Principles and Applications of Liquid Scintillation Counting

A Primer for Orientation

– National Diagnostics Laboratory Staff

Principles

Applications

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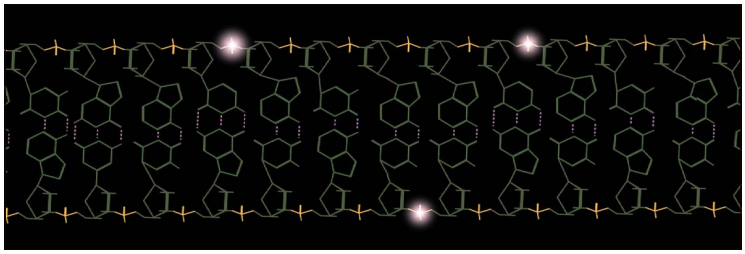
1 Fundamentals of Liquid Scintillation Counting

Liquid Scintillation Counting...Making Light of the Situation

The chemical properties of an element are determined by its atomic number - the number of protons in the nucleus (and electrons within neutral atoms of that element). Uncharged neutrons, within the nucleus along with protons, do not contribute to the atomic number, but will alter the atomic mass. This makes possible the existence of isotopes, which are atoms of the same element with different atomic weight. Most isotopes are stable, and do not undergo any spontaneous nuclear changes. A subset of isotopes possess too few or too many neutrons to be stable. These are radioactive. Radioactive atoms spontaneously rearrange their nuclei, emitting energy or particles in the process.



Radioactive isotopes of common elements are extremely useful in life science disciplines, among others, because radioactive atoms can be substituted for their nonradioactive counterparts in chemical formulations. The resulting radioactive compound is easily detectable but still chemically identical to the original material. Two detection methods predominate for assaying such incorporated radioactivity. In autoradiography, labeled material is allowed to expose a photographic emulsion. Development of the emulsion reveals the distribution of labeled material. In the second detection method, the amount of radioactivity in labeled samples is directly measured, either by a Geiger counter or by a scintillation counter. In scintillation counting, the sample is mixed with a material that will fluoresce upon interaction with a particle emitted by radioactive decay. The scintillation counter quantifies the resulting flashes of light.

1.1 Radioactive Emissions

1.1.1 Types of Radioactive Emission

Radioactive decay occurs with the emission of particles or electromagnetic radiation from an atom due to a change within its nucleus. Forms of radioactive emission include alpha particles (α), beta particles (β), and gamma rays (γ). α particles are the least energetic, most massive of these decay products. An α particle contains two protons and two neutrons, and thus comprises a stable helium nucleus. α particles only weakly penetrate whatever matter they encounter. They are unable to penetrate even 10 cm of air.

β particles are high energy electrons. These are produced during the conversion of a neutron to a proton in the nucleus. β particles are emitted in concert with a neutrino (Neutrinos are almost impossible to detect). The sum of the energies of the neutrino and β particle is a constant for a given isotope, and defines the maximum energy (E_{max}) which can be observed for any particle emitted from that isotope. E_{max} is approached only for particles emitted with a low energy neutrino. In practice a distribution of energies is observed, which is characteristic for the emitting isotope.

γ -rays differ from α and β emissions in that γ-rays are electromagnetic radiation, not particles. γ -rays are quite penetrating, in many cases passing through up to 5 cm of lead. Additionally, γ -rays are capable of generating secondary β emission from material they pass through. An electron in the material may absorb the energy of the γ-ray, and be promoted to an excited state which is no longer bound to its nucleus. Such an electron escapes from the atom as a free β-particle.

1.1.2 Characteristics of Useful Isotopes

The list of known radioisotopes is extensive, but the number of isotopes used in research is fairly small. To be useful as a label in research, an isotope must meet a restrictive set of qualifications. First of all, it must be an element that is already a part of the experimental system. For biological research, for example, isotopes of carbon, hydrogen, oxygen, and phosphorus are widely used. Alternatively, an element which can be substituted for another in the system may be used: sulfur isotopes can be used in place of oxygen, for example.

