\)C, calculate the measuring efficiency at each setting. Plot a graph of the results with the ordinate as \(^3\)H efficiency and the abscissa as \(^14\)C efficiency. Each value is the \(^3\)H and \(^14\)C efficiency with the same region limits (Figure 4-3).

It is seen from the graph that lowering the upper limit of Region A significantly reduces the spill of \(^14\)C into the region, while sacrificing little \(^3\)H efficiency. Below the knee of the graph the loss of \(^14\)C efficiency in Region A produces a corresponding loss in \(^3\)H efficiency. The suggested optimum region settings are those through the knee of the curve, as indicated by the dotted region of Figure 4-3.

![Figure 4-3. Efficiency of Both Radionuclides in Dual Label Measurements in the Region A.](image)
Operating the liquid scintillation system with AEC will maintain the separation determined above. Refer to Figure 4-4 for the 4 dual label efficiency curves using AEC.

The preceding explanations offer methods of determining sample activity by plotting the results of measuring standards and using data to manually calculate sample activity. The liquid scintillation systems can be provided with the dpm option which automatically computes the sample activity for single and dual labeled samples. Measurement of a series of quenched standards automatically establishes the efficiency correlation, stores this data and also plots the curves with the printout device. Subsequent sample measurements produce a QIP and by interpolation fits the value to the stored data by a "dual fixed point least square quadratic." The calculated efficiency factor allows the dpm of the radionuclide to be obtained and presented to the user on the system's printer.

Within the limits of the correlation, the computer fits the sample QIP by a least square calculation of a quadratic equation forcing the curve produced through the fixed points either side of the sample QIP. This interpolation procedure is more accurate than polynominal expression which gives equal weight to all calibration points of the correlation curve. At the extremities of the efficiency curves, the calculation of efficiency is by linear interpolation or linear extrapolation for samples with a QIP beyond the last point of the calibration.

To achieve the highest precision over the complete range of quenching, it is recommended that 10 standards be used and the QIP spacing of these standards should be fairly even.

![Figure 4-4. Dual Label Efficiency Curves Using AEC.](image-url)
LIQUID SCINTILLATION ANALYSIS

Calculation of DPM Error With Dual Label Samples

In the measurement of a sample containing two radionuclides, inevitably the regions or channels of the instrument will record events from both radionuclides. The contribution from each radionuclide to the recorded counts depends upon the relative activities of the two radionuclides and the degree of quenching in the scintillation cocktail. The following is an explanation of the statistical uncertainty in the computed activity of the lower energy radionuclide in dual label counting.

To simplify the explanation, let us assume the two radionuclides involved are $^3$H and $^{14}$C, the beta maximum energies of which are 18.6 and 156 keV, respectively. The same reasoning will apply to any pair of radionuclides, provided the $\beta$-max energies are different by at least 3:1.

If a sample containing $^3$H and $^{14}$C label is counted on a Tri-Carb liquid scintillation analyzer, the Spectralyzer spectrum analyzer will store the counts according to the amplitude of the pulses. As the spectrum of pulse heights is a continuum extending from zero to the maximum beta energy for each radionuclide, a composite spectrum is produced. Counts are, therefore, stored from 0 to 156 keV. The liquid scintillation process does not distinguish between the source ($^3$H or $^{14}$C) of these counts. Thus, between 0 and 18.6 keV all $^3$H events are stored and all the $^{14}$C events which have energies between 0 and 18.6 keV are also stored. Beyond 18.6 keV, that is, from 18.6 to 156 keV, the store will contain only $^{14}$C events. One approach to the separation of the events according to the radionuclide is to set the lower limit of Region B at 18.6 keV and, thus, exclude all $^3$H from Region B. For several reasons this is not necessarily the optimum setting.

We will consider the more general case where Region A and Region B both include counts from $^3$H and $^{14}$C. The number of counts in each region will, of course, depend upon how much $^3$H and $^{14}$C is contained in the sample. If all samples were unquenched, theory would tell us the percentage of $^{14}$C contribution to the total counts below 18.6 keV. To unravel this puzzle with quenched samples, it is necessary to conduct a second measurement to determine the percentage of each radionuclide in the two energy regions used.

This measurement is to determine the level of quenching and involves the use of the external standard to obtain the QIP value. By counting a series of quenched $^{14}$C standards the measured efficiency of $^{14}$C in Region A and in Region B can be determined. The efficiency is related to QIP for each of the standards and the percentage of $^{14}$C in each region is calculated. Similarly, counting a series of quenched $^3$H standards will produce an efficiency correlation of the percentage of $^3$H in the two regions at different levels of quenching. This efficiency correlation data is stored. The measurement of a dual label sample produces the SIE and the counts per minute in Region A and Region B. Now we have the data necessary to calculate the DPM of each radionuclide.

Let us assume:

(a) The instrument is perfect; i.e., it is efficient and stable.

(b) The efficiency correlations are valid with the settings used.

(c) The mathematical interpolation of the efficiency is not subject to error.

Thus, the following is based only on statistics.

The calculation requires the solution to four equations; the count rate contribution of $^3$H in Region A, $^3$H in Region B, $^{14}$C in Region A and $^{14}$C in Region B. Efficiency is obtained from the stored correlations by a Dual Fixed Point Least Square Quadratic interpolation.
Perhaps the most realistic approach is to consider the uncertainty of determining the \(^3\)H activity in the sample. It is this value which has the greatest apparent uncertainty.

The uncertainty in calculating \(^3\)H activity is directly related to the ratio of counts in Region B and Region A and the efficiency of measuring \(^3\)H and \(^14\)C in Region B.

There are two major factors in the statistical uncertainty in calculating \(^3\)H activity:

1. the ratio of counts per minute in the two regions (K), and
2. the \(^14\)C measuring efficiency in the two regions R(C). These values are defined as:

\[
K = \frac{\text{Region } B \text{ cpm}}{\text{Region } A \text{ cpm}} \quad \text{for dual label sample}
\]

\[
R(C) = \frac{\text{Measuring efficiency for } ^{14}C \text{ in Region } A}{\text{Measuring efficiency for } ^{14}C \text{ in Region } B}
\]

The following graph is plotted using these parameters. Thus, to determine the uncertainty of the \(^3\)H dpm, calculate K from the measured data and look up the value of R(C) at the sample quench level (tSIE) from the efficiency correlation curves. With this data, determine the F value. The F value is a relationship between the value of K and R(C) and provides a factor used in calculating the uncertainty in determining the tritium activity. The \(^3\)H uncertainty expressed as a percentage is as follows:

\[
\text{UNCERTAINTY } ^3H = \frac{F}{\sqrt{A}} \times 100\%
\]

where A is the cpm in Region A x Time

**REMEMBER, This Is The Theoretical And Neglects Other Errors Associated With Counting.**

The uncertainty in calculating \(^3\)H activity is also subject to the precision of determining the region cpm and QIP values, i.e., the normal statistics of a measurement.

Let us consider a hypothetical example of a measurement where a level of quenching is indicated by a tSIE of 450. Using the Automatic Efficiency Control and the preset \(^3\)H/\(^14\)C setting, the following values are typical.

(a) \(^14\)C measuring efficiency in Region A is 13%,
(b) \(^14\)C measuring efficiency in Region B is 77%.

This will yield an R(C) value of 0.169.

Let us further assume the count rate obtained with a five minute measurement from this hypothetical sample is:

5675 cpm in Region A and 2125 cpm in Region B

This gives a K value of 0.375

Referring to the graph (Figure 4-5), the F factor is between 1.05 and 1.1 and will be interpreted as 1.09.

Substituting this value in the equation gives the uncertainty of:

\[
\frac{1.09 \times 100\%}{\sqrt{5675 \times 5}} = 0.65\% \text{ in the DPM of } ^3H
\]
A sample of similar quenching and, therefore, similar R(C) value but where the Region A cpm = 2837 and Region B cpm = 2672 where K = 0.942 gives a F value of 1.20 and an uncertainty of

\[
\frac{1.20 \times 100\%}{\sqrt{2837 \times 5}} = 1.0\%
\]

**Figure 4-5.** Chart to Calculate F Value for Uncertainty of \(^3\)H DPM Value.

**Full Spectrum Dual Label DPM**

Full Spectrum DPM is a patented technique developed by Packard Instrument Co. to calculate the activities of the individual radionuclides of dual labeled samples by using a Spectrum Unfolding technique.

Rather than counting in discrete regions to separate the two radionuclides, spectrum unfolding decomposes the composite spectrum into two components. Each component represents the count contribution of the particular radionuclide to the total activity of the dual labeled sample.
Spectrum Unfolding

The key to spectrum unfolding is the Spectral Index of the Sample (SIS). At a given level of quenching, each radionuclide has a very definite pulse height distribution and hence, a unique SIS index can be calculated. When two radionuclides are combined in a single sample, the resulting pulse height distribution is the sum of the two individual distributions (refer to Figure 4-6).

![Composite Spectrum of a Dual Labeled Sample (³H and ¹⁴C).](image)

The SIS of the total distribution is a function of the SIS of the individual distributions and the fractional counts of each radionuclide. If SISL and SISH are the spectral index values of the low and the high energy radionuclide, then the SIS of the total distribution (SIST) can mathematically be calculated as follows.

\[
SIST = \frac{SIS_L \cdot \sum N_L(E) + SIS_H \cdot \sum N_H(E)}{\sum N_L(E) + \sum N_H(E)}
\]  

(1)

\[\sum N_L(E) = \text{accumulated counts from the low energy radionuclide} \]

\[\sum N_H(E) = \text{accumulated counts from the high energy radionuclide}.\]

This can be rewritten as:

\[
SIST = SIS_L \cdot \frac{\sum N_L(E)}{T} + SIS_H \cdot \frac{\sum N_H(E)}{T}
\]

(2)

where \(T = \sum N_L(E) + \sum N_H(E) = \text{total counts accumulated from sample}\)

or

\[
SIST = SIS_L \cdot F_L + SIS_H \cdot F_H
\]

(3)
LIQUID SCINTILLATION ANALYSIS

In this equation:

\[ F_L = \frac{\sum N_L(E)}{T} \] : count fraction of low energy radionuclide

\[ F_H = \frac{\sum N_H(E)}{T} \] : count fraction of high energy radionuclide

\[ F_H + F_L = \frac{\sum N_H(E) + \sum N_L(E)}{T} = 1 \] (4)

\[ F_H = 1 - F_L \] (5)

Substituting (5) in (3) gives:

\[ SIS_T = SIS_L \cdot F_L + SIS_H \cdot (1 - F_L) \]

\[ = SIS_H - F_L (SIS_H - SIS_L) \] (6)

\[ F_L = \frac{SIS_H - SIS_T}{SIS_H - SIS_L} \] (7)

\[ F_L \] can also be expressed as:

\[ F_L = \frac{CPM_L}{CPM_T} \] (8)

Substituting (8) in (7) yields:

\[ CPM_L = \frac{SIS_H - SIS_T}{SIS_H - SIS_L} \cdot CPM_T \] (9)

The CPM of the high energy component can be derived in a similar way:

\[ CPM_H = \frac{SIS_T - SIS_L}{SIS_H - SIS_L} \cdot CPM_T \] (10)

For a particular quench level, the count rates of the individual radionuclides of a dual labeled sample can be derived from the measured CPM and SIS when the individual SIS values for each radionuclide are known. When the counting efficiencies for each radionuclide at that particular quench level are also known, then the individual DPM can be calculated.

DPM calculations are thus very straightforward, and are performed at maximum efficiency levels for each radionuclide. No spillover corrections are required since spectrum unfolding gives the actual CPM for each radionuclide.
Determining Unknown DPM

In order to be able to calculate CPM and DPM over the entire quench range, SIS versus tSIE and efficiency versus tSIE correlation curves must be stored for each radionuclide (refer to Figure 4-7). These quench correlation curves are established by counting a quench series of reference standards for each radionuclide.

To determine the individual activity (CPM and DPM) of each radionuclide of a dual labeled sample, the quench level must be measured (tSIE) and this value is used to interpolate the SIS and efficiencies of the individual radionuclides from the respective quench correlation curves. The latter values are used to calculate the count rates and the disintegration rates of both radionuclides of the dual labeled sample.

*Figure 4-7. Quench Correlation Curves for Full Spectrum DPM.*
Full Spectrum DPM Versus Conventional DPM

Spectrum unfolding introduces the concept of regionless counting. This eliminates user defined energy regions or windows for each radionuclide of interest. Optimization of counting regions is no longer required and errors due to nonreproducible settings are eliminated. Complicated automatic region tracking schemes to maintain optimal settings are obsoleted. A detailed understanding of the principles of liquid scintillation counting is no longer required to accurately measure the radioactivity in dual labeled samples. The zero spillover concept maximizes the counting efficiencies for each radionuclide. At each quench level, optimum counting accuracy for each radionuclide is obtained since they are counted at maximum efficiency (refer to Figure 4-8).

Spectrum unfolding offers the capability to compute both CPM and DPM for the individual radionuclides. Actual radionuclide activity can be monitored in real time, error calculations are based on the individual activities and the preset error condition reflects the actual total accumulated counts for the radionuclide. Only one calibration curve per radionuclide is required and errors in DPM calculations due to user modified regions/windows are eliminated.

Spectrum unfolding eliminates a significant limitation to the present conventional state of-the-art techniques for dual label counting. These techniques require a substantial working knowledge of nuclear physics and chemical interaction to produce correct final results. Full Spectrum DPM on the other hand ensures precise results with little or no prior knowledge of the technique. The spectrum unfolding approach is capable of determining dual label activities simultaneously without regard to multiple discrete energy thresholds. This technique gives the most accurate DPM values in the range of 50:1 to 1:8 ratios of lower to higher energy radionuclides.
Statistics of Liquid Scintillation Counting
Statistics Of Liquid Scintillation Counting

Introduction

The reason that statistics are considered in nuclear counting using a liquid scintillation counting system is that radionuclide decay, by its very nature, can emit its beta particle at any time (random decay). While it is impossible for us to determine when a nuclear decay will occur, statistics allow us to describe the average behavior of the all nuclear decays within a sample. In order to deal with this random decay for radionuclides, counting statistics are used. Statistics are used to express the probability of obtaining a given count within a certain defined confidence limit. Thus, the counts (cpm or dpm) are expressed in terms of a percent two sigma value (%2s standard deviation) from a mean count.

Statistics Of Nuclear Counting

Statistics in liquid scintillation counting can be most easily explained by counting a single radioactive sample ten times. The data from this experiment are shown in Table 5-1. As can clearly be seen, the cpm results are not identical for any of the counting times. How can we obtain the statistics for these 10 counts? If one goes to basic statistics, the distribution of these values can be expressed as normal or Gaussian distribution.

<table>
<thead>
<tr>
<th>Count # (i)</th>
<th>cpm(x)</th>
<th>x-(x)</th>
<th>(x-(x))^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>46,704</td>
<td>89</td>
<td>7,921</td>
</tr>
<tr>
<td>2</td>
<td>46,685</td>
<td>70</td>
<td>4,900</td>
</tr>
<tr>
<td>3</td>
<td>46,627</td>
<td>12</td>
<td>144</td>
</tr>
<tr>
<td>4</td>
<td>46,495</td>
<td>-120</td>
<td>14,400</td>
</tr>
<tr>
<td>5</td>
<td>46,827</td>
<td>212</td>
<td>44,944</td>
</tr>
<tr>
<td>6</td>
<td>46,566</td>
<td>-49</td>
<td>2,401</td>
</tr>
<tr>
<td>7</td>
<td>46,514</td>
<td>-101</td>
<td>10,201</td>
</tr>
<tr>
<td>8</td>
<td>46,587</td>
<td>-58</td>
<td>3,364</td>
</tr>
<tr>
<td>9</td>
<td>46,548</td>
<td>-67</td>
<td>4,489</td>
</tr>
<tr>
<td>10</td>
<td>46,625</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>Sum</td>
<td>466,148</td>
<td></td>
<td>92,864</td>
</tr>
<tr>
<td>Mean ((\bar{x}))</td>
<td>46,615</td>
<td></td>
<td>(92,864 / (10 - 1))</td>
</tr>
</tbody>
</table>

These data can be described by calculating two parameters: the mean and the standard deviation. The mean (\(\bar{x}\)) is defined as the sum of the cpm (defined as x) values divided by the number of times the sample was counted (i). Thus from Table 5-1, 466,148 cpm/10=46,615 which is the mean \(\bar{x}\) cpm. Next, the standard deviation of counting these 10 samples must be obtained. This is done by using the following equation:

\[
\text{Standard deviation} = s = \sqrt{\frac{\text{Sum} \ (x-\bar{x})^2}{i-1}}
\]
Example of Standard Deviation Calculation

Example 1: Analysis of Data from Table 5-1

\[ S.D. = \sqrt{\frac{92,864}{9}} \]
\[ S.D. = 101.58 \ (102.0) \]

Thus, the counting results for this sample counted ten times would be expressed as the (mean) 46,615 ± S.D. or 46,615 ± 102 cpm.

Now that the standard deviation value can be calculated, what does this S.D.(± 102 cpm) really mean for nuclear counting?

This can be easily demonstrated in Figure 5-1, which displays a normal (Gaussian distribution) superimposed on the actual count data (Table 5-1). The count data is expressed as a histogram or bar chart.

![Figure 5-1: Normal Distribution and Count Data for a Radioactive Sample Counted Ten Times.](image)

The normal distribution curve is calculated by the equation:

\[ y = e^{-(x-x_0)^2/2\sigma^2} \quad \text{where } x_0 = 46,615 \ \text{cpm (mean)} \]
\[ \text{and the standard deviation (s) is 102 cpm.} \]

Thus, the probability of the true count occurring within ±s of the mean is 68%. The 68% is called the confidence level for s (standard deviation). Since nuclear counting requires a higher confidence level than 68%, a 2s value is used and defined as a 95.5% confidence limit.

Now, how can these values be used in nuclear counting? From basic statistics, we know that the expected standard deviation is equal to the square root of the total number of counts. This is the master equation for calculation of counting statistics.

\[ S.D. = s = \sqrt{\text{Total Counts}} \]
Calculation of Counting Statistics

As was shown earlier, the standard deviation is calculated as the square root of the total counts detected in the sample. This result has a confidence limit of 68%, but nuclear counting uses the $2s$ value (95.5% confidence limit). A typical calculation is shown in Example 2.

Example 2: If 9,500 counts are counted in 1.0 minutes, what is the $2s$ value?

\[
\text{If } s = \sqrt{\text{Total Counts}} \quad \text{then} \\
2s = 2\sqrt{\text{Total Counts}} = 2\sqrt{9,500} = 2(97.5) = 195 \\
\]

Therefore, with a confidence limit of 95.5%, the resultant counts (Example 2) would be expressed 9,500 ± 195 counts. This is a rather cumbersome method of expressing the results (very large numbers can be obtained). In order to make it more convenient, a %$2s$ value is calculated. The %$2s$ can be calculated for either Equation 1 or Equation 2 (a mathematical reduction of Equation 1). The %$2s$ for the data of Example 2 is shown in Example 3.

\[
\begin{align*}
\text{Equation 1: } \%2s &= \frac{(100) \times 2 \times s}{\text{Total Counts}} \\
\text{Equation 2: } \%2s &= \frac{200}{\sqrt{\text{Total Counts}}} \\
\end{align*}
\]

Example 3: Calculation of %$2s$ for a sample count

\[
\begin{align*}
\%2s &= \frac{(100)(97.5)}{9,500} = 2.05\% \quad \text{(Equation 1)} \\
\%2s &= \frac{200}{\sqrt{9,500}} = 2.05\% \quad \text{(Equation 2)} \\
\end{align*}
\]

Thus, the final result can be expressed as 9,500 counts ± 2.05% with a 95.5% confidence level.

Count Rate Statistics

Since most methods in nuclear counting analyze the cpm value rather than the total counts in a sample, it is important to determine the count rate statistics. How does this effect the statistics of counting with reference to count rates?

If you now count the same sample three minutes, you increase the total counts threefold. The increased sample size of 28,500 total counts from 9,500 increases the accuracy of the measurement. This is indicated by the smaller percentage of the $2s$ value of 338 counts for 28,500 total counts.

Example 4: Calculation of $2s$ for count rate

\[
\begin{align*}
2s &= 2\sqrt{\text{Total Count}} = 2\sqrt{\text{cpm} \times \text{time}} \\
&= 2\sqrt{9,500 \times 3} = 2\sqrt{28,500} = 338 \text{ counts} \\
\end{align*}
\]
The total counts can be reduced to cpm ± 2s by dividing both the total counts and its 2s value by the counting time of 3 minutes:

\[
\frac{cpm \pm 2s}{\text{Counting Time}} = \frac{\text{Total Count}}{\text{Counting Time}} + \frac{2s}{\text{Counting Time}}
\]

\[
= \frac{28,500 \pm 338}{3}
\]

\[
= 9,500 \pm 113 \text{ cpm}
\]

Mathematically, the 2s calculation for any net cpm can be reduced to:

\[
2s = \pm 2 \sqrt{\frac{cpm}{\text{time}}}
\]

\[
= \pm 2 \sqrt{\frac{9,500}{3}} = \pm 2 \sqrt{3,166}
\]

\[
= \pm 113 \text{ cpm}
\]

Thus, the final results would be expressed as 9,500 ± 113 cpm. Now, that the 2s value has been calculated for a count rate, how can the %2s (value normally printed out for a sample analyzed by a nuclear counter) be calculated? This can be calculated using Equation 3.

\[
\text{Equation 3: } \%2s = \frac{100 \times 2}{\sqrt{cpm \times \text{counting time (min.)}}}
\]

Example 5: Calculation of %2s for count rate for 9,500 cpm counted for 3 minutes.

\[
\%2s = \frac{(100) \times 2}{\sqrt{(9,500 \text{ cpm}) (3 \text{ min.})}} = \frac{200}{\sqrt{28,500}} = 1.18%
\]

Thus, in counting the sample for three minutes compared to the one minute count, the %2s has been reduced from 2.05% (Example 3) to 1.18% (Example 5). This value (%2s) is inversely proportional to the total counts and to the count time.

**Determination of Counting Time for Certain %2s Value**

The next question is how are these parameters used in liquid scintillation counting (LSC)? Normally in LSC, there are two different methods of terminating the counting of a sample. The first method is called preset time. This method stops counting the sample after the user-defined time. The second method is to terminate the counting based on a certain %2s value, so that, all of the samples are counted with the same statistical precision. This counting time can be calculated using the following Equation 4:

\[
\text{Equation 4: } T(\text{time = minutes}) = \frac{1}{\text{cpm}} \left[ \frac{200}{\%2s \text{ desired}} \right]^2
\]
Example 6: Calculation of the Time Required to obtain a %2s of 0.5% for a sample containing 9,500 cpm.

\[
T = \frac{1}{9,500} \left[ \frac{0.5}{200} \right]^2 \\
T = 16.84 \text{ minutes}
\]

Counting a sample containing 9,500 cpm to a %2s value of 0.5%, the time required to count the sample would be 16.84 minutes. In modern liquid scintillation counters, a preset %2s value can be entered into the counting conditions and the instrument automatically terminates when enough counts have been accumulated.

From this information described above, a nomogram can be prepared from which the count time and/or %2s can be calculated if the cpm of the sample are known. The relationship between all three parameters is shown in Figure 5-2.

---

*Figure 5-2: Nomogram Used to Calculate Statistics in Nuclear Counting.*
The following examples demonstrate how to use this nomogram. If a %2s of 0.5% for a sample containing 9,500 cpm is required, the count time can be obtained from the nomogram. One determines the count time by placing an X at 9,500 on the cpm scale and an X at 0.5 on the %2s scale (Figure 5-3).

Figure 5-3: Nomogram to Determine Counting Time for a Defined %2s Level-Single Isotope.
A line is drawn between these two points (to the A-line). A line is then drawn from the A-line to zero% crossover (single label). A line is drawn between these two points. The point (B) where the line crosses the minute line (16.8 min) is the time required to obtain 0.5% 2s statistics for this sample. Second, a sample containing 85,000 cpm is counted for 10 minutes, what is the %2s of this measurement (Figure 5-4)?

![Nomogram to Determine %2s of Measurement - Single Isotope.](image)

In summary, this nomogram can be used to determine the %2s, cpm, or counting time for a single label sample if two of three parameters are known.
Statistics of Net Count Rate

The next case is that of determining the statistics of net count rate. This is used most commonly for samples in which the count rate is near the background level or for dual-labeled samples. First, the case of a low level sample (counts of sample and background similar) will be discussed. As often is the case in certain specialized applications, such as assessment of $^3$H in ground water, $^{14}$C dating, and low level environmental monitoring, the sample and the background count rates can be very similar. For these samples, a net count value must first be obtained using Equation 5.

Equation 5: \[ \text{Net count} = \text{counts (sample)} - \text{counts (background)} \]

Thus for net counts, two separate parameters (background and sample count) must be considered as each having its own associated uncertainty. From statistics, it is known that the uncertainty of two parameters can be expressed using the rules for the propagation of error. This states that the error (standard deviation) for these parameters is equal to the square root of the sum of the squares of the two uncertainties (Equation 6).

Equation 6: \[ \text{S. D. (net counts)} = \sqrt{s^2 \text{ sample} + s^2 \text{ background}} \]

This equation can be further reduced by algebraic manipulation and substitution to obtain Equation 7:

Equation 7: \[ \frac{\text{S. D. (net cpm) expressed as cpm}}{\text{cpm}} = \sqrt{\frac{\text{CPM}_{\text{sample}}}{T_{\text{sample}}} + \frac{\text{CPM}_{\text{bkg}}}{T_{\text{bkg}}}} \]

Thus the S.D. for a net cpm can be expressed as the square root of the sum of the cpm divided by counting time of sample, and the cpm divided by the counting time of the background. This is illustrated in Example 7. A background sample is counted for 10 minutes, and 10 cpm is printed out. An unknown sample is counted for 200 minutes with 50 cpm being obtained.

Example #7: Now consider this sample which could only be found commonly in a laboratory situation

\[ s = \sqrt{\frac{50 \text{ cpm}}{200 \text{ min.}} + \frac{10 \text{ cpm}}{10 \text{ min.}}} = \sqrt{0.25 + 1} \]

\[ s = 1.12 \]

The result for this sample would thus be expressed as the net cpm \( (\text{CPM}_{\text{sample}} - \text{CPM}_{\text{bkg}}) \pm s \), or for this example, \( (50 \text{ cpm} - 10 \text{ cpm}) \) or \( 40 \pm 1.12 \text{ cpm} \).
Now in order to calculate the %2s for this sample, Equation 8 must be used.

Equation 8: Calculation of %2s, for net cpm calculation.

\[
\%2s = \frac{100 \times 2 \times s}{cpm_{sample} \times cpm_{BKG}}
\]

Using this equation the %2s can be calculated for the Example 7 data.

\[
\%2s = \frac{100 \times 2 \times 1.12}{40} = 5.60\%
\]

Thus the result would be expressed as:

\[40 \text{ cpm} \pm 5.6\%\]

As can be seen, this is a much larger error than the results obtained with background not included in the calculation (40 cpm ± 2.0%).

\[
\%2s = \frac{200}{\sqrt{\text{Total Counts}}} \text{ or } 2.0\%
\]

Using this example, it is clear that the background can contribute considerably to the error in the result if number of counts in the sample is low and close to those found in the background. Instead of going through all of these calculations, the following expression can be used:

\[
\%2s \text{ of net count rate} = \%2s \text{ (sample)} \times F
\]

\[
F = \frac{\sqrt{1 + RP}}{1 - R}
\]

\[
R = \frac{CPM_{BKG}}{CPM_{sample}}
\]

\[
P = T_s/T_{BKG}
\]

\[
F = \frac{\sqrt{1 + (0.2/20)} - 2.80}{1 - 0.2}
\]

\[
\%2s \text{ of net count rate} = \%2s \text{ gross count rate} \times F
\]

\[
\%2s(S - B) = 5.60\% = (2.0)(2.80)\%
\]

If optimized conditions are desired, the sample and the background should be counted for the same time period. Take for example the previous sample counting both for 10 minutes (gross count rate of sample= 50 cpm and Background= 10 cpm). In order to use the nomogram, we must first calculate the % crossover (amount contributed to total counts by the background). The % crossover is calculated by the following equation:

\[
\% \text{ crossover} = \frac{CPM_{BKG} \times 100\%}{CPM_{sample}} = \frac{1,000}{50} = 20\%
\]
Now, go to the far right-hand scale of the nomogram to the 20% crossover and place an X. Next, place an X at the length of time the sample was counted (10 min.). Draw a line between these two X’s until it crosses the A line. Place an A at this point and place an A on the cpm scale (total cpm = 50) and connect these two A’s with a line. Where the line crosses the % 2s line (B) is the % error of counting (12.24%) this sample (Figure 5-5).

*Figure 5-5. Special Nomogram for Statistics of Samples with Low Count Rates - Single Isotope (Example of Calculation of %2s).*
Nothing in the previous discussion states that background is the only source of activity which could be considered. The background activity could be due to cosmic radiation or an unrelated radiolabeled material present in the preparation. Counting dual label ($^3$H and $^{14}$C) samples is very similar to the previous discussion of a blank or background sample. In dual label counting, we can have crossover of $^3$H into the $^{14}$C region and $^{14}$C into the $^3$H region. The interference of the $^3$H into the $^{14}$C region (lower energy isotope into high energy isotope region) can be eliminated by choosing a counting region which lies above the endpoint of the low energy spectrum (isotope exclusion method). The interference of $^{14}$C into the $^3$H region cannot be eliminated or avoided, and must be compensated for in the calculations. The net count rate of the low energy label in region A can be calculated by subtracting the contribution of spillover from the total count rate:

$$\text{cpm (low energy)} = \text{cpm Total - cpm (spillover $^{14}$C and $^3$H)}$$

The % crossover can be calculated using the efficiency of $^{14}$C in the $^3$H region ($E_{hA}$), the efficiency of $^{14}$C in the $^{14}$C region ($E_{hB}$) and the CPM$^b$.

$$\text{cpm (crossover)} = \frac{\text{CPM (high energy)}}{\text{EhA}} \times \frac{\text{EhB}}{\text{CPM}^b}$$

$$\% \text{ crossover} = \frac{\frac{\text{CPM}^b \times \text{EhA}}{\text{CPM}^b}}{\text{EhB}}$$

Now let’s use the nomogram to do the following example for a previously counted sample:

$$\begin{align*}
\text{EhA} &= 12.2 \\
\text{EhB} &= 82.4 \\
\text{CPM}^a &= 15,818 \\
\text{CPM}^b &= 87,800
\end{align*}$$

$$\% \text{ crossover} = \frac{100\% \times \frac{12.2}{82.4} \times 87,800}{15,818}$$

$$\% \text{ crossover} = 82.2$$

The nomogram is then used to calculate the time required to obtain %2s of 1% for the low energy isotope. First, the far left axis (cpm) is marked with an X at CPM$^A = 15,818$. Next, the %2s axis is marked with an X at 1%. A line is then drawn between these two points until the line crosses the A line; at this point an A is marked. Next, an A is placed at the far right axis for % spillover (82.2). A line is connected between the two A’s, and the counting time (B) is then determined (145 minutes) (Figure 5-6).
In addition to using the nomogram for postrun calculations, prerun determinations can be done if the dpm of the two radioisotopes are known (DPM_A and DPM_B). This is important, since the scientist may desire to know how good the statistics for counting are and how long must this dual label sample be counted to obtain a certain confidence level. This can be done by doing the following calculations:

\[ \text{CPM}_A = DPM_B \times E_h A + DPM_A \times E_l A \]

\[ = \text{cpm contributed by higher energy isotope in region A} + \text{cpm contributed by lower energy isotope in region A} \]

\[ \% \text{ spillover} = \frac{DPM_B \times E_h A}{\text{CPM}_A} \times 100\% \]

\[ = \text{cpm contributed by higher energy isotope in region A divided by total cpm (cpm lower & cpm higher) in region A} \]
Now, let's do an example of the use of the nomogram for dual label sample where the dpm of both radio-
uclides \(^3H/^{14}C\) is known and calculate the %2s with a count time of 10 minutes.

\[
\begin{align*}
\text{Ela} &= 55.0 \text{ (efficiency of } ^3H \text{ in region A)} \\
\text{EhA} &= 12.2 \text{ (efficiency of } ^{14}C \text{ in region A)} \\
\text{DPM}_A &= 50,000 \\
\text{DPM}_B &= 50,000 \\
\text{CPM}_A &= \text{DPM}_B \times \text{EhA} + \text{Ela} \\
\text{CPM}_A &= 50,000 \times 0.122 + 50,000 \times 0.55 = 33,600 \\
\% \text{ Spillover} &= \frac{\text{DPM}_B \times \text{EhA}}{\text{CPM}_A} \times 100\% \\
\% \text{ Spillover} &= \frac{50,000 \times 0.122}{33,600} \times 100\% = 18.2\%
\end{align*}
\]
Now, one first goes to the % spillover line (far right) to a value of 18.2 and places an X. Next, an X is placed at the count time of 10 minutes. Two points are connected and extended to the A line. An A is placed where this line crosses the A axis. Now go to the far left to a CPM = 33,600 and place an A. Connect the two A's with a line. Where this line crosses the %2s axis (0.45%) is the statistical accuracy of the count (B) (Figure 5-7).