Useful isotopes must also have a reasonable half-life. The half-life of an isotope is a measure of the rate at which it decays to a nonradioactive state. As each nucleus emits its radiation, it eventually reaches a stable configuration which will not emit again. Thus a given quantity of isotope will eventually yield a finite total amount of radiation. Each nucleus decays independently, so the probability of a decay event occurring at any time is equal to the probability of any one nucleus decaying multiplied by the total number of radioactive nuclei present. This number changes as decay progresses, always proportional to the number of radioactive nuclei remaining. To express a rate of radioactive decay which is independent of the amount of material involved, the time required for the decay of 50% of the starting material, the half-life, is a useful quantity. Half-lives may range from milliseconds to thousands of years, a value characteristic of the particular isotope. Assuming that a 24 hour period is required for an experiment, an isotope with a half life ($t_{1/2}$) of 6 hours would undergo 2^4 fold reduction in emissions, or a loss of 94%. Most experimentally used isotopes have $t_{1/2}$ values of 10 days or more.

$$\lambda = \frac{0.693}{t_{1/2}} \qquad N = N_0 e^{-\lambda t}$$

λ = decay constant
$t_{1/2}$ = half-life
N = number of radioactive atoms
N_0 = initial number
t = elapsed time

To determine the number of radioactive atoms present within a sample at a given time, use the equations above.

Radioisotopes: Decay Products and Energies			
Isotope	**Emission**	**Energy (MeV)**	**Half Life ($t_{1/2}$)**
Tritium (^{3}H)	β	0.019	12.3 yrs
Carbon (^{14}C)	β	0.156	5730 yrs
Sulfur (^{35}S)	β	0.167	87.2 days
Phosphorus (^{32}P)	β	1.710	14.3 days
Phosphorus (^{33}P)	β	0.249	25.3 days
Iodine (^{125}I)	γ	0.178	59.9 days

Table 1.1.2a

1.1.3 The Use of Isotopes in Research

For many kinds of research, the utility of radioisotopes stems from their chemical identity with their nonradioactive counterparts. This allows their incorporation into "tracers", radiolabeled components which can be followed and detected through a series of reaction steps. Tracers are invaluable in metabolic studies, where they allow the determination of the catabolic and/or anabolic fates of nutrient compounds. Animals are fed diets containing labeled molecules, such as sugars or amino acids, and the radioactivity is followed through the system until excretion or incorporation. Another use for isotopes has been protein and DNA analysis studies, where probes which bind to specific macromolecules can be radiolabeled without interfering with their activity.

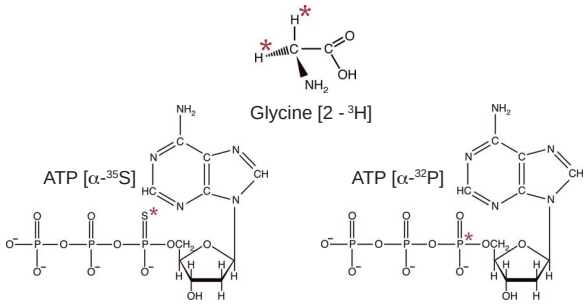


Figure 1.1.3a *Examples of radiolabeled biological molecules including tritiated glycine and ATP labeled with either ^{35}S or ^{32}P.*

1.2 Measurement of Radiation and Isotope Quantitation

Most research applications of radioisotopes, at some stage, require quantitation of the isotope, which is done by measuring the intensity of radiation emitted. Common nomenclature expresses this intensity as disintegrations per minute (DPM). The SI unit for radiation, the Becquerel (Bq), corresponds to 60 DPM (one disintegration per second). The curie, an earlier and still prevalent measure, is equal to 3.7 x 10^{10} Bq. Truly accurate measurement of DPM would require that every emission event be detected and counted, which is not possible in most situations. Additionally, naturally occurring isotopes and cosmic radiation contribute significant "background" radiation. Corrections for efficiency and background are needed to convert CPM, the counts per minute measured, into DPM, the number of decay events which actually occurred. Techniques have been developed for applying these corrections, and a great deal of research has been carried out to improve the efficiency of counting, using various detection systems.

1.2.1 Ionization Detection

Alpha, beta & gamma radiation all fall into the category of ionizing radiation. Alpha & beta particles directly ionize the atoms with which they interact, adding or removing electrons. Gamma-rays cause secondary electron emissions, which then ionize other atoms. The ionized particles left in the wake of a ray or particle can be detected as increasing conductivity in an otherwise insulating gas, which is done in electroscopes, ionization chambers or proportional counting chambers. These devices measure the pulse of conductivity between two electrodes when a particle or ray ionizes the gas between them. If a sufficiently high voltage is applied between the electrodes, an amplification of the signal can be obtained, and such counters can be quite sensitive. Their utility is severely limited by the fact that for most research applications only gas phase isotopes can be detected. This greatly complicates sample preparation (requiring the combustion of ^{14}C to $^{14}CO_2$, for example) and may preclude the analysis of some compounds entirely.