In summary, the nomograms provide an accurate method for determining counting statistics (%2s) and/or counting time for low level samples when the sample count and background are similar, and for dual-labeled samples.
Performance Assessment

The final two areas to be considered are associated with the performance and stability of the instrumentation for liquid scintillation counting. The first is Figure of Merit (FOM), a term used to characterize the sensitivity of a system. When comparing two liquid scintillation counting systems at high sample activity, when background is insignificant, counting efficiency is the measure of performance.

Figure of Merit \((E^2/B)\)

When the background count rate approaches the gross count rate then the sample and background counting can be expressed using Equation 8:

\[
\text{Equation 8: } \frac{\text{background count time}}{\text{sample count time}} = \sqrt{\frac{\text{background count rate}}{\text{sample count rate}}}
\]

When the net sample contribution to the gross count rate is small (i.e. Background sample count rate). The total count time is twice the sample time.

When comparing two liquid scintillation counters, we want to analyze the effect of efficiency and background on the time required to obtain a specified accuracy in the net count rate. Since the gross count rate depends on the sample, both instruments should be compared using the same sample. And since we usually compare testing of similar instruments for performance, we need to consider two cases: high level activity and low level activity.

For high level activity samples, where gross count rate is much larger than the background, the count is time inversely proportional to efficiency for each instrument as expressed in Equation 9:

\[
\text{Equation 9: } \frac{\text{time (1)}}{\text{time (2)}} = \frac{\text{Efficiency (instrument 2)}}{\text{Efficiency (instrument 1)}}
\]

Thus, for this type of sample, the instrument with the best performance has the highest counting efficiency.

For low level activity sample, where the background count rate is fairly close to the gross count rate, then performance can be expressed by Equation 10:

\[
\text{Equation 10: } \frac{\text{time (1)}}{\text{time (2)}} = \left[ \frac{\text{efficiency (2)}}{\text{background (2)}} \right] + \left[ \frac{\text{efficiency (1)}}{\text{background (1)}} \right]
\]

Thus, the instrument with the highest Efficiency squared divided by background \((E^2/B)\) has the best performance.
**CHI-SQUARE TEST**

The next area to be discussed is that of the assessment of instrument stability. The instrument stability is assessed using the CHI-SQUARE test. Until now, we have only considered the %2s (counting statistics) due to the random nature of the nuclear decay. This CHI-SQUARE test compares this %2s to the actual %2s obtained from a series of counts (ie. 20 measurements). The CHI-SQUARE value is calculated using the relationship in Equation 11:

\[
\chi^2 = \sum_{i=1}^{M} \frac{(x - \bar{x})^2}{\bar{x}}
\]

where \( \bar{x} = \text{mean value} \)

\( i = \text{number of measurement} \)

\( x = \text{value of each individual measurement} \)

This can be further illustrated by counting a sample 20 times for 1.0 min. (see Table 2).

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>46,704</td>
<td>148</td>
<td>21,904</td>
</tr>
<tr>
<td>2</td>
<td>46,685</td>
<td>129</td>
<td>16,641</td>
</tr>
<tr>
<td>3</td>
<td>46,627</td>
<td>71</td>
<td>5,041</td>
</tr>
<tr>
<td>4</td>
<td>46,495</td>
<td>-61</td>
<td>3,721</td>
</tr>
<tr>
<td>5</td>
<td>46,827</td>
<td>271</td>
<td>73,441</td>
</tr>
<tr>
<td>6</td>
<td>46,666</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>7</td>
<td>46,514</td>
<td>-42</td>
<td>1,764</td>
</tr>
<tr>
<td>8</td>
<td>46,557</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>46,548</td>
<td>-8</td>
<td>64</td>
</tr>
<tr>
<td>10</td>
<td>46,625</td>
<td>69</td>
<td>4,761</td>
</tr>
<tr>
<td>11</td>
<td>46,879</td>
<td>323</td>
<td>104,329</td>
</tr>
<tr>
<td>12</td>
<td>46,384</td>
<td>-172</td>
<td>29,584</td>
</tr>
<tr>
<td>13</td>
<td>46,255</td>
<td>-301</td>
<td>90,601</td>
</tr>
<tr>
<td>14</td>
<td>46,003</td>
<td>-553</td>
<td>305,809</td>
</tr>
<tr>
<td>15</td>
<td>46,814</td>
<td>258</td>
<td>66,564</td>
</tr>
<tr>
<td>16</td>
<td>46,583</td>
<td>7</td>
<td>49</td>
</tr>
<tr>
<td>17</td>
<td>47,000</td>
<td>444</td>
<td>197,136</td>
</tr>
<tr>
<td>18</td>
<td>46,355</td>
<td>-201</td>
<td>40,401</td>
</tr>
<tr>
<td>19</td>
<td>46,608</td>
<td>52</td>
<td>2,704</td>
</tr>
<tr>
<td>20</td>
<td>46,110</td>
<td>-446</td>
<td>198,916</td>
</tr>
<tr>
<td>sum</td>
<td>931,119</td>
<td>-1</td>
<td>1,163,531</td>
</tr>
<tr>
<td>( \bar{x} )</td>
<td>46,556</td>
<td>( \chi^2 = \frac{1,163,531}{46,556} )</td>
<td>( \chi^2 = 24.99 )</td>
</tr>
</tbody>
</table>

*Table 5-2. Chi-Square Analysis of Repeat Measurements of a \(^{14}\)C Sample, Preset count time = 1.0 min.*

For this example, the CHI-SQUARE value is 24.99. If for 20 counts, the CHI-SQUARE value falls in the range of 12.44 to 28.41 (mathematical table reference 2,3), the fluctuation in the data is due only to counting statistics of the sample. The counter is stable for the time range over which the sample was taken. Since these 20 counts were taken for 1.0 min. each consecutively, then the count was stable over 20 minutes. If you wish to check the stability of the system over a day, then every 60 minutes a one minute count could be taken. For modern liquid scintillation counters, the CHI-SQUARE for both \(^3\)H and \(^{14}\)C is determined daily using unquenched standards. Thus, the investigator is able to determine and document that the system was performing properly when a particular assay or count was determined. This is especially important when a particular government agency, such as the FDA, requires that the data and system performance be monitored during specific assays.
References

Sample Preparation

Introduction

Probably one of the most important steps in the scintillation counting process is the sample preparation procedure. Most biologically radiolabeled samples, such as a piece of a plant (leaf, stem, root), a culture of cells, or an aqueous sample, cannot be placed in a vial and quantitated directly. The sample must be prepared so that the liquid scintillation analyzer is quantitating a homogeneous mixture of sample and scintillation solution. Therefore, it is extremely important to understand the different methods of sample preparation. This chapter and the next will cover the various methods of sample preparation; including aqueous samples, carbon trapping methods, proteinaceous tissue solubilizers, and sample oxidation (automatic and manual).

Components Of A Liquid Scintillation Solution

In order to understand what components are present in a liquid scintillation solution, it is necessary to understand the liquid scintillation process. This is shown in Figure 6-1. The process involves the transfer of kinetic energy from the beta particle (β− emitted by the radionuclide to solvent molecules. These excited solvent molecules then transfer their energy to scintillator molecules. When these molecules become excited, they return to their stable energy state by emitting photons with a certain intensity. The intensity of the light is directly proportional to the energy of the emitted beta particle.

![Figure 6-1. Basic Scintillation Process.](image)

A classical aqueous-accepting scintillation solution is composed of several components: solvent, primary and secondary scintillators, and surfactant(s). Each of these components will be further explained:

1) Solvent - a chemical which converts the kinetic energy (β−) of radiations into excitation energy and 'transfers' this energy to a scintillator molecule.

2) Primary scintillator (solute) - a chemical which converts excitation energy into photons.

3) Secondary scintillator (solute) - a chemical which shifts the light wavelength of emitted photons into an optimal wavelength response range of the photomultiplier tubes.

4) Surfactant (emulsifier)-a chemical which forms a stable, homogeneous emulsion through the formation of micelles with aqueous samples and organic solvents.
Solvent

The solvent acts as both a vehicle for dissolving the sample and scintillator, and the location of the initial energy transfer from the nuclear decay products to the solvent. The solvent must be able to transfer the energy without significant loss. The type of solvent molecules which are best able to do this are molecules which have nonbonding pi (π) electrons which can easily be excited to higher energies without the loss of energy. Typical molecules which display this π electron structure are aromatic molecules. In addition to the ability to transfer energy efficiently, the solvent must have the following characteristics:

1) It must readily dissolve the radioactive analyte and the scintillator molecules.
2) It must be a low content of natural ¹⁴C.
3) It must have a high transparency to the photons of light emitted by the scintillator molecule.

A list of the most common solvents and their structure is shown in Table 6-1. Two characteristics of these solvents are worth investigating further: Relative Pulse Height (RPH) and flash point. The RPH is the pulse height measured for a radionuclide in toluene compared to an unknown solvent with the RPH for toluene referenced as 100. The flash point is defined as the lowest temperature at which the vapor of a combustible liquid can be made to ignite momentarily in air.

Table 6-1. Common Scintillation Solution Solvents.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Chemical Structure</th>
<th>Relative Pulse Height</th>
<th>Flash Point °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2,4-Trimethylbenzene</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>112</td>
<td>50</td>
</tr>
<tr>
<td>(pseudocumene)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-Xylene</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>110</td>
<td>30</td>
</tr>
<tr>
<td>Toluene</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>100</td>
<td>5</td>
</tr>
<tr>
<td>Benzene</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>85</td>
<td>-11</td>
</tr>
<tr>
<td>1,4-Dioxane</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>65</td>
<td>12</td>
</tr>
<tr>
<td>m-Xylene</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>107</td>
<td>25</td>
</tr>
<tr>
<td>o-Xylene</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>98</td>
<td>29</td>
</tr>
<tr>
<td>Alkylbenzene</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>91</td>
<td>150</td>
</tr>
<tr>
<td>(high flash point)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The relative pulse height (RPH) is important because if this number falls below 100, the energy transfer and resultant output of photons is reduced. Thus, the higher the RPH, the better the solvent is for scintillation solution preparation. The flash point is also important. If the flash point is very low, the sample can easily be ignited and should be considered a potential fire hazard. If the solvent has a low flash point, it is normally more toxic (possibly even carcinogenic). Now let us examine each of these solvents individually.

One of the earliest solvents to be used was benzene but, because of its high toxicity, extremely low flashpoint, and the inability to quantitate aqueous samples in this solution, its use quickly became replaced with toluene and dioxane. Today, a special application for benzene is its use in low activity carbon dating. It is used in conjunction with the benzene synthesizer (converts sample completely to benzene for quantitation). By using this technique, large sample volumes can be incorporated into the scintillation solution.

Next, the solvent dioxane was used for counting aqueous samples using Bray’s solution cocktail. However, dioxane-based cocktails exhibit many problems. First, they were not useful for aqueous solutions containing proteins, since dioxane will precipitate proteins. Second, sample types are limited because many materials will cause the naphthalene used in the Bray’s cocktail to precipitate. Third, the dioxane is subject to auto-oxidation which produces peroxides (strong quenching and chemiluminescence production). These problems, together with a low counting efficiency, a low flash point and high toxicity (suspicous of being carcinogenic), have resulted in the mainly discontinued use of dioxane for liquid scintillation counting.

Next toluene was the solvent of choice. Toluene was readily available in high purity and at moderate cost. Together with TRITON X-100® (a nonionic surfactant) it formed one of the first emulsifier (colloidal) cocktails for counting aqueous-type samples. Toluene has several disadvantages as a solvent. The flash point for toluene is lower than room temperature; it can represent a significant fire hazard if not handled properly. The solvent toluene has also been proven to be highly carcinogenic, especially in the liver.

Xylene, a frequently used solvent, gives high energy conversion efficiencies and high relative pulse heights. The more common practice for using xylene as a solvent is to use one of the pure xylenes (ortho, meta, or para) or to mix two of them in a known ratio. Para-xylene is not useful by itself in controlled temperature liquid scintillation counters because it will freeze at 12-13˚C. Although the xylenes are classified as flammable liquids, their flash points are above normal room temperatures, so they represent less fire hazard than the previous solvents.

Pseudocumene (1,2,4-trimethylbenzene) has become a popular solvent for newly developed ready-for-use scintillation cocktails. First, it offers the highest energy conversion efficiency of the solvents known. Second, it is classified only as a combustible liquid by U.S. Department of Transportation regulations and, therefore, has few restrictions on shipping and storage. Third, it can be used with plastic vials, because the diffusion rate of this solvent through the vial walls is lower compared to the solvents mentioned previously.

Due to the toxicity, waste disposal, shipping and storage problems associated with the low flash point solvents previously discussed, a high flash point solvent was developed. This high flash point solvent, a long chain alkylbenzene derivative, has a low toxicity level and a high flash point (≥150˚C), with only a slightly lower RPH (91). In conjunction with new emulsifiers necessary for aqueous samples, this new solvent gives excellent performance. It is also environmentally benign and, in many locations, is drain disposable. This high flash point solvent is found in Packard’s Opti-Fluor® scintillation cocktail.

*Triton X-100 is a Registered Trademark of Rohm and Haas
Scintillator - Primary

The scintillator molecules accept energy from the excited solvent molecules. The scintillator molecule then becomes excited to a higher energy state. The excited scintillators will then relax and return to the ground state with the concomitant release of energy. This energy is released in the form of a photon (light flash) which can be quantitated by the liquid scintillation analyzer. Thus, the scintillator must effectively accept the energy from the solvent and produce photons. The transfer must be relatively quantitative with the energy of the beta particle being directly proportional to the intensity of the photons released by the scintillator.

Several different types of primary scintillator are available for liquid scintillation counting (Table 6-2). The most popular are PPO, PBD, Butyl-PBD, and BBOT.

Four characteristics of each of the primary scintillators are presented. The first is the optimum concentration used in the preparation of the scintillation solution. If a higher scintillator concentration is used, quenching may result from the presence of excess scintillator. The second characteristic is that of the peak fluorescence of the scintillator. This value should be between 300 and 425 nm. This is the wavelength response of the photomultipliers (PMTs) which are used to detect the photons produced by the scintillator. The spectrum of PPO relative to the sensitivity of the photomultiplier sensitivity is shown in Figure 6-2.

The third characteristic is that of the decay time (t). This is very important, in that the photon peak must be very sharp, and not have tailing, this is most important at high count rates, when photons of various intensities can occur in very short periods of time. If the decay time is too long, then the photons created by beta particles at a high rate can be lost in the counting circuit. All modern scintillators typically have decay times of less than two nanoseconds (Table 6-2). The earlier scintillators, napthalene and diphenylantrachene, have decay times of 96 and 9.4 nanoseconds.
The fourth characteristic is the fluorescent yield, $\phi$. This is defined as the fraction of excited molecules which emit photons.

$$\phi = \frac{\text{number of photons}}{\text{number of excited molecules}}$$

The closer the number is to 1.0, the better energy transfer and photon yield. In summary, each of the following primary scintillators have the following characteristics.

PPO (2, 5-diphenyl oxazole) is currently the most widely used primary scintillator. The reasons for the popularity of PPO are good scintillation efficiency (quantum yield) in moderate concentrations, good solubility in scintillation solvents, relatively low cost, and nonreactivity with most chemicals measured by liquid scintillation counting. For routine liquid scintillation applications, concentrations of five to six grams per liter will produce a cocktail with great resistance to chemical quenching. This enables higher counting efficiencies with lower concentrations of scintillator. In concentrations exceeding 10 grams per liter, PPO will act as a quenching agent of the scintillation process (self-quenching).

Butyl-PBD [2-(4-tert-Butylphenyl)-5-4 Biphenyl)-1,3,4-Oxadiazole] is another efficient primary scintillator. It has three disadvantages. First, its cost is about double that of PPO. Second, the concentration required to achieve high efficiency is about twice that of PPO (recommended concentration: 12 grams/liter). Third, Butyl-PBD is chemically reactive. It will react with acids, bases, amines and other compounds. This reduces its effective concentration in the final cocktail. With basic samples Butyl-PBD produces a brownish color in the scintillation cocktail. This causes both chemical and color quenching in the sample.

BBOT [2,5-bis-2-(tert-Butylbenzoxazolyl)-Thiophene] is a primary scintillator which emits light (446 nm) mostly in the visible region. This property makes it less affected by optical quenching in the ultraviolet region. It has several disadvantages. First, it is only about 80% as efficient as PPO in converting excitation energy into light (quantum yield). Second, BBOT costs about twice as much as PPO and it is used in higher concentrations (recommended concentration: 7 grams/liter). Third, BBOT is chemically reactive. BBOT can react with acids producing a yellow to green color.

<table>
<thead>
<tr>
<th>Solute</th>
<th>Optimum solute concentration (g/liter)</th>
<th>Fluorescence maximum (nm)</th>
<th>Decay time (nsec)</th>
<th>Quantum yield $\phi$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPO</td>
<td>5-7</td>
<td>375</td>
<td>1.4</td>
<td>0.83</td>
</tr>
<tr>
<td>PBD</td>
<td>8-10</td>
<td>375</td>
<td>1.0</td>
<td>0.69</td>
</tr>
<tr>
<td>Butyl-PBD</td>
<td>12</td>
<td>385</td>
<td>1.0</td>
<td>0.69</td>
</tr>
<tr>
<td>BBOT</td>
<td>7</td>
<td>446</td>
<td>1.6</td>
<td>0.61</td>
</tr>
</tbody>
</table>
Scintillators - Secondary

Secondary scintillators (the third component in a liquid scintillation solution) were used in the early days of liquid scintillation counting in order to shift the wavelength of the photons emitted from 370 nm to 420 nm. The major reason for this was that the spectral response of the early photomultipliers was near 400 to 420 nm. Thus, the secondary scintillator acted as a wavelength shifter to change the wavelength of the photons emitted in the scintillation process. The exact mechanism of the second scintillator was not clearly understood. It was clear, however, that with the secondary scintillator present, the photons produced by the scintillation solution came only from the secondary scintillator. This was providing that the secondary scintillator concentration was sufficient to give 100% energy transfer from the primary to the secondary scintillator. Thus, a direct energy transfer from the primary to the secondary scintillator was necessary.

Today, a secondary scintillator is not absolutely necessary to shift the emitted wavelength because the modern photomultipliers are sensitive to the wavelength of the primary scintillator. The primary use today for secondary scintillators occurs when a large amount of color quenched sample is placed in the scintillation solution. The secondary scintillator provides a method to more effectively transmit the energy from the beta particle to produce light flashes which are directly proportional to the energy of the beta particle.

Three secondary scintillators are commonly used in preparing liquid scintillation solution: bis-MSB, POPOP, and dimethyl POPOP. The structures, wavelength average, and optimal concentration are given in Table 6-3.

Table 6-3: Properties of Secondary Scintillators.

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>ABBREVIATION</th>
<th>CONCENTRATION (g/liter)</th>
<th>FLUORESCENCE MAXIMUM WAVELENGTH (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="POPOP Structure" /></td>
<td>POPOP</td>
<td>0.05-0.2</td>
<td>415</td>
</tr>
<tr>
<td><img src="image" alt="M2POPOP Structure" /></td>
<td>M2POPOP</td>
<td>0.1-0.5</td>
<td>427</td>
</tr>
<tr>
<td><img src="image" alt="bis-MSB Structure" /></td>
<td>bis-MSB</td>
<td>1.5</td>
<td>425</td>
</tr>
</tbody>
</table>

The best general purpose secondary scintillator is bis-MSB [p-bis(o-Methylstyril)Benzene]. It is used as the secondary scintillator in most ready-to-use scintillation cocktails. Bis-MSB is readily soluble in solvents for liquid scintillation counting. It has a fast rate of dissolution. This makes it an extremely convenient secondary scintillator for laboratory-prepared cocktails. In high concentrations, it can function as a primary scintillator, supplementing the PPO concentration to increase resistance to quenching, and does not react chemically with most liquid scintillation samples (recommended concentration: 1.5 grams/liter).
POPOP (1,4-bis-[2-(5-Phenylloxazolyl)]-Benzene) was one of the earliest secondary scintillators used. It is still the most widely used in laboratory-made cocktails. It has low solubility, which limits the amount which can be incorporated into a scintillation cocktail. Its rate of dissolution is slow, thus resulting in long mixing times. POPOP is chemically nonreactive with most chemicals used in liquid scintillation counting (recommended concentration: 0.1 grams/liter).

Dimethyl-POPOP [1,4-bis-[2-(4-Methyl-5-Phenylloxazolyl)]-Benzene] is a derivative of POPOP which has higher solubility and faster dissolution rates in scintillation solvents. Dimethyl-POPOP is chemically reactive. It can react with acids to produce a yellow to greenish color (recommended concentration: 0.2 grams/liter).

**Surfactants (Emulsifiers)**

The fourth component of a liquid scintillation solution which accepts aqueous samples is a surfactant or emulsifier. This is probably the most important component of the aqueous-accepting scintillation solution because it provides the method for making a homogeneous solution between the aqueous sample and the organic scintillation solution. These emulsifiers hold the sample in intimate contact with the solvent by the formation of micelles. These surfactants are normally of two types: ionic and nonionic. Examples of each type are shown in Figure 6-3.

The nonionic surfactants are normally used to form micelles with water and certain salt solutions. The ionic surfactants have sample capacity for certain types of salt solutions.

The use of a nonionic emulsifier in a scintillation solution may result in up to three sample/scintillation solution types. The first is a clear homogeneous single phase which is liquid in nature. This is normally present for small sample loads. The next phase is a heterogeneous, two-phase solution, which cannot be counted effectively in a liquid scintillation analyzer. The two phases are a water phase and an organic phase.
The third phase is a gel phase in which the sample and the scintillation solution form a clear gel. This is an effective solution for liquid scintillation counting. A typical phase diagram for a scintillation solution with various amounts of sample (sample load) is illustrated in Figure 6-4.

![Phase Diagram for Insta-Gel Scintillation Cocktail.](image)

In order to effectively analyze aqueous samples, it is important to use a phase diagram to determine whether the sample is being counted under homogeneous conditions necessary for liquid scintillation analysis.

**SPECIAL APPLICATIONS USING SCINTILLATION SOLUTIONS**

With the procedure described in the previous section, aqueous samples can be counted accurately if a single fluid phase or gel is formed, but many samples are not simple aqueous samples. These include:

1. Counting radioactivity from paper chromatograms.
2. Counting gamma or x-rays in a liquid scintillation analyzer.
3. Counting trapped 14C-labeled carbon dioxide.
4. Counting tissue samples.
5. Counting samples on glass fiber filters.
6. Counting samples on membrane filters.
7. Counting TLC plate scrapings.
8. Counting slices obtained from gel electrophoresis.
10. Counting insoluble particulates.
The major problem with most of the ten applications listed above is that the sample is not homogenous in nature. How does this affect the counting efficiency of the sample? This is illustrated in the following manner (Figure 6-5).

When a radionuclide decays it can emit a beta particle in any direction. If the radiolabel is dissolved in the scintillation cocktail, the radionuclide is completely surrounded by the detector and $4\pi$ counting geometry is attained. (Solid angles are measured in steradians, $4\pi$ steradians is the solid angle of a sphere). If the radioactive compound has precipitated, or is adsorbed to the vial wall or on a solid support (filter paper, etc.), the counting geometry is reduced to something less than $4\pi$. For adsorption and precipitation, the geometry is reduced to essentially $2\pi$, resulting in a loss of half of the detectable counts of the radionuclide. For higher energy nuclides, some of the beta particles can penetrate the solid support and be quantitated. If the labeled compound is on the surface of a solid support, the counting geometry is approximately $2\pi$, if the compound has penetrated into the solid support, then counting geometry can be even less than $2\pi$. For samples on solid supports, geometry is to some degree energy-dependent. If the beta particles emitted have adequate energy to pass through the solid support and interact with the scintillation cocktail, the geometry will be greater than $2\pi$. For very energetic beta particles, such as from $^{32}\text{P}$, the geometry may approach $4\pi$. Thus, it is important especially for tritium (low energy beta emitter) to count with $4\pi$ geometry (a homogeneous solution). The following procedures are methods which enable good counting geometries for the applications described below.

1) **Counting of Radioactivity from Paper Chromatography.**

In order to quantitate samples from paper chromatography, it is important to elute the radioactivity from the paper chromatographic strip. In order to do this, it is important to assess the nature of the molecule on the paper chromatograph. Usually, paper chromatography is done with a particular solvent system. If the molecule moves rapidly on the paper chromatograph, then an easy method to elute the molecule is to use the same solvent used to develop the chromatograph. If the molecule moves very slowly on the paper chromatograph, then a solvent with the opposite polarity of the developing solvent should be used. The paper strips containing the molecule of interest are combined with the solvent and vortexed in the scintillation vial. If this solvent contains no heavy quenching agent such as chlorinated hydrocarbons, chloroform or carbon tetrachloride, scintillation solution can be added and the sample counted directly.
2) Counting Gamma and x-rays in a Liquid Scintillation Analyzer
Since many gamma and x-rays have very high energies compared to most beta particles, they can pass through the scintillation solution without transferring their energy to a solvent or a scintillator molecule. In order to quantitate these radionuclides, it is important to add a heavy metal compound such as tetrabutyl tin or tetraethyllead to the scintillation cocktails. For higher energy gamma and x-rays, not even the addition of these heavy metals makes possible their quantitation.

3) Counting Trapped ¹⁴C-Labeled Carbon Dioxide
There are two basic applications for the use of a carbon dioxide trapping agent: metabolism assessment and automatic sample oxidation. Two types of methods are used for trapping labeled carbon dioxide; inorganic and organic bases.

Solutions of inorganic bases have been used for a long time to trap carbon dioxide for liquid scintillation counting. Aqueous solutions of up to 1N sodium hydroxide or potassium hydroxide and methanolic solutions of 2N potassium hydroxide have been used routinely. The disadvantages of inorganic bases are three-fold. First, inorganic bases are strong quenching agents. Second they provide low trapping capacities. Third, they produce severe chemiluminescence. The major reasons for the use of inorganic bases is that their smell is preferred over the more pungent organic bases, and they are less expensive.

Organic bases, such as Hydroxide of Hyamine 10-X®, have been used to trap carbon dioxide for liquid scintillation counting. (Hydroxide of Hyamine 10-X is commercially available in a concentration of one normal in methanolic solution. It will trap about 0.5 millimole of carbon dioxide per mL. The proteinaceous tissue solubilizers are all organic bases and can be used to trap carbon dioxide.) These tissue solubilizers are toluene solutions with normalities of about 0.5N. These bases are fine for trapping small amounts of radiolabeled carbon dioxide.

For trapping large quantities of carbon dioxide, organic amines offer the best trapping capacities. Carbo-Sorb® is an amine which will absorb up to 5.8 millimoles of C0₂ per mL. Carbo-Sorb and the carbamate which results when C0₂ is trapped, are soluble in scintillation solutions containing toluene, xylene, or pseudocumene. These chemicals exhibit less quenching than inorganic bases. They will still produce chemiluminescence. In order to eliminate this chemiluminescence, one of three methods can be used.

First, the user can allow the chemiluminescence to decay to an acceptable level. Second, chemiluminescence can be corrected for by the instrument (see page 2-22). Third, chemiluminescence can be eliminated by selecting a cocktail which inhibits this chemiluminescence quickly to background levels (Hionic-Fluor™-Packard).
The main applications of these trapping agents are summarized in Table 6-4.

Table 6-4. Carbon Dioxide Trapping Agents.

<table>
<thead>
<tr>
<th>Trapping Agent</th>
<th>Properties</th>
<th>Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>KOH ) NaOH solution</td>
<td>Low speed of trapping. Max. capacity (0.5-1.0 mmol/mL) depending on strength of solution.</td>
<td>Expired air. Low volumes of (^{14})CO(_2) to be trapped in 2-4 M solution.</td>
</tr>
<tr>
<td>Hydroxide of Hyamine(^{10})-X</td>
<td>Max. capacity of 0.5 mmol CO(_2)/mL.</td>
<td>Expired air. Reacts faster than KOH/NaOH</td>
</tr>
<tr>
<td>Soluene -350</td>
<td>Lower maximum capacity than Hyamine 10-X. Reacts somewhat slower than Hyamine 10-X.</td>
<td>For trapping small volumes of (^{14})CO(_2).</td>
</tr>
<tr>
<td>Methoxy-Ethyl-Amine</td>
<td>Reacts fast. Maximum capacity of 5-6 mmol CO(_2)/mL.</td>
<td>For trapping large volumes of (^{14})CO(_2). Not preferred for expired air experiments because of irritating smell. Used in Packard's sample oxidizer.</td>
</tr>
</tbody>
</table>

4) Counting Tissue Samples

Since tissue and proteinaceous materials cannot be counted directly, they must be solubilized. This can be done either with a tissue solubilizer or with sample oxidation. The use of the sample oxidizer for this purpose will be discussed in detail in the next chapter of this manual. Now, the use of tissue solubilizers for digesting tissues, cells, and proteinaceous material are presented.

Packard Instrument Company offers the tissue solubilizer, Soluene -350. It is a toluene solution containing 0.5N of a specific quaternary ammonium hydroxide compound. Many samples can be solubilized at room temperature in less than two hours with 1.0 mL of solubilizer. Solubilization times can be shortened by heating the samples to 40 to 50°C.

Soluene -350 solubilizer contains additional ingredients to improve water holding capacity and stability of the solubilizer. One mL of Soluene -350 solubilizer can hold up to 0.45 mL of water. This makes it an ideal solubilizer for tissue homogenates. Soluene -350 solubilizer has a rapid tissue-dissolving rate. Because of its greater stability, solubilizations can be performed at temperatures up to 50°C, thus accelerating the solubilization process. Many samples can be solubilized at room temperature within a few hours. Soluene -350 exhibits the least chemical quenching of any of the known solubilizers.

In addition to tissue solubilizers to digest tissues, early work was done using a methanolic solution of hydroxide of Hyamine 10-X. Hydroxide of Hyamine 10-X is chemically identified as p-(diisobutylcresoxyethoxyethyU dimethyl benzyl ammonium hydroxide. Its strength as a tissue solubilizer is relatively low at room temperature compared to the Soluene tissue solubilizer. It also exhibits a large amount of chemical quenching due to its methanolic base. On the other hand, this methanolic base will reduce the chemiluminescence resulting from this basic ammonium compound.