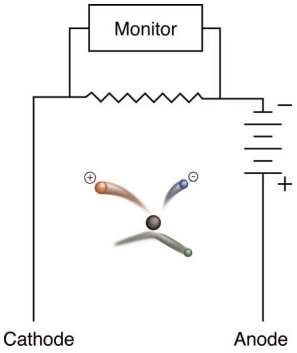


Figure 1.2.1a *The stylized representation of an ionization chamber above shows a beta particle colliding and ionizing a neutral particle.*

1.2.2 Scintillation Detection

Some irradiated atoms are not fully ionized by collision with emitted particles, but instead have electrons promoted to an excited state. (A sub population of ionized atoms can recombine with an ion of opposite sign and also produce an excited state.) Excited atoms can return to ground state by releasing energy, in some cases as a photon of light. Such scintillation phenomena form the basis of a set of very sensitive radiation detection systems. In solid scintillation systems, a crystal of inorganic or organic material, the scintillator, is irradiated by the sample. The light emitted in response to this irradiation is taken as a measure of the amount of radioactivity in the sample. Solid scintillation is excellent for γ radiation which is highly penetrating and can cause scintillation throughout a large crystal. An advantage of these techniques is that the same crystal is used for each sample, which enhances reproducibility. Unlike ionization counting, a gas phase sample is not required. For α or β counting, however, solid scintillation has severe limitations. The crystal must be protected from contamination by the sample, which means that the α & β particles must traverse a barrier prior to reaching the scintillator. α-rays in particular are severely attenuated by even 0.05mm of aluminum or copper, and so cannot be expected to reach a scintillator crystal through even the thinnest shielding.

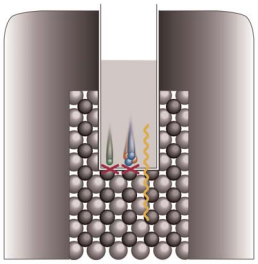


Figure 1.2.2a *In a solid scintillator, beta and alpha particles cannot penetrate the barrier between the sample well and the NaI crystal, but gamma rays pass through easily.*

Liquid scintillation (LSC), detailed in the next section, was evolved to provide a usable method for counting organic isotopic compounds. These materials are most often water soluble β-emitters. LSC addresses the need for convenience, reproducibility, and high sensitivity in these assays. It also offers solutions to the problem of counting aqueous (i.e. biological) samples in a nonaqueous environment.

1.3 Mechanism of Liquid Scintillation Counting

By eliminating the combustion steps needed for gas phase analysis, the introduction of liquid scintillation counting (LSC) reduced the time required to analyze radioactive samples from hours to minutes. For low energy ("soft") β emitters, LSC offers unmatched convenience and sensitivity. LSC detects radioactivity via the same type of light emission events which are used in solid scintillation. The key difference is that in LSC the scintillation takes place in a solution of scintillator, rather than in a solid crystal. This allows close contact between the isotope atoms and the scintillator, which is not possible with solid scintillation. With LSC the short path length of soft β emissions is not an obstacle to detection.

Liquid scintillation cocktails absorb the energy emitted by radioisotopes and re-emit it as flashes of light. To accomplish these two actions, absorption and re-emission, cocktails contain two basic components, the solvent and the phosphor(s). The solvent carries out the bulk of the energy absorption. Dissolved in the solvent, molecules of phosphor convert the absorbed energy into light. Many cocktails contain additional materials to extend their range of use to different sample compositions, but the solvent and the phosphor provide the scintillation of the mixture.

1.3.1 The Role of the Solvent

The solvent portion of an LSC cocktail comprises from 60 - 99% of the total solution. When a radioisotope dissolved in the cocktail undergoes an emission event, it is highly probable that the particle or ray will encounter only solvent molecules before its energy is spent. For this reason, the solvent must act as an efficient collector of energy, and it must conduct that energy to the phosphor molecules instead of dissipating the energy by some other mechanism. The solvent must not quench the scintillation of the phosphor, and, finally, the solvent must dissolve the phosphor to produce a stable, countable solution.