*Registered Trademark of Rohm and Haas
Special care should be taken when using tissue solubilizers. They are designed to solubilize proteins and tissues; therefore, they will cause severe burns when they come in contact with living tissue. Solubilizations should be performed in tightly capped vials, because air will react with the solubilizers, causing color formation.

5) Counting Samples on Glass Fiber Filters
The use of glass fiber filters is useful in applications using a cell harvester (receptor binding, cell propagation studies, disease diagnostics, and release studies) and DNA/RNA, DNA/DNA hybridization studies. In order to quantitate samples on glass fiber filters, it is necessary to elute the radioactive material from the filter. In order to do this, it is necessary to know the chemical nature of the radiolabeled material. If the sample is completely soluble in the scintillation fluid, then the sample can be quantitated directly. Examples of soluble samples are some drugs, lipids, and fatty acids. If the sample is not soluble, then place the wet filter in a glass scintillation vial Add 10 mL of Insta-Fluor/Soluene -350 (9:1) and quantitate in a liquid scintillation counter. The Insta-Fluor is an organic based scintillation solution, and the Soluene -350 is a powerful tissue solubilizer. This procedure can also be used efficiently on cellulose acetate, Teflon®, Duralon®, and Fluoropore® filters. In addition, a specialized scintillation solution, Filter-Count, can be used for membrane filters of cellulose nitrate. A detailed description of the procedure will be described in the next section on counting membrane filters.

6) Counting Samples on Membrane Filters
In many biochemical applications, special membrane filters are used to quantitate various radiolabeled products. These membrane filters include cellulose nitrate, mixed cellulose esters, and PVC filters. In order to effectively quantitate radioactivity on these membranes, the procedure for glass fiber filters could be used, but for some membranes the Soluene -350 turns the solution yellow and makes accurate quantitation difficult. An alternative to this procedure is the use of Filter-Count specialized scintillation solution, which does not cause color in solution. This scintillation solution can dissolve many membrane filters and also solubilize the radioactive samples. The wet or dry membrane filter is placed in a scintillation vial and 4 to 10 mL of Filter-Count is added. After 15 minutes, the sample is shaken and quantitated in the liquid scintillation counter.

7) Counting Thin Layer Chromatographs (TLC)
Two methods of TLC can be performed in order to separate various radiolabeled components: glass and plastic TLC. For glass backed TLC plates, the sample is first applied, the chromatography performed, and the glass TLC allowed to dry. After this, the lanes containing the radiolabeled samples, are divided into 0.5 to 1.0 mm slices with a pencil A razor blade or scoring device is then used to remove each 0.5 to 1.0 mm section from the glass backing. The scraped area can then be removed and placed into a scintillation vial. This process is very tedious and time-consuming. If a plastic backed TLC plate is used, each section 0.5 to 1.0 mm can be cut out and placed directly in a scintillation vial. The next step is to dissolve the radiolabeled material from the thin layer material. This can be done by assessing the nature of the radiolabeled material and determining what solvent is necessary to remove the radioactivity from the TLC plate. Once this is done, then 1 to 2 mL of the solvent is added to
the scintillation vial and allowed to set for 10 to 15 minutes. At this point, an aqueous scintillation solution, Hionic-Fluor or Insta-Fluor, can be added (10-15 mL) to each sample. The radiolabeled material is then quantitated in a liquid scintillation counter.

Another method of counting TLC scraping is suspension counting for ¹⁴C or higher energy isotopes. Excellent results can be obtained. For complete details see next page.

8) Counting Polyacrylamide Gel Slices Obtained from Electrophoresis Samples

Many applications involve the use of polyacrylamide gel electrophoresis (PAGE) to separate DNA, RNA, proteins, peptides, and other molecules. In order to quantitate radioactivity from the PAGE separated sample, four different procedures can be used:

1. Solubilizing
2. Macerating
3. H₂O₂
4. H₂O₂/HC10₄

First, the procedure using a tissue solubilizer will be described. A gel slice (1 to 3 mm) is placed in the bottom of a glass scintillation vial A solution containing 9 mL of Insta-Fluor and 1 mL of Soluene - 350 is added to the vial and it is closed tightly with a polyethylene lined cap. The vial is placed in a waterbath at 50°C for 2 to 4 hours or overnight at room temperature. The gel will appear swollen and transparent. The sample is then counted in a liquid scintillation analyzer after temperature and chemiluminescence stabilization.

Second, the gel slice can be macerated (broken up with a glass rod) in 5 mL of water and incubated at 50 °C for four hours. After the incubation 10 mL of Insta-Gel is added to the sample before quantitation.

Third, the gel slice is incubated with 2 mL of 30% H₂O₂ for 5 hours at 50 °C. After the incubation 12 mL of Hionic-Fluor is added to the sample.

Fourth, the gel slice is incubated in 1 mL of 30% H₂O₂ and 1 mL HC10₄ for 2 hours at 80°C. After the incubation 15 mL of Pico-Fluor 15 is added to the sample before quantitation.

In summary, the Insta-Fluor/Soluene method gives high counting efficiency, high recoveries, fast results, and is easy to perform. This is the best of the four procedures. The wet oxidation method (three and four), gives high recoveries but reduces counting efficiency. Also, H₂O₂ may react and alter some of the radiolabeled material, and causing loss of radioactivity in gaseous forms and under some circumstances explosion hazard may result in the glass scintillation vial. This treatment results in lower counting efficiencies. Macerating and elution with water give good results for water soluble radioactive compounds, but are counted with lower efficiencies.

9) Counting Blood Samples

Many experiments require quantitation of radiolabeled material in blood. These experiments are used to monitor clearance rate studies of new drugs and other applications. The procedure involves adding 0.1 to 0.4 mL of whole blood to a 1 mL mixture of Soluene -350/isopropanol,1:1. This mixture is incubated at 40 °C for 15 to 30 minutes. At the end of the incubation, 0.5 mL of 30% hydrogen peroxide is added slowly (dropwise) with constant stirring. This mixture is then allowed to set for 30 minutes at room temperature. At the end of this time, 10 mL of Hionic-Fluor are added to the sample.
The cocktail counting efficiency (Ec) is determined using a soluble standard in the organic phase of the cocktail (e.g., $^3$H-toluene). The chemical and optical quenching should be the same in the two samples. The ratio of these efficiencies provides a quantitative measure of phase contact (P.C.).

$$\text{P.C.} = \frac{\text{C.E.}}{\text{Ec}}$$

The closer the value of P.C. is to 1.0, the better the phase contact. At a value of 1.0, the radionuclide is being counted as a true solution. Values < 1.05 or > .95 give questionable results.

The amount of sample a scintillation cocktail will hold is its sample holding capacity, expressed as the sample load (SL). For liquid analytes, sample load is usually expressed as a percentage, and is the ratio of the volume of sample to the volume of the sample plus cocktail.

$$\% \text{ Sample Load} = \frac{\text{mL sample}}{\text{mL sample} + \text{mL cocktail}} \times 100$$

The relationship between sample volume, cocktail volume and sample load is shown in Figure 6-6.

For aqueous types of samples, a hydrophilic scintillation cocktail is required. The sample holding capacity of these types of cocktails is determined by the emulsifier system and the solvent. Together they form a specific capacity for a certain analyte.
This sample contains the labeled compound, but may also contain concentrations of other solutes (e.g., salt/buffer solutions). If these solutes exceed the maximum sample holding capacity of the cocktail, then precipitation, phase separation, or a milky appearance of the sample may occur, resulting in incorrect counting performances.

Visual inspection of the homogeneity of the sample may not always be adequate. In case of doubt, apply the phase-contact method for checking homogeneity, or use the sample homogeneity monitor of the liquid scintillation analyzer.

For nonaqueous organic solutions, lipophilic scintillation cocktails can be used, although these types of samples cannot be counted in the emulsifier-type cocktails (universal scintillation cocktails). The lipophilic cocktails will normally give higher counting efficiency and are less expensive.

**Counting Performance**

The second criterion which affects quality of the measured data is the counting performance of the scintillation cocktail. The better the counting performance of the cocktail, the shorter is the counting time required to achieve a desired statistical precision, or the greater the statistical precision which can be achieved in a given period of time. Also, depending on the type of experiment, less radioactivity can be used with the more efficient cocktail to achieve the same statistical precision.

Counting performance is related to both the efficiency of measuring the radioactivity in the analyte and the sample holding capacity of the cocktail. Cocktails do have correct counting efficiencies with a certain amount of analyte if the Phase-Contact value, with that specific amount of analyte, is equal to one (P.C. = 1.0).

To evaluate the counting performance of a particular cocktail, the Figure of Merit (FOM) is used. The most useful formula of the FOM for evaluation of counting performance is shown in the equation below (E = counting efficiency and V = sample load).

\[
FOM \propto E \times V
\]

In this form, the FOM can be used to compare the counting performance of scintillation cocktails. The cocktail which has the largest FOM will give the best counting performance for that analyte. For any single cocktail, the sample load (V) which gives the largest FOM for that analyte will give the best counting performance. If the volume of analyte is limited, the cocktail which has the highest counting efficiency for that volume of the analyte will give the best counting performance.

To evaluate cocktails according to the FOM requires measurements of the counting efficiency at different sample loads of specific samples. For cocktails which are used for a variety of analytes, a more convenient criterion for comparative evaluation is the quench resistance of the scintillation cocktail. Cocktails which are most resistant to chemical or optical quenching will exhibit a small loss of counting efficiency with increased volumes of the quenching material. Quench resistance can be defined as the slope of the correlation curve for efficiency versus volume of the quenching agent. The lower the value of the slope of this curve, the greater is the quench resistance for the cocktail. Generally, the scintillation cocktail with the greatest quench resistance will give the best counting performance for any analyte over its range of sample holding capacity.
The sample is then allowed to equilibrate in the instrument for 30 minutes before counting, in order to stabilize temperature and chemiluminescence.

10) Counting Insoluble Particulates Samples

Cab-0-Sil®, a thixotropic gel powder, can be added to a scintillator solution in concentrations of 3 to 4% (w/v) in a vial to form stable gels. Under mechanical agitation the mixture becomes a fluid, returning to a gel when agitation is stopped.

This preparation is used to suspend insoluble particulates (e.g. radiolabeled barium carbonate samples) for counting. In lower concentrations (1 to 2%) Cab-0-Sil is used to provide a large surface area to minimize adsorption of samples to the vial wall.

The previous ten applications provide methods necessary to form a homogeneous counting solution from a heterogeneous sample. This homogeneity provides 4 π counting geometry and, consequently, accurate DPM determination of the hard-to-quantitate sample.

Choosing A Scintillation Cocktail

Choosing the appropriate scintillation cocktail is very important in liquid scintillation analysis. The combination of the sample and the scintillation solution is a major determining factor of the quality data (DPM) in the counter. Several factors are important in the quality data:

1. Sample compatibility
2. Counting performance
3. Cost
4. Convenience
5. Safety and disposability

These factors are all interrelated, and each will be discussed separately.

Sample Compatibility

The primary criterion for choosing a scintillation cocktail is sample compatibility (the compatibility between the scintillation cocktail and analyte or sample to be measured). One factor affecting sample compatibility is the solubility of the sample in the cocktail. The optimum counting performance is achieved when the sample is in solution with the scintillation cocktail. The radionuclide in the analyte is then in intimate contact with the primary solvent. If the analyte is in solution, the sample is in a homogeneous phase; if the analyte is not in solution, the sample is in a heterogeneous (multiphase) state. The term ‘phase contact’ is used to describe the degree of homogeneity of the sample; ‘good phase contact’ describes those samples which count as solutions. In a scintillation cocktail, the organic phase is the medium in which the energy conversion process occurs. Radionuclides not in the organic phase (described earlier - precipitated, adsorbed, or in a separate liquid phase) will yield a lower counting efficiency than the potential counting efficiency of the cocktail. Phase contact can be quantified using standards (samples of known activity). The counting efficiency (C.E.) is determined using a known activity of the labeled compound in the analyte or by using a radioactive compound that will distribute in the cocktail the same as the analyte (e.g., ³H-water).
The quench resistance of different ready-to-use cocktails, is illustrated in Figure 6-7.

![Figure 6-7. Quench Resistance of Packard Pico-Fluor™ 15 Versus Conventional LSC cocktails.](image)

**Cost**

Cost may be an important consideration in choosing a scintillation cocktail. Economy can be achieved by using lower cost materials or by reducing the amount of materials used. Generally, the lower cost scintillation cocktails produce lower counting performance. Often, greater economies can be achieved without sacrificing counting performance by choosing a higher performance cocktail which could result in a reduction of the volume of scintillation solution. The volume reduction will depend upon the sample holding capacity of the high performance cocktail. For example, consider tritiated analytes of 1.0-2.0 mL diluted aqueous salt solutions counted in 10 mL of a TritonX-100/Toluene solution with counting efficiencies of 42 to 37% respectively. In 5 mL of Pico-Fluor 40, these analyte volumes would count with efficiencies of 40 to 35%. Thus, by changing to Pico-Fluor 40, the volume of cocktail used can be reduced by 50% without sacrificing efficiency.

This volume reduction also results in a lower volume of radioactive waste. This, together with the use of small vials, results in a significant cost reduction per sample, even if Pico-Fluor 40 were to cost twice as much as a low price, low performance cocktail.

When comparing costs of scintillation cocktails, it is important to include all the costs involved. Commercially prepared cocktails generally cost more than the cost of the materials for similar laboratory-prepared cocktails. For laboratory prepared cocktails, there are a number of hidden costs which should be added to the material cost of the cocktail. The largest of these costs is the cost of preparation of the cocktail. This includes the labor to make the cocktail plus the expense of any special labware required for the preparation of the cocktail. The preparation cost of a laboratory-prepared cocktail usually includes a minimum of two hours of a technician's time per lot (typically four liters only).

An expense which applies to both commercial cocktails and laboratory-prepared cocktails is the cost of quality assurance. Each lot of cocktail should be tested for counting performance and sample compatibility, to ensure that correct and consistent results are obtained, if compared to previous batches.
This cost is usually higher on laboratory-prepared cocktails, since lot sizes are typically only one gallon. In laboratories which use large quantities of cocktails, lot sizes may be five gallons, which requires special labware to prepare the cocktail and requires more preparation time. An advantage of buying commercially prepared cocktails in large quantities is that the quality of the cocktail from a single lot will be assured and quality assurance time will be minimized. In addition, the manufacturers of commercial cocktails must meet quality assurance standards and will stand behind their product.

Another cost associated with laboratory-prepared cocktails is defective material or material being unsuitable for liquid scintillation counting. A cocktail that does not perform as required must either be reworked to improve performance, used as is, or scrapped. Reworking or scrapping a cocktail is expensive. Using the defective cocktail is perhaps the most expensive alternative, since this may lead to incorrect experimental results and may require repeating the entire experiment.

Except for the material cost, the expenses of a scintillation cocktail are hidden in the operating expenses for the laboratory. They are, nevertheless, real expenses, since time spent making the cocktail is time lost doing more productive projects. When all costs are included, commercial cocktails are usually more economical than comparable laboratory-prepared cocktails.

**Convenience**

Convenience is another important consideration in choosing the scintillation cocktails. Convenience usually translates into a time (cost) savings or improved quality of data. For example, the use of a single, universal cocktail is a convenience, and it provides improved quality of data, since there is no chance of using the wrong cocktail in an experiment. The users become familiar with its performance, its sample holding capacity and, if DPM is calculated, the confidence of efficiency determination applicable to all experiments. Costs can be reduced, since more of a common cocktail is used (purchasing in larger quantities usually reduces the unit cost on any item), less time is spent finding the appropriate cocktail, and experiments have to be repeated less frequently.

Commercial cocktails are convenient, since no time is required to prepare the cocktail. These cocktails are usually packaged in containers which are convenient to use. Cost savings have already been discussed for the commercially produced cocktails. These ready-for-use cocktails may also provide improved counting performance, since impurities which affect counting performance and sample holding capacity are removed during manufacture, enabling a comparison with previously obtained results.

**Safety**

An important aspect for choosing an LSC cocktail today is safety. Safety should always be a concern in the laboratory. With scintillation cocktails two aspects of safety are important: fire and health. Most solvents used in scintillation cocktails are flammable and also have toxic properties. Whenever scintillation cocktails are used or handled, procedures should be adopted to reduce or eliminate the hazards.

The hazard of fire is a major concern, since many materials in the laboratory will readily burn. The Department of Transportation has divided liquids which will bum into two categories for regulatory purposes: 1) flammable liquids, which have flash points below 100°F (TCC) and 2) combustible liquids, with flash points of 100°F or higher.
To evaluate the relative fire hazard of scintillation cocktails, it is necessary to understand what flash point means. The flash point of a liquid is the lowest temperature required to evolve an adequate vapor concentration so that the vapors can be ignited by an open flame near the surface of the liquid. The fire hazard is decreased for any liquid with a flash point above the laboratory’s ambient temperature.

Table 6-5 lists the flash points for a number of commonly encountered liquids. Common solvents for scintillation cocktails are marked with an asterisk. As can be seen from this table, scintillation cocktails have flash points comparable to or higher than many other commonly used liquids. Special consideration should be given to the high flash point solvent used in Opti-Fluor scintillation cocktail.

**Table 6-5. Flash Points of Some Common Liquids.**

<table>
<thead>
<tr>
<th></th>
<th>°F</th>
<th>°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>High flash point solvent of Opti-Fluor* and Opti-Fluor O.</td>
<td>302</td>
<td>150</td>
</tr>
<tr>
<td>Pseudocumene*</td>
<td>118-122</td>
<td>48-50</td>
</tr>
<tr>
<td>Xylene*</td>
<td>78-84</td>
<td>25-29</td>
</tr>
<tr>
<td>Isopropyl alcohol</td>
<td>72</td>
<td>22</td>
</tr>
<tr>
<td>p-Dioxane</td>
<td>54</td>
<td>12</td>
</tr>
<tr>
<td>Ethyl alcohol (absolute)</td>
<td>48</td>
<td>9</td>
</tr>
<tr>
<td>Toluene*</td>
<td>41</td>
<td>5</td>
</tr>
<tr>
<td>Benzene</td>
<td>12</td>
<td>-11</td>
</tr>
<tr>
<td>Gasoline (isooctane)</td>
<td>10</td>
<td>-12</td>
</tr>
</tbody>
</table>

Liquid scintillation cocktails with higher flash points generally offer the following advantages:

- Safer to use
- Fewer regulations for usage and storage.
- Fewer restrictions for transportation
- Lower vapor pressures, resulting in less vapor released in the instrument and working area.
- Less diffusion through plastic vials, resulting in a more constant counting volume, a lower "wall-effect," etc. Especially with the very high flash point solvents, as used in Opti-Fluor, no diffusion is observed; therefore, no 'wall effect' occurs.

The health hazard may be a more important safety consideration with scintillation cocktails. Excessive exposure to the solvent vapors may cause headaches, nausea and dizziness. Prolonged exposure may cause fainting. Table 6-6 provides data to compare the relative health hazard of some primary solvents used in liquid scintillation cocktails.

**Table 6-6. Toxicity Values on Some Primary Solvents.**

<table>
<thead>
<tr>
<th></th>
<th>Vapor pressure(^1) at 25°C in mm. of Hg.</th>
<th>Equilibrium(^2) Vapor Concentration at 25°C, ppm</th>
<th>Threshold Limit Value (TLV)(^3) ppm</th>
<th>Toxicity Ratio(^4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High flash-point solvent(^5)</td>
<td>0.075</td>
<td>2.90</td>
<td>25</td>
<td>116</td>
</tr>
<tr>
<td>Pseudocumene</td>
<td>2.2</td>
<td>11,600</td>
<td>100</td>
<td>116</td>
</tr>
<tr>
<td>p-Xylene</td>
<td>8.8</td>
<td>10,800</td>
<td>100</td>
<td>108</td>
</tr>
<tr>
<td>m-Xylene</td>
<td>8.2</td>
<td>8,700</td>
<td>100</td>
<td>87</td>
</tr>
<tr>
<td>o-Xylene</td>
<td>6.6</td>
<td>37,100</td>
<td>100</td>
<td>371</td>
</tr>
<tr>
<td>Toluene</td>
<td>28.2</td>
<td>28.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

2. Calculated for barometric pressure of 760 mm Hg.
4. Toxicity ratio is the ratio of equilibrium concentration to TLV.
5. For the high flash point solvent in Opti-Fluor, no TLV value is assigned.
The Threshold Limit Value (TLV) of a substance is defined as the maximum concentration of a substance in air to which most humans can be exposed for a period of time without exhibiting any toxic effects. Lower TLV values indicate greater risk of toxic effects. The lower the vapor pressure of a liquid, the lower is the rate of evaporation and the less is the exposure to vapors. These two factors must both be considered when evaluating the toxicity hazard of solvents. A toxicity ratio, the ratio of the equilibrium vapor concentration to the TLV, can be used. This provides a convenient means for evaluating the toxicity hazard for liquids. A low toxicity ratio indicates a lower toxic hazard.

The toxicity ratios for solvents used in liquid scintillation cocktails are all relatively high; the only way to ensure a low toxicity hazard with these solvents is to provide adequate ventilation in areas where the solvents are used and stored. Use of dispensing devices will also limit the amount of vapors which can escape into the air. The high flash point solvent used in Packard Opti-Fluor cocktail exhibits the lowest toxicity when compared with commonly used solvents.

**Volume of Cocktail Used**

In selecting the volume of cocktail used, the same criteria apply as for choosing a cocktail. Traditionally, 10 to 15 mL of scintillation cocktail are used. With the advent of small vials, as little as 3 mL of scintillation cocktail can be used with good counting performance. For small volumes of cocktail, chemical quenching becomes more of a problem because quenching is dependent upon the analyte concentration.

Reducing the cocktail volume to one-half for a given volume of analyte nearly doubles the concentration of quenching agents in the sample. Low cost cocktails, either laboratory-made or commercially prepared, usually have low scintillator concentrations and are therefore more subject to quenching (they have less quench resistance).

For good counting performance, cocktails in high scintillator concentrations and an optimum emulsifier system (having a relatively high quench resistance together with the analyte), are needed.

The major advantage of converting to small vials is cost savings. Small vials cost less than conventional vials of the same material Often the reduction in volume of scintillation cocktail will result in savings, even if a more expensive cocktail is required to achieve the desired counting performance. A major cost savings also comes from reduction of radioactive waste disposal.

The major limitation of using small vials is the volume of analyte which can be measured. In a conventional vial, analyte volumes up to 10 mL can be measured with good counting performance. For small vials, which have total capacities of 6 to 7 mL, the largest practical volume of analyte is 3 mL.

**Summary**

Choosing a cocktail for liquid scintillation counting is a very important part of the experiment design. For correct counting data, the cocktail must be compatible with the analytes to be counted. Deciding which cocktail to use is therefore a compromise between counting performance, cost, and convenience. Cost is not necessarily determined by the price of the scintillation cocktail alone. The volume of cocktail used, the cost of vials, disposal cost, and other costs associated with the cocktail would be included when evaluating cost per sample.

Safety is always an important concern in the laboratory. A scintillation cocktail with a flash point above the laboratory temperature is less of a fire hazard than a cocktail with a low flash point. Health hazards are the most significant hazards associated with scintillation cocktails. These hazards can be minimized by proper handling of the scintillation cocktails and good ventilation.

The use of the new generation of LSC cocktails, showing a much lower vapor pressure, will certainly improve safety in the work area.
SAMPLE OXIDATION
Sample Oxidation

Introduction
Since the 1940's investigators have been attempting to eliminate various liquid scintillation sample counting problems by oxidizing the samples. Within the last decade oxidation has become practical with commercial oxidizers. As will become apparent, the Tri-Carb 306 sample oxidizer (Packard) is currently the most efficient and convenient means of oxidizing any sample containing $^3$H and/or $^{14}$C.

Wet Oxidation
Over the last forty years, various wet oxidation reagents have been used to liberate $^{14}$CO$_2$, $^3$H$_2$O, and $^{35}$SO$_3$ generated from oxidizing samples containing $^{14}$C, $^3$H, and $^{35}$S, respectively (1, 2). The following is a list of some of the reported reagents used to achieve oxidation: nitric acid, nitric acid with perchloric acid, fuming sulfuric acid with periodate and chromic acid, and perchloric acid with hydrogen peroxide (1, 2, 3). These strong oxidizing reagents were used either to enhance solubility of samples, decolorize samples, separate radionuclides, or for a combination of these reasons. A consequence of using strong oxidizing reagents is the production of chemiluminescence. In addition, these reagents impair counting efficiency since they are strong chemical quenching agents. Recovery of radioactivity is often low because untrapped radioactive gases have escaped, or oxidation was incomplete. Logistically, wet oxidation is slow as well as potentially explosive (1, 2).

Dry Oxidation
Dry oxidation refers to combusting a sample in an atmosphere of oxygen to yield the highest oxides such as H$_2$O, CO$_2$, SO$_3$, oxides of nitrogen, etc. (3). Dry oxidation can be subdivided into two categories: static and dynamic. The underlying difference is that static oxidation uses a fixed quantity of oxygen, where as dynamic uses a continuous flow of oxygen during combustion. As sample sizes increase, a fixed quantity of oxygen may result in incomplete oxidation, and low, nonreproducible recoveries.

Static Oxidation
The methods of static oxidation that have been used are: oxygen flask (Schoniger method), and bomb methods (1, 2, 3, 4). A fixed volume of oxygen within a sealed flask is the common feature in both methods. Following ignition by electric current or an infrared source, the resulting gaseous products are usually cooled and collected in the same flask (2). The methods are time-consuming. Figure 7-1 illustrates a typical Schoniger oxygen flask.

![Figure 7-1. A Typical Oxygen Combustion Flask.](image)
Dynamic Oxidation

Dynamic oxidation uses a continuous flow of oxygen to ensure complete oxidation and to force the gaseous products through the H₂O and CO₂ collecting regions and the untrapped gases into vented waste. The instruments fall into two types of combustion: catalytic and noncatalytic. Two catalytic instruments, the Oxymat (Intertechnique), based upon the Peterson prototype, and the Harvey Material Oxidizer, yield higher recoveries of ³H and ¹⁴C than the static oxidation methods. However, the catalysts (commonly copper oxide and/or copper manganese) suffer from poisoning by retaining S, P, and halogen compounds (2). Poisoning occurs particularly with liquid samples. Other disadvantages include limited sample size (<1.0 gm) and time-consuming methods (1, 2).

Two types of noncatalytic dynamic oxidizers have been developed, one by Noakes (1, 4) and the other by Kaartinen in conjunction with Packard Instrument Company. The former method utilizes the Schoniger oxygen flask concept but with a continuous flow of O₂ through a steel tube into the combustion chamber. The sample is combusted by means of spark ignition. The gaseous products are swept into a cooled condenser and then through a H₂O exchange column (1, 4). The noncondensed gases, including CO₂, are bubbled into a CO₂ absorber and exchange column. The appropriate scintillation cocktails are then pumped down the separate collection columns into the vials. The recoveries are reported to be 97.8 ±1.5% for ³H and 98.5 ±1.0% for ¹⁴C, while the spillover of ¹⁴C into ³H is 0.8% and ³H into ¹⁴C is 0.07% (1). The memories are likewise low: 0.2% for ³H and 0.05% for ¹⁴C (1). Up to 0.73 gm of wet sample has been claimed to be burned and ready for LSC in 3 minutes (1). The Kaartinen technique, adopted into the Packard Tri-Carb 306 sample oxidizer is discussed on the following pages.
Tri-Carb 306 Sample Oxidizer

The Packard Tri-Carb 306 sample oxidizer is designed for automatic operation and offers simplicity while maximizing performance. The sample oxidizer can burn up to 1.2 gm sample, wet or dry, usually in less than 3 minutes. The recoveries for $^3$H and $^{14}$C are 99 ±1.0%. The memory for $^3$H and $^{14}$C is less than 0.05%, while the spillover of $^3$H into the $^{14}$C sample is below 0.01% and $^{14}$C into the $^3$H sample is 0.04%.

Figure 7-2 shows a schematic of the Tri-Carb 306 sample oxidizer, which for illustrative purposes has been divided into three sections: OX, $^3$H and $^{14}$C. Section OX is where the sample is loaded and combusted. Section $^3$H is where H$_2$O and $^3$HOH are collected, while Section $^{14}$C traps CO$_2$ and $^{14}$CO$_2$ and vents the untrapped gases. Sections OX, $^3$H, and $^{14}$C are shown separately in Figures 7-3, 7-4, and 7-5, respectively.
The pathway of sample combustion and evolved gases is traced below. The sample, which can be wet or dry, is placed in the platinum-rhodium wire basket. After the desired combustion time has been selected, the automatic operation is started by depressing the Program Start button. This raises and seals the sample and basket into the combustion flask.

Oxygen begins to flow as electric current passes through the wire basket to ignite the sample. The continuous flow of O₂ sweeps the gaseous products into the air-cooled condenser.

As the gaseous products pass through the condenser (section ³H shown in Figure 7-4), most of the water vapor condenses and drips into the scintillation vial below. The remaining small quantity of water vapor is trapped in the primary and secondary tritium exchange columns.

![Figure 7-4. Water Collection Section.](image-url)
During combustion, gaseous products, other than water vapor, pass through the $^3$H Section into the $^{14}$C Section of the oxidizer (shown in Figure 7-5). As the sample basket assembly is raised, the CO$_2$ absorber is injected into the reaction column. The flow of gases through the reaction column spreads the absorber on the walls of the convoluted column, thus increasing the surface area. The absorption of CO$_2$ is an exothermal reaction which liberates heat and causes a small amount of CO$_2$ to evolve; this is trapped by the carbon exchange column. The other remaining gases from combustion are vented into a waste bottle containing methanol, which absorbs the fumes of the CO$_2$ absorption agent.

Packard recommends either Carbo-Sorb or Carbo-Sorb II as the CO$_2$-absorber to use in the Model 306 sample oxidizer. Unlike most other CO$_2$-absorbers, these two absorbers are soluble in a toluene-base scintillation cocktail without the aid of a secondary solvent.

At the end of combustion, the flask is purged with steam and nitrogen. This steam and N$_2$ purge is a unique feature of the Tri-Carb 306 sample oxidizer. The purge step washes the water trapped in the condenser into the vial below, increasing recovery and decreasing memory. This is followed by the injection of tritium scintillation cocktail through the primary exchange column, flushing the trapped water into the $^3$H vial. Nitrogen and Carbon scintillation cocktail are injected in the $^{14}$C Section through the carbon exchange and reaction columns. This washes the trapped and absorbed $^{14}$CO$_2$ into a second vial.
Upon completion of sample preparation, the vials are lowered automatically. Following sample and scintillation cocktail collection, the vial holder automatically tilts to allow for pressurized deionized water and N2, to cleanse the two tritium exchange columns. A small quantity of tritium scintillation cocktail remains on the walls of the exchange columns to enhance absorption for the next sample burn. A final flush of the carbon exchange and reaction columns cleanses the system and establishes conditions ready for the next sample combustion.

The Model 306 sample oxidizer is an instrument designed to give highly reproducible recoveries of ³H and ¹⁴C while eliminating chemiluminescence and various quenching problems.

**Advantages of Sample Oxidation**

As the methods of sample oxidation have become refined, the advantages of the technique have increased. Sample oxidation eliminates many liquid scintillation counting problems that frustrated earlier investigators.

**All Methods**

Regardless of the method, sample oxidation eliminates insolubility problems which ordinarily lead to sample self-absorption and other associated counting geometry problems that reduce counting efficiencies. As discussed in a previous section, sample self-absorption occurs whenever the label is not in intimate contact with the primary solvent molecules. This occurs with precipitates, phase separation, and inert supports such as filters, gels, TLC scrapings, etc. In addition, certain labeled materials, such as many amino acids and proteins, tend to adhere to the walls of the scintillation vial resulting in another geometry problem in counting. Oxidation of labeled samples to ³H₂O and/or ¹⁴CO₂ yields soluble samples without the above problems.