Aromatic organics have proven to be the best solvents for LSC. The prototypical LSC solvent is toluene (The solvents used in National Diagnostics scintillation fluids are safer and less toxic than toluene). The π cloud of the toluene ring (or any aromatic ring) provides a target for β-interaction, which captures the energy of the incident particle. This captured energy is generally lost through transfer to another solvent molecule, as toluene has little tendency to emit light or undergo other alternate decay modes. Thus, a β-particle passing through a toluene solution leaves in its wake a number of energized toluene molecules. The energy from these molecules passes back and forth among the solvent ring systems, allowing efficient capture by dissolved phosphors.

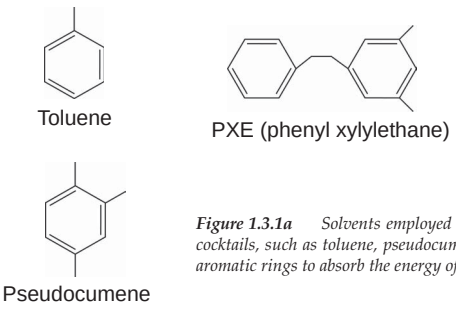


Figure 1.3.1a *Solvents employed in liquid scintillation cocktails, such as toluene, pseudocumene, or PXE, possess aromatic rings to absorb the energy of incident radiation.*

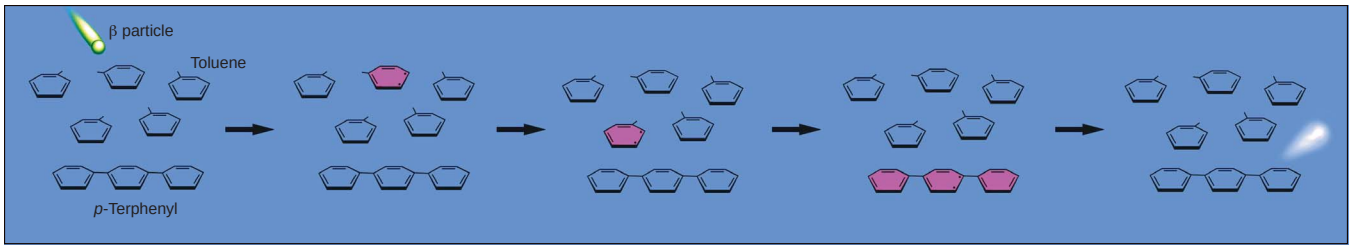


Figure 1.3.1b *The solvent molecules in a scintillation cocktail absorb a portion of an alpha or beta particle's energy. The energy passes between solvent molecules until the energy reaches a phosphor, which absorbs the energy and re-emits it as light.*

1.3.2 The Role of Phosphors (Scintillators)

Phosphors are broadly divided into two classes: primary and secondary scintillators. Included at 0.3-1% of the solution volume, primary scintillators provide the conversion of captured energy to the emission of light. The molecules of scintillator appear to induce a dipole moment in their solvation shell, allowing direct transfer of energy between the scintillator and excited solvent molecules separated by up to 10 other solvent molecules. Primary scintillators must be capable of being excited to a light emitting state by excited solvent molecules, and they must be soluble in the solvent at a sufficient concentration to give efficient energy capture.

Secondary scintillators, or wavelength shifters, were originally included in scintillation cocktails to compensate for the narrow spectral response of early photomultiplier tubes. Most primary scintillators emit light below 408nm, but the response of early photomultiplier tubes drops significantly in this range. A secondary scintillator captures the fluorescence energy of the excited primary scintillator, and re-emits it as a longer wave length signal. The process by which this energy exchange takes place is not clear. (Although the emission spectrum of the primary scintillator and the absorption spectrum of the secondary scintillator generally overlap, the kinetics of the exchange suggest direct contact rather than an emission-absorption event.) While modern phototubes are generally capable of counting the light pulses from the primary scintillator, secondary scintillators have been found to improve efficiency in many cases and are still included in most cocktails.

It has been found that linked benzene rings, rather than larger aromatic systems, generally make superior scintillators. PPO is the most commonly used primary, and Bis-MSB the most common secondary scintillator. Napthalene is somewhat unique, in that it can serve as a low efficiency scintillator and as a solvent, in concert with other organics.

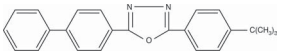
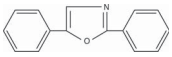
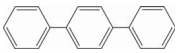
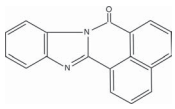
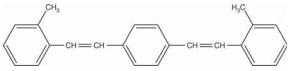
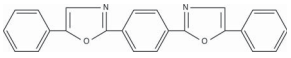
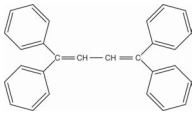
Primary Scintillators		
Scintillator	**Structure**	**Emission Wavelength**
Butyl PBD 2-[4-biphenylyl]-5-[4-*tert*-butyl-phenyl]-1,3,4-oxadiazole) **Order No. SFC-20**		363nm
Naphthalene **Order No. SFC-40**		322nm
PPO 2,5-diphenyloxazole **Order No. SFC-10**		357nm
p-Terphenyl **Order No. SFC-50**		340nm
Secondary Scintillators		
BBQ (7H-benzimidazo[2,1-a]benz [de]isoquinoline-7-one) **Order No. SFC-13**		477nm
Bis-MSB (1,4-bis[2-methylstyryl]-benzene) **Order No. SFC-90**		420nm
POPOP (1,4-bis[5-phenyloxazol-2-yl]benzene) **Order No. SFC-60**		410nm
TPB (1,1,4,4-tetraphenyl-1,3-butadiene) **Order No. SFC-15**		455nm

Table 1.3.2a

1.4 Liquid Scintillation Signal Interpretation

1.4.1 Patterns of Light Emission

A β particle, passing through a scintillation cocktail, leaves a trail of energized solvent molecules. These excited solvent molecules transfer their energy to scintillator molecules, which give off light. Each scintillator molecule gives off only one photon on activation, (and the wavelength of that photon is characteristic of the scintillator, not the β-particle), but multiple scintillators are activated by the energized molecules generated by one β-particle. The path of a β-particle in a cocktail is generally less than 0.1 cm; and the half life is correspondingly short, which means that the burst of photons from an emission event derives from a small space, and reaches the PMT with sufficient simultaneity to be read as one pulse of light.

The number of photons generated is directly proportional to the path length of the β particle, which is in turn determined by its emission energy (the β particle rebounds from solvent molecule to solvent molecule, until its incident energy is exhausted). The intensity of each light pulse corresponds to the emission energy and the number of pulses per second corresponds to the number of radioactive emissions.

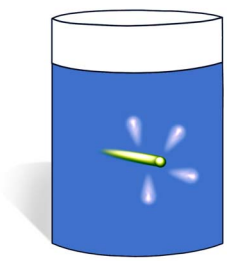


Figure 1.4.1a *Passing through scintillation fluid, a single beta particle gives rise to multiple, nearly simultaneous emissions of light. These photons are registered by the photomultiplier tube as one pulse of energy. The magnitude of this light pulse corresponds to the number of photons.*

1.4.2 Pulse Analysis

The scintillation counter classifies each pulse of photons according to the number of photons in the pulse, which corresponds to the energy of the individual β emission event. Pulses are collated into channels, and the counts per minute (CPM) in each channel is recorded. Each channel corresponds to a specific range of β energies (channels are also known as counting windows), and counts with energies above or below set limits are excluded from a particular channel. The usual practice is for three channels to be selected, which divide the energy spectrum of emissions into low, medium and high energy. The lowest channel corresponds to the energy of ^{3}H emissions, the highest to ^{32}P. When the counts have all been collated, the researcher knows the intensity of radiation, expressed as CPM, and its energy distribution, or spectrum. CPM is proportional to the amount of isotope in the sample, and the spectrum indicates the identity of the isotope.

Within a theoretically ideal cocktail, all of the energy from each β particle would be collected and converted into light. The spectrum of emitted β energy and the DPM values could then be taken directly from the data. The highest energy emissions would be compared with the E_{max} (maximum emission energies) for known radioisotopes to confirm the isotope identity. Real cocktails, however, are less than 100% efficient in energy collection and conversion, especially with lower energy β emissions. This makes data interpretation somewhat more complex.

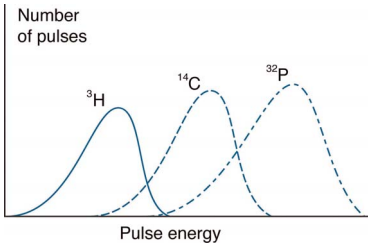


Figure 1.4.2a *A scintillation counter collating the energy spectrum of β emissions into three channels would read the majority of ^{3}H emissions in the low energy channel, ^{14}C in the intermediate channel, and ^{32}P in the high energy channel.*

1.4.3 Counting Efficiency

While the effectiveness of a scintillation cocktail may be expressed a number of ways, it is most often given as the percentage of emission events that produce a detectable pulse of photons, referred to as the counting efficiency. In other words, counting efficiency is equal to CPM/DPM, the ratio of Counts per Minute (CPM) to Disintegrations per Minute (DPM) expressed as a percentage. Counting efficiency varies for different isotopes, sample compositions and scintillation counters. Poor counting efficiency can be caused by an extremely low energy to light conversion rate, (scintillation efficiency) which, even optimally, will be a small value. It has been calculated that only some 4% of the energy from a β emission event is converted to light by even the most efficient scintillation cocktails. Fortunately, this number does not vary greatly across a wide range of β–energies, which avoids an additional level of complexity in signal interpretation. However, the low efficiency in energy conversion means that low energy β particles will only generate a few photons. ^{3}H, for example, has a maximum β energy of 0.019 MeV, which at 4% scintillation efficiency will generate about 240 photons. The average emission energy is generally 30-40% of E_{max}, which would give 70-100 photons in this case. Most phototubes used in scintillation detection only detect 1 in 4 photons, so the average ^{3}H β-emission event will produce only a 20-25 photon pulse in the counter. Clearly many emissions of below average energy, or emissions which lose photons due to sample characteristics, will fall below the level of a 1 photon event and will not register as a count on the instrument. The loss of CPM due to absorption of β-energy or photons by sample components is known as quenching. Quenching can easily reduce pulses below the detection limit of the counter, thus reducing the overall counting efficiency.

1.4.3 Quenching

Quenching is the loss of counts due to sample or cocktail characteristics and may result from a variety of components in a sample. Quenchers are customarily divided into the categories of chemical quenchers or color quenchers. Chemical quenchers absorb radioactive energy before it is converted to light. Therefore, chemical quenchers reduce the number of photons generated by each β-particle. Color quenchers absorb light in the range of the wavelength emitted by the scintillator. In this case the number of photons emitted is not changed, but the number reaching the photomultiplier tube is reduced.

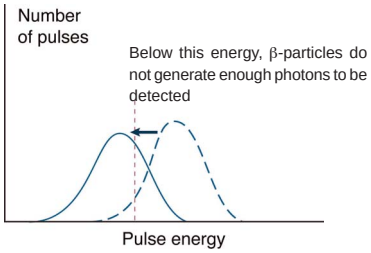


Figure 1.4.3a *Strong quenching can shift the majority of pulses below the threshold of detection (marked by the dashed red line).*

In both types of quenching, the energy of all light pulses is reduced, and the total CPM is reduced by the number of pulses quenched to below detectable levels. This leads to an underestimate of the total counts, and thus of the isotope present. It also leads to an apparent shift in the energy spectrum of the sample.

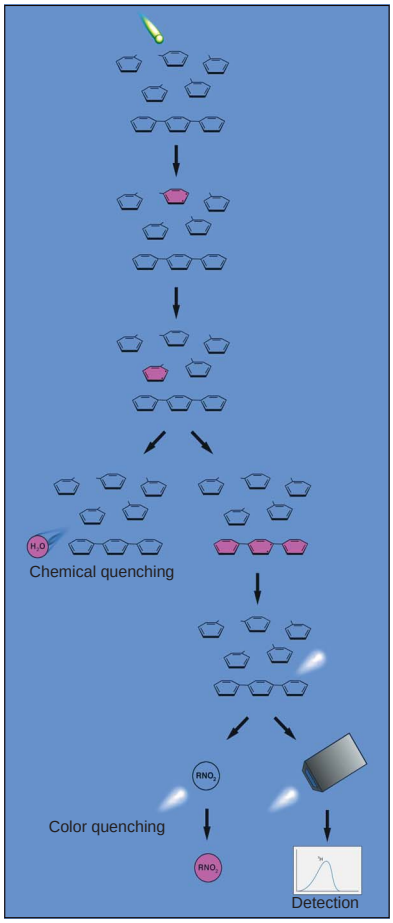


Figure 1.4.3b *Chemical quenching and color quenching can keep energy from a radioactive event from making its way through the scintillation mechanism to the photomultiplier tube. The stylized diagram above presents chemical quenching by water and color quenching by an organic nitrate as two possible obstacles to efficient counting.*

Quench Correction

Various methods are available for quench correction. The most straightforward, but most laborious, is the use of an internal standard. A known amount of radioactivity, added to an unknown sample, will increase the DPM by a predictable amount. The difference between the increase in DPM observed and that expected is due to quenching, and allows the determination of counting efficiency for that sample. The drawback to the use of internal standards is that each sample must be counted twice. It is also inconvenient to add an internal standard to many vials.

Many scintillation counters offer the use of an external standard to correct for quenching. After initial counting, a strong γ emission source is placed next to the vial and the sample is counted again. The γ rays cause secondary emission of Compton electrons, which scintillate in the cocktail like β particles. The counts due to sample radioactivity are subtracted, leaving only the Compton electron counts. The theoretical energy distribution of the Compton electrons is compared with the measured energy spectrum to determine the extent of quenching. The samples must still be counted twice, but nothing need be added to the vials, and the process may be carried out automatically by the counter.

The analysis of the energy spectrum is commonly done by computing a "channels ratio". The detected counts are divided into channels based on their relative energies, and the number of high energy counts (with two channels, the "B" channel) is compared to the number of low energy counts (channel "A"). The ratio, calculated as B/A or B/B+A, will change if the sample is quenched. Quenching reduces the intensity of each light pulse, so counts will appear to be of lower energy. This will shift counts from high to low energy channels, and decrease the channels ratio. In practice, a set of quenched standards is created by adding a quenching agent to reduce the CPM of an internal standard. The channels ratio of the external standard is then determined, and a correlation is established between quench and channels ratio. The channels ratio analysis may also be applied to the sample itself to determine quenching. Again, a set of quenched standards is assembled, and a known amount of radioactivity is added to each. A curve is constructed, relating CPM/DPM to B/B+A. Once this curve has been generated, the quench of any subsequent sample can be determined from its channels ratio. This quenching factor is then used to correct CPM to DPM.

1.5 The Complete Scintillation Cocktail

Living creatures contain both hydrophobic and hydrophilic compounds, any of which may be labeled during the course of a radioactive experiment. As discussed in earlier sections, the best solvents for scintillation counting are the aromatic organics, such as toluene and xylene. Hydrophobic compounds can be counted directly in such solvents, but hydrophilic materials, which include many biological samples, are completely insoluble in simple cocktails. This requires the engineering of complex cocktails, capable of bringing hydrophilic sample molecules into close proximity to organic solvents and the dissolved scintillators.

Most scintillation cocktails designed for aqueous samples contain surfactants, which emulsify the sample into the organic solvent. Toluene containing the detergent Triton-X100 is a prototypical example of an emulsion cocktail (Figure 1.5a). When water is added to a solution of Triton-X100 in toluene, the detergent molecules orient to form micelles with their hydrophobic alkane chains facing out into the solvent, and their hydrophilic polyethylene glycol chains facing in, "dissolved" in a small amount of trapped water. Various other components are added to the cocktail in small amounts, which regulate the size of the micelles to maintain overall solution clarity.

Because the surfactants and other additives are generally less effective at energy capture than the solvent, emulsion cocktails are less efficient than pure solvent cocktails. In addition, the partitioning of the aqueous samples into micelles means that the radioactive emissions must escape from the micelle before beginning the scintillation process. Energy is lost while the emitted particle traverses the micelle, resulting in fewer photons per particle reaching the counter. The result is an effective quench, which can be corrected by the means given in the previous section. This quenching is dependent upon the size of the micelles, which in turn depends upon the ratio of sample to cocktail. It is important to use a correction curve which accounts for this volume dependence.

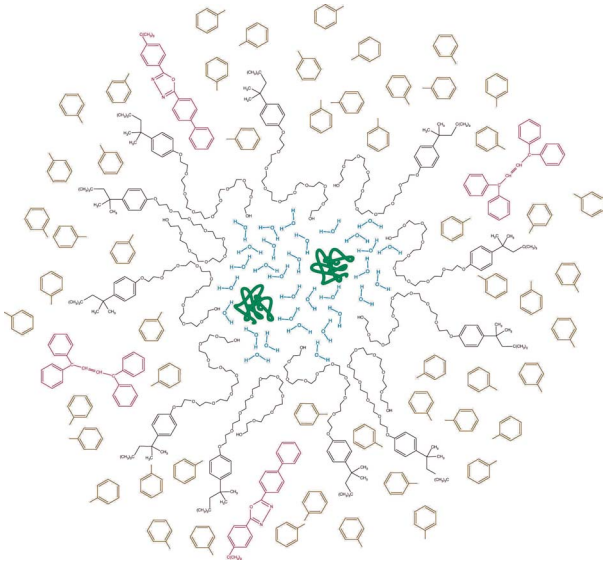


Figure 1.5a *Micellar structure in a scintillation cocktail. Hydrophilic proteins (green) and water (blue) are emulsified by Triton X-100 (black). Radioactive emissions from the labeled protein must pass through the micelle to encounter the toluene solvent (brown) before energy can be passed to the primary and secondary phosphors (red) and be re-emitted as light.*

Sample Capacity

Increasing the amount of water dispersed in an emulsion cocktail will increase micellar size, decreasing the energy in any given β particle when it finally escapes the micelle and begins to generate light. At some point, the amount of water added causes a micellar inversion, in which the organic solvent is surrounded by the surfactant, while the water makes up the bulk solution. Efficiency will decrease drastically at this point. The inversion process generally does not yield a clear solution. Above the cocktail sample holding capacity, the mixture is cloudy or opaque, and photons emitted within such a solution are lost to internal reflectance. Sample holding capacity is dependant upon sample composition and upon temperature. In planning scintillation counting experiments, it is crucial to ensure that the sample volume not be too close to the capacity of the cocktail. Such samples may turn opaque with a 1-2° change in temperature, and give falsely low readings. With the use of translucent plastic scintillation vials this type of artifact can be very difficult to detect.

Sample Holding Capacities of National Diagnostics Ecoscint A

Sample	Capacity (ml sample /10ml cocktail)
Water (20°C)	4.5
Water (25°C)	4.0
Water (15°C)	5.0
0.05M Tris-HCl	4.5
0.15M NaCl	4.0
10% Sucrose	3.0
8M Urea	1.0

Table 1.5a

1.6 Chemiluminescence and Static Electricity

Another commonly encountered artifact is chemiluminescence. This is caused by any chemical reaction which generates an excited product molecule, which decays to emit light. These reactions generate only a single photon, which may be quenched, or may reach the counter to register as a low energy emission event.

Such reactions can generate 10^5-10^6 cpm, skewing both total cpm data and counts ratio information. Chemiluminescence is generally diagnosed by counting the samples twice with a period of about an hour between counts. As the chemiluminescent reaction consumes its substrate, the rate of photon production decreases noticeably over an hour, and will usually decrease to zero over the course of 2-24 hours. By contrast, even a short-lived isotope like ^{32}P will decrease its emissions by only 5% over 24 hours.

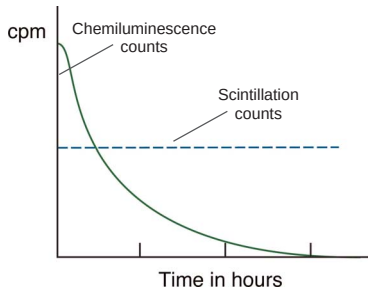


Figure 1.6a *Spurious counts due to chemiluminescence (green) dissipate over the course of hours, while the true count stays nearly constant. With the common isotopes used in life sciences research, the rate of radioactive decay is much slower than the decay of chemiluminescent reactions.*

Many scintillation counters use coincidence counting to eliminate counts due to chemiluminescence. This system uses two photomultiplier tubes, generally mounted opposite each other. Because chemiluminescence only generates one photon at a time, only one photomultiplier tube will be activated. In contrast, the burst of photons from a genuine decay event will activate both photomultiplier tubes. Coincidence counting eliminates those emission events which do not appear at both photomultiplier tubes, thus eliminating chemiluminescence counts. However, coincidence counting will also cause some low energy emission events to be missed.

A further source of spurious counts is static electricity. The energy from a static electric buildup can be released as a burst of light from the cocktail. In dry environments, with plastic vials and latex gloves, high levels of static can build up, sufficient to give 10^4 cpm or higher from an affected sample. Static is