**Oxidation With Trapping of Evolved Gases or Vapors**

Any oxidation method, wet or dry, that traps the evolved labeled gas(es) and/or vapor(s) offers additional advantages. These include: 1) an elimination of optical quenching, 2) an elimination of chemiluminescence, 3) reduced variation in chemical quenching, 4) reduced background count rate, and 5) separation of radionuclides in dual label experiments. The process can be designed to eliminate chemicals, including colored pigments, bleaches, tissue solubilizers, etc., which cause chemical and optical quenching as well as chemiluminescence. Although chemical quenching is not eliminated, it is minimized and relatively constant. In addition, the chemical quenching, such as oxygen quenching, is consistent regardless of the original state or composition of the sample. The trappings procedure can be used to isolate each radionuclide of a dual label sample into separate samples for counting. This separation provides improved sensitivity in the detection and quantification of dual labels.

**Dynamic Oxidation**

The advantages of dynamic oxidation over other methods are twofold: more complete oxidation of samples and the ability to use larger sample sizes. The sample size limitations imposed on static oxidation go hand in hand with the fixed volume of oxygen available for combusting a sample of a given mass. Unless there is a continuous flow of oxygen, as in dynamic oxidation, oxidation may be incomplete, increasingly so as the sample size increases. As the previous recovery values indicated, dynamic oxidation will give better recovery of label than static oxidation.
Tri-Carb 306 Sample Oxidizer-Noncatalytic, Dynamic Oxidation

The Tri-Carb 306 sample oxidizer eliminates chemiluminescence and several quenching problems. It also provides complete oxidation of large sample sizes, is convenient, rapid and completely automatic. The setup and operation of the instrument is simple. Preparation of samples for combustion is also simple and rapid. The recovery is 99% ±1%, spillover is negligible, and the memory effect is less than 0.05%.
References


GLOSSARY
OF TERMS
GLOSSARY OF TERMS

ACCURACY · Accuracy is an expression of the correctness of a measurement when compared to the true value of the quantity being measured. Radioactive decay is a random phenomenon hence the true value cannot be stated. It is preferable to refer to UNCERTAINTY in a measurement of radioactivity. (See also PRECISION.)

ALPHA PARTICLE · [Symbol: α (alpha)] A doubly positively-charged particle emitted by certain radioactive materials. It is made up of two neutrons and two protons bound together, hence is identical with the nucleus of a helium atom. It is the least penetrating of the three common types of radiation (alpha, beta, gamma) emitted by radioactive material, being stopped by a sheet of paper. It is not dangerous to plants, animals or man unless the alpha-emitting substance has entered the body. (See DECAY, RADIOACTIVE.)

AMPLIFICATION · The change in amplitude of a signal, i.e., increase of amplitude of pulses from the PMTs. In liquid scintillation counting, amplification also refers to a decrease in pulse amplitude.

AUGER ELECTRON · An electron originating in the cloud of electrons surrounding a nucleus that has acquired sufficient energy to break away from the atom. An Auger electron is produced when an inner shell electron is removed by mechanisms such as electron capture and an outer shell electron loses energy to fill the vacancy. The excess energy is frequently transferred to another outer electron of the atom which then breaks away and becomes an Auger electron with a kinetic energy equal to the excess energy minus the electron's binding energy.

AUTOMATIC EFFICIENCY CONTROL (AEC) · A method used by the Tri-Carb liquid scintillation analyzer to compensate for the effect of quenching on the sample spectrum. AEC extracts from the Spectralyzer spectrum analyzer the equivalent keV data for the region entered into the measurement program. AEC is based on SIE or tSIE.

BACKGROUND RADIATION · The radiation in man's natural environment including cosmic rays from space and radiation from radionuclides present in the local environment. Sources of the latter include materials of construction, e.g., metals, glasses, ceramics and concrete.

BECQUEREL · [Symbol: Bq] The basic unit of radioactivity in the International System of Units. One becquerel is equal to one disintegration per second. Since one curie is $3.7 \times 10^{10}$ dps, it is also $3.7 \times 10^{10}$ becquerels.

BETA PARTICLE · [Symbol: β (beta)] An elementary particle emitted from a nucleus during radioactive decay, with a single electrical charge and a mass equal to 1/1837 that of a proton. A negatively charged beta particle is identical to an electron. A positively-charged beta particle is called a positron.

BIOLOGICAL HALF-LIFE · [Symbol: T_b] The time required for a biological system, such as a man or an animal, to eliminate, by natural processes, half the amount of a substance (such as radioactive material). (Compare HALF-LIFE.)

CERENKOV RADIATION · Light emitted when charged particles pass through a transparent material at a velocity greater than that of light in that material. It can be seen, for example, as a blue glow in the water around the fuel elements of pool reactors. Pavel A. Cerenkov was the Russian scientist who first explained the origin of this light.
CHEMILUMINESCENCE · Random single photon events which are generated as a result of the chemical interaction of sample components. The coincidence circuit excludes most chemiluminescent events except at high rates.

CHEMICAL QUenchING · A reduction in the scintillation intensity seen by the photomultiplier tubes due to materials present in the scintillation solution that interfere with the processes leading to the production of light. The result is fewer photons per keV of beta particle energy and usually a reduction in counting efficiency. (Also refer to OPTICAL and QUENCHING.)

CHI-SQUARE TEST · A general procedure for determining the probability that two different distributions are actually samples of the same population. In nuclear counting measurements, this test is frequently used to compare the observed variations in repeat counts of a radioactive sample with the variation predicted by statistical theory.

COCKTAIL · The solution in which samples are placed for measurement in a liquid scintillation counter. Solvents and scintillators are the major components of a scintillation cocktail.

COEFFICIENT OF VARIATION (C.V.) · The ratio of the standard deviation of a distribution to its arithmetic mean.

COLLISION · A close approach of two or more particles, photons, atoms or nuclei, during which such quantities as energy, momentum and charge may be exchanged.

COINCIDENCE RESOLVING TIME · [Symbol: \( \tau \)] The maximum time interval allowed between two or more input signals for the production of an output signal. Input signals separated by more than the coincidence resolving time produce no output signal. The coincidence resolving time of Tri-Carb spectrometers is approximately \( 20 \times 10^9 \) second.

COINCIDENCE CIRCUIT · A portion of the electronic analysis system of the liquid scintillation counter which acts to reject pulses which are not received from the two PMTs within the COINCIDENCE RESOLVING TIME.

COINCIDENCE THRESHOLD · The minimum decay energy required for a liquid scintillation counter to detect a radioactive event. It is approximately \( 1/3 \) keV when the sample being measured is unquenched.

COMPENSATED REGIONS · Regions that are compensated for different levels of quenching. (Also refer to AUTOMATIC EFFICIENCY CONTROL.)

COMPTON EFFECT · Elastic scattering of photons (x-ray or gamma ray) by electrons. In each such process the electron gains energy and recoils, and the photon loses energy. This is one of three ways photons lose energy upon interacting with matter, and is the usual method with photons of intermediate energy and materials of low atomic number. It is named for Arthur H. Compton, American physicist, who discovered it in 1923.

COSMIC RAYS · Radiation of many sorts, but mostly atomic nuclei (protons) with very high energies, originating outside the earth’s atmosphere. Cosmic radiation is part of the natural background radiation. Some cosmic rays are more energetic than any man-made forms of radiation.

COUNTER · A general designation applied to radiation detection instruments that measure radiation in terms of individual ionizations, displaying them either as the accumulated total or their rate of occurrence.
LIQUID SCINTILLATION ANALYSIS

CURIE · [Symbol: Ci] A basic unit of radioactivity. The curie is equal to $3.7 \times 10^{10}$ disintegrations per second, which is approximately the rate of decay of 1 gram of radium. A curie is also a quantity of any nuclide having 1 curie of radioactivity. Named for Marie and Pierre Curie, who discovered radium in 1898. (See BECQUEREL.)

DAUGHTER · A nuclide, stable or radioactive, formed by radioactive decay of a PARENT.

DEAD TIME · The length of time immediately following the sensing of a signal pulse that the instrument remains insensitive and unable to process another pulse.

DECAY, RADIOACTIVE · The spontaneous transformation of one nuclide into a different nuclide or into a different energy state of the same nuclide. The process results in a decrease, with time, of the number of original radioactive atoms in a sample. It involves the emission from the nucleus of alpha particles, beta particles, or gamma rays; or the nuclear capture or ejection of orbital electrons; or fission. Also called radioactive disintegration.

DECONTAMINATION · The removal of radioactive contaminants from surfaces or equipment by cleaning and washing with a solvent or chemical.

DETECTOR · Material or a device that is sensitive to radiation and can produce a response signal suitable for measurement or analysis. A radiation detection instrument.

DISCRIMINATOR · An electronic circuit which distinguishes signal pulses according to their pulse height or voltage. It is used to exclude extraneous radiation counts or background radiation, or as the basis for pulse height analysis.

DISINTEGRATION · See DECAY.

DPM · Disintegrations Per Minute.

DUAL LABEL · Two different radionuclides in a sample.

EFFECTIVE HALF-LIFE · [Symbol: $T_{\text{eff}}$] The time required for a radioactive element in a biological system, such as man or an animal, to be reduced by one-half as a result of the combined action of radioactive decay and biological elimination.

EFFICIENCY · The ratio of measured observations (ie. counts) to the number of decay events which occurred during the measurement time. It is usually expressed as a percentage.

EFFICIENCY CORRELATION · The relationship between the measuring efficiency in a region of analysis and the quench indicating parameter. (QIP)

EFFICIENCY TRACING · A technique which uses spectral distribution analysis to calculate absolute activities of the sample at 100% efficiency.

ELECTROMAGNETIC RADIATION · A general term to describe an interacting electric and magnetic wave that propagates through vacuum at the speed of light. It includes radio waves, infrared light, visible light, ultraviolet light, x-rays and gamma rays.

* Triton x-100 is a Registered Trademark of Rohm and Haas
ELECTRON  ·  [Symbol: e⁻] An elementary particle with a unit negative electrical charge and a mass 1/1837 that of the proton. Electrons surround the positively charged NUCLEUS and determine the chemical properties of the atom. Positive electrons, or POSITRONS, also exist.

ELECTRON CAPTURE (EC)  ·  A mode of radioactive decay of a nuclide in which an orbital electron is captured by and merges with the nucleus, thus forming a new nuclide with the mass number unchanged, but the atomic number decreased by 1. (See K-CAPTURE.)

ELECTRON VOLT  ·  [Symbol: eV] The amount of kinetic energy gained by an electron when it is accelerated through an electric potential difference of 1 volt. It is equivalent to 1.603 x 10¹² erg. It is a unit of energy, or work, not of voltage.

EXCITED STATE  ·  The state of molecule, atom, or nucleus when it possesses more than its normal energy. Excess molecular or atomic energy may appear as light or heat. Excess nuclear energy is often released as a gamma ray.

EXTERNAL STANDARD  ·  A radioactive source placed adjacent to the liquid sample to produce scintillations in the sample for the purpose of monitoring the sample's level of quenching. The external standard is commonly ²²⁶Ra, ¹³⁷Cs or ¹³³Ba.

EXTERNAL STANDARD RATIO (ESR)  ·  The ratio of the counts produced in two channels of pulse-height analysis when a sample is irradiated with an external standard source. It is used as an indicator of the level of quenching in the scintillation solution.

FIGURE OF MERIT  ·  A term applied to a numerical value used to characterize the performance of a system. In liquid scintillation counting, specific formulas have been derived for quantitatively comparing certain aspects of counter performance and cocktail performance.

FLUORESCENCE  ·  The emission of light resulting from the absorption of incident radiation and persisting only as long as the stimulating radiation is continued.

FULL SPECTRUM DPM  ·  Advanced spectrum analysis technique used to separate the individual radionuclide spectra in dual-labeled samples to calculate DPM results.

GAIN CONTROL  ·  A control used to adjust the height of a pulse received from the detecting system.

GAMMA RAYS  ·  [Symbol: γ (gamma)] High energy, short-wavelength electromagnetic radiation originating in the nucleus of an atom. Gamma rays are very penetrating and are best stopped by dense materials, such as lead or depleted uranium. Gamma radiation frequently accompanies alpha and beta emissions and always accompanies fission.

GROSS COUNTS  ·  The total number of counts accumulated in the established counting region during the measuring period.

GROUND STATE  ·  The state of a nucleus, atom, or molecule at its lowest (normal) energy level.

HALF-LIFE  ·  [Symbol: T ¹/₂] The time in which one-half the atoms of a particular radioactive substance disintegrate to another nuclear form. Measured half-lives vary from millionths of a second to billions of years.
HALF-THICKNESS  ·  The thickness of any given absorber that will reduce the intensity of a beam of radiation to one-half its initial value.

INTENSITY (Radiant Beam)  ·  The amount of energy, the number of photons, or the number of particles of any radiation incident upon a unit area per unit time.

INTERNAL STANDARD  ·  A known amount of radioactivity which is added to a sample in order to determine the counting efficiency of that sample. The radionuclide used must be the same as that which is in the sample.

ION  ·  An atom or molecule that has lost or gained one or more electrons. By this ionization it becomes electrically charged. Examples: 1) an alpha particle, which is a helium atom minus two electrons, 2) a proton, which is a hydrogen atom minus its electron.

IONIZATION  ·  The process of adding one or more electrons to, or removing one or more electrons from, atoms or molecules, thereby creating ions. High temperatures, electrical discharges or nuclear radiations can cause ionization.

IONIZING RADIATION  ·  Any radiation displacing electrons from atoms or molecules, thereby producing ions. Examples: alpha, beta, gamma radiations, short-wave ultraviolet light. Ionizing radiation may produce severe skin or tissue damage.

IPA (Integrated Photomultiplier tube Assembly)  ·  A combination of a conventional PMT and a portion of the associated electronics in a single package.

ISOTOPE  ·  One of two or more atoms with the same atomic number (the same chemical element) but with different atomic weights. An equivalent statement is that the nuclei of isotopes have the same number of protons but different numbers of neutrons. Thus, $^{13}$C and $^{14}$C are isotopes of the element carbon (the superscript denoting the differing mass numbers) of approximate atomic weights. Isotopes usually have very nearly the same chemical properties, but somewhat different physical properties. (See NUCLIDE.)

K-CAPTURE  ·  The capture by an atomic nucleus of an orbital electron from the first (innermost) orbit or K-shell, surrounding the nucleus.

keV (kiloelectron Volt)  ·  One thousand electron volts.

KILO  ·  [Symbol: k] A prefix that multiplies a basic unit by $10^3$.

KINETIC ENERGY  ·  The energy which a body possesses by virtue of its motion. In classical mechanics, it is one-half the product of mass times the square of velocity.

LIMIT OF DETECTION  ·  The minimum amount of the characteristic property being measured that can be detected with reasonable certainty by the analytical procedure being used. The term "sensitivity" is sometimes incorrectly used to mean LIMIT OF DETECTION.

LINEAR AMPLIFICATION  ·  A form of pulse modification which increases the height of all pulses by a common factor. This preserves the relative height of all pulses.

LIQUID SCINTILLATION ANALYZER  ·  A liquid scintillation counter which stores the complete sample spectrum and uses applied spectrum analysis to calculate count rates or disintegrations per minute (absolute activity).
LOGARITHMIC AMPLIFICATION · A form of pulse modification in which pulse height is made proportional to the logarithm of the original height. This form of amplification does not preserve the relative height of all pulses.

LOWER LIMIT · Defines lower energy level of a region or channel.

LUMINESCENCE · A general term applied to the emission of light by causes other than high temperature. Light produced by the latter cause is called incandescence.

MASS NUMBER · The sum of the neutrons and protons in a NUCLEUS. It is the nearest whole number to an atom's atomic weight. For instance, the mass number of $^{235}$U is 235.

MEAN · The average of a list of numbers.

MEGA · [Symbol: M] A prefix that multiplies a basic unit by $10^6$.

MeV (Megaelectron Volts) · One million electron volts.

MICRO · [Symbol: µ] A prefix that divides a basic unit by $10^6$.

NANO · [Symbol: n] A prefix that divides a basic unit by $10^9$.

NET COUNTS · The total counts minus the background counts during the sample counting time.

NEUTRINO · [Symbol: $\nu$ (nu)] An electrically neutral elementary particle with a negligible mass. It interacts very weakly with matter and hence is difficult to detect. It is produced in many nuclear reactions, for example, in beta decay, and has high penetrating power. Neutrinos from the sun usually pass right through the earth.

NEUTRON · [Symbol: n] An uncharged elementary particle with mass slightly greater than that of the proton, and found in the nucleus of every atom heavier than hydrogen. A free neutron is unstable and decays with half-life of about 13 minutes into an electron, proton and neutrino. Neutrons sustain the fission chain reaction in a nuclear reactor.

NUCLEUS · The small, positively charged core of an atom. It is only about $1/10,000$ the diameter of the atom, but contains nearly all the atom's mass. All nuclei contain both protons and neutrons, except the nucleus of ordinary hydrogen, which consists of a single proton.

NOISE PULSE · A spurious signal appearing in the electronics of the system.

NUCLIDE · A general term applicable to all isotopes of all elements. It includes both stable and radioactive forms. Frequently, the term "isotope" is erroneously used to mean nuclide. (See ISOTOPE.)

OPTICAL QUENCHING · A reduction in the scintillation intensity seen by the photomultiplier tubes due to absorption of the scintillation light either by materials present in scintillation solution or deposited on the walls of the sample container or optic (e.g., dirt). The result is fewer photons per keV of beta particle energy and usually a reduction in counting efficiency. (See CHEMICAL QUENCHING.)

PARENT · A radionuclide that upon radioactive decay or disintegration yields a specific nuclide (the DAUGHTER) either directly or as a later member of a radioactive series.
**Phase Contact** · A phrase used to describe the degree of contact between two phases of heterogeneous samples. In liquid scintillation counting, better phase contact usually means higher counting efficiency.

**Phosphor** · A luminescent substance; a material capable of emitting light when stimulated by radiation.

**Phosphorescence** · See Photoluminescence.

**Photoelectron** · An electron emitted within the PMT upon exposure to photons of light. Photons are thus converted into an electrical signal.

**Photoluminescence** · Delayed and persistent emission of single photons of light following activation by radiation such as ultraviolet.

**Photomultiplier Tube (PMT)** · A device for the detection and measurement of low levels of light. It consists of a photocathode and a series of dynodes between the photocathode and the output electrode. The photoelectrons generated at the photocathode are multiplied by secondary emissions at each dynode. The resultant output pulse represents amplification of the photoelectric emission. The amplification is a function of the number of dynodes and the voltage applied to the dynodes. Photomultiplier tubes are used in liquid scintillation counting. Also named Multiplier Phototube (MPT).

**Photon** · In the quantum theory of Electromagnetic Radiation light is propagated in discrete packets of energy called Photons. The quantity of energy in each packet is called a Quantum.

**Pico** · [Symbol: p] A prefix that divides a basic unit by $10^{12}$.

**Positron** · [Symbol: $\beta^+$ (beta-plus)] An elementary particle with the mass of an electron but a positive charge. It is the "antielectron." It is emitted by some radionuclides and is also created in pair production by the interaction of high-energy gamma rays with matter.

**Precision** · Precision is an expression of the repeatability of a measurement. It is an expression of the Uncertainty in the measurement that is usually attributable to random variations which can be treated statistically.

**Primary Scintillator** · The substance in the scintillation cocktail which absorbs decay energy transferred from the solvent and emits light (photons) approximately proportional in intensity to the decay energy.

**Primary Solvent** · The medium in which both sample and scintillator are dissolved. It plays a major role in the transfer of decay energy to the scintillator (See Secondary Solvent.)

**Proton** · An elementary particle with a single positive electrical charge and a mass approximately 1837 times that of the electron. The nucleus of an ordinary or light hydrogen atom. Protons are constituents of all nuclei. The atomic number ($Z$) of an atom is equal to the number of protons in its nucleus.

**Pulse** · The electrical signal resulting when photons are detected by the PMT.

**Pulse Height Analyzer (PHA)** · An electronic circuit which sorts and records pulses according to height or voltage.

**Pulse Index** · The number of afterpulses following a detected coincidence pulse (used in three-dimensional or pulse height discrimination) to reduce the background of a liquid scintillation analyzer.
QUANTUM · The unit quantity of energy according to the quantum theory. It is equal to the product of the frequency of the electromagnetic radiation and Planck's constant (6.6256 x 10^-37 erg-sec.).

QUENCH INDICATING PARAMETER (QIP) · A value indicating the level of quenching in a sample (may be SIE, SIS, SCR, ESR or tSIE).

QUENCHING · Anything which interferes with the conversion of decay energy to photons emitted from the sample vial. This usually results in a reduction in counting efficiency. (See also CHEMICAL QUENCHING and OPTICAL QUENCHING.)

RAD · The unit of absorbed radiation dose. One rad is equal to 100 ergs/g of medium.

RADIATION · The emission or propagation of energy through matter or space by electromagnetic disturbances which display both wave-like and particle-like behavior. In this context, the "particles" are known as photons. The term radiation has been extended to include streams of fast-moving particles (alpha and beta particles, free neutrons, etc.) Nuclear radiations include alpha particles, beta particles, gamma rays and neutrons emitted from atomic nuclei during nuclear transformations.

RADIOACTIVITY · The property of an unstable nuclide of emitting radiation by spontaneous disintegration. The term is often shortened to "activity".

REGION · Similar to what is termed a "window" or "channel" in conventional liquid scintillation counters. The regions in a three region Tri-Carb system are referred to as "region A, B, or C."

ROENTGEN EQUIVALENT MAN (REM) · The unit of dose equivalent, which is a quantity used in radiation protection. The dose equivalent in rems is numerically equal to the absorbed dose in rads multiplied by the quality factor, the distribution factor and any other necessary modifying factors. For external sources of electrons and beta particles these last three factors are assumed to be 1.0.

SAMPLE COUNTING CHANNELS RATIO (SCR) · The ratio of net counts in two selected regions or channels.

SAMPLE SELF-ABSORPTION · The absorption of radiation, emitted by radioactive atoms, by the material in which the atoms are located. In particular, the absorption of radiation within the sample being assayed. SAMPLE SELF-ABSORPTION is eliminated when the sample being assayed is dissolved in the liquid scintillation solution.

SAMPLE OXIDATION · The combustion of organic samples, in the presence of oxygen, to produce H2O and CO2. It is advantageous in liquid scintillation since it can be used to convert colored or insoluble samples to colorless and soluble materials.

SCATTERING · A process that changes a particle's trajectory. Scattering is caused by particle collisions with atoms, nuclei and other particles or by interactions with electric or magnetic fields. If there is no change in the total kinetic energy of the system, the scattering is called elastic. If the total kinetic energy changes due to a change in internal energy the process is called inelastic scattering.
SCINTILLATION · A flash of light produced in a scintillator by an ionizing event. The scintillation is the sum of all photons produced by the decay event.

SCINTILLATION COUNTER · An instrument that detects and measures ionizing radiation by counting the light flashes (scintillations) resulting from the transfer of the energy of the radiation to scintillators.

SECONDARY SCINTILLATOR · Material in the scintillation cocktail which absorbs the emitted light of the primary scintillator and reemits it at a longer wavelength. It is added to improve the counting efficiency of the sample.

SECONDARY SOLVENT · A chemical included in the scintillation cocktail to improve sample or scintillator solubilities or to improve energy transfer.

SIGMA, PERCENT (% σ) · An expression of the standard deviation as a percentage. It is numerically equal to 100 times the STANDARD DEVIATION divided by the mean.

SINGLE LABEL · Only one radionuclide in a sample.

SOLUTE · A substance dissolved in a solution.

SOLVENT · Any substance that dissolves other substances.

SPECIFIC ACTIVITY · The quantity of radioactivity per unit mass, e.g., dpm/g, Ci/g.

SPECTRAL ENDPOINT · The maximum pulse height in the pulse-height distribution produced by the sample. It is of particular interest in the measurement of beta emitting radionuclides, since it corresponds to the maximum energy (E_{max}) emitted by a particular radionuclide.

SPECTRAL INDEX OF EXTERNAL STANDARD (SIE) · A number obtained from the Spectralyzer spectrum analyzer that is calculated from the spectral distribution of the external standard and is used as an index of the level of quenching in the sample.

SPECTRAL INDEX OF THE SAMPLE (SIS) · A number obtained from the Spectralyzer spectrum analyzer that is calculated from the spectral distribution of the sample, and is used as an index of the level of quenching in the sample.

SPECTRALYZER™ Spectrum Analyzer · The "spectral-analytical" section of the Packard Tri-Carb liquid scintillation counter.

SPECTRUM UNFOLDING · A technique of separating a composite dual label spectrum into its single label components.

SPILLOVER · A term used to describe the situation in dual label counting where a portion of the spectrum from one radionuclide is included in the REGION (window) used to count the other radionuclide.

STABLE ISOTOPE · An isotope that does not undergo radioactive decay.

STANDARD DEVIATION (σ) · A measure of the dispersion about the mean value of a series of observations. It is expressed in the same units as the mean value.

STATIC ELECTRICITY · An accumulation of electric charge on an insulated body such as a scintillation vial. In liquid scintillation counting, a discharge of static electricity may result in spurious pulses from the photomultiplier tubes.
SUMMATION · The addition of the amplitudes of the pulses received from each PMT.

TERMINATORS · The parameters used to end a sample measurement (i.e., preset time and preset count).

THREE-DIMENSIONAL SPECTRUM ANALYSIS · The analysis of the pulse energy distribution in function of energy, counts per energy, and pulse index. Allows auto optimization of a liquid scintillation analyzer for maximum performance.

THRESHOLD LIMIT VALUE (TLV) FOR TOXICITY · The airborne concentration of a substance which produces no adverse effects which will be experienced by workers repeatedly exposed to the substance.

TOXICITY RATIO · The ratio of the equilibrium concentration of a substance to the TLV for that substance.

TRANSFORMED SPECTRAL INDEX OF THE EXTERNAL STANDARD (tSIE) · A number obtained from the Spectralyzer spectrum analyzer that is calculated from the spectral distribution of the external standard and is used as an index of quenching in the sample.

TRITIUM · A radioactive isotope of hydrogen with two neutrons and one proton in the nucleus. It is man-made and is heavier than deuterium (heavy hydrogen). Tritium is used as a label in experiments in chemistry and biology. The nucleus of tritium is called a “triton.”

UNCERTAINTY · In a nuclear decay measurement, UNCERTAINTY refers to the lack of complete knowledge of a sample's decay rate due to the random nature of the decay process and the finite length of time used to count the sample.

VECTOR QUALITATIVE ANALYSIS · The mathematical expression of the relation between the external standard quench parameter (tSIE) and the Sample Spectrum Endpoint (SEP) used in monitoring the homogeneity of the sample.

X-RAY · A penetrating form of electromagnetic radiation emitted during electronic transitions in an atom. Usually involved are transitions in which outer orbital electrons give up some energy to replace missing inner orbital electrons. X-rays can also be produced by bombarding a target with high speed electrons. In this case they are called bremsstrahlung.
The Beta Ray Spectrum and the Average Beta Energy of Several Isotopes of Interest in Medicine and Biology

J. MANTEL

Wayne State University, School of Medicine, Department of Radiology, Detroit, Michigan 48207, U.S.A.

(Received 23 February 1972)

The beta ray spectra and the average energy has been calculated for 59 isotopes of interest in medicine and biology. For each isotope, the average energy, the beta ray spectrum, and a table including the pertinent input data used for computation, is given.

IN BETA ray dosimetry and beta ray counting, a knowledge of the beta ray spectrum and its average energy is important.

The average energy $E_{av}$ as a function of atomic number $Z$ and maximum energy $E_{max}$ for allowed spectra have been computed by MARINELLI et al.$^{(1)}$ and LOEVINGER$^{(2)}$ and for allowed and forbidden spectra by SHIMANSKAYA and ZALETSKII$^{(3)}$, WIDMAN et al.$^{(4)}$, DILLMAN$^{(5)}$, and MURTHY$^{(5)}$.

The computed beta ray spectrum and, in general, the composite beta ray spectrum (if more than one beta group is present), are seldom found in the literature.$^{(6)}$

The composite spectrum is calculated from the relation

$$N(E) = \sum_{i=1}^{k} P_i N_i(E),$$

where $N(E)$ is the number of particles of energy $E$ in the composite spectrum, $K$ is the number of beta spectra emitted by the isotope under consideration, $P_i$ is the frequency of emission for the spectrum $i$, $E_i$ is the maximum beta particle energy of the spectrum $i$ and $N_i(E)$ is the number of particles of energy $E$ in the spectrum $i$.

The average kinetic energy $E_{av}$ of the isotope under consideration is calculated from the
relation

\[ E_{\alpha\beta} = \frac{\int_0^{E_{\text{max}}} E N(E) \, dE}{\int_0^{E_{\text{max}}} N(E) \, dE}, \]

where \( E_{\text{max}} \) is the maximum beta particle energy for which there is emission in the composite spectrum. The number of particles of energy \( E \) in the spectrum \( i \) is calculated from the Fermi theory of beta decay.

\[ N_i(E) = f_i(Z,E)(E_i - E)^2(E_i + 1)(E_i^2 + 2E)^{\alpha/2} \]

where \( E \) is in units of \( mc^2 \) and \( f_i \) is the tabulated Fermi function \(^7\) multiplied by screening correction \(^8\) and shape factor. \(^9\) The same screening correction has been used in this work for allowed and forbidden transition. \(^10\) The shape factor correction is calculated from the relation

\[ S_n = \sum_{s=0}^{s=n} \frac{(2s + 1)!}{4^n(2s)!} \left( \frac{1}{2s - 2\nu + 1} \right) q^{2s-\nu} L_s, \]

where \( n \) is the degree of forbiddenness (for allowed transition \( n = 0 \)), \( q \) is the neutrino momentum in units \( mc \) and \( L_s \) are tabulated functions. \(^11\) Presently it is not possible to calculate the shape for non-unique forbidden spectra. However, it has been shown empirically that the non-unique first forbidden spectra have a shape similar to allowed transition \(^12\) and the non-unique second forbidden spectra have a shape similar to unique first forbidden transition. \(^5\)

The average energy computed by DILLMAN \(^5\) for a few radionuclides agrees with the results obtained in this work.

In the tables accompanying each spectrum, the following notation is used for the type of transition: for allowed transition \( -0 \), unique first forbidden transition \( -1 \), unique second forbidden transition \( -2 \), non-unique first forbidden transition \( -1' \) and non-unique second forbidden transition \( -2' \).

Acknowledgement-The author wishes to thank Mrs. CELIA JAROSZ for her clerical assistance.
LIQUID SCINTILLATION ANALYSIS

J. Maniel

H3
Avg. β energy 571 keV

C11
Avg. β energy 389-3 keV

C14
Avg. β energy 493 keV

N13
Avg. β energy 487 keV

Na 22
Avg. β energy 214-7 keV

Na 24
Avg. β energy 552.9 keV

P32
Avg. β energy 692.9 keV

S 35
Avg. β energy 486 keV

Cl 36
Avg. β energy 321.3 keV

AR 41

K 43
Avg. β energy 2953 keV
LIQUID SCINTILLATION ANALYSIS
References

Radioactivity Decay Tables

General Decay Table

The General Decay Table is used to determine the fractions of activity remaining for any radionuclide. Divide the time elapsed \((t)\) by the half-life \((T)\) of the radionuclide to calculate the number of half-lives expired \((t/T)\). Using the General Decay Table, find the fraction of activity remaining.

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HALF LIVES \((t/T)\)
Tritium Decay Tables (Fraction Remaining)

Half-life: 12.43 Years

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²1 year = 365.25 days
³1 month = 365/12 = 30.44 days
# Decay Data For Selected Radionuclides

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<th>Energy (keV)</th>
<th>Emanations per 100 Disintegrations</th>
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Notes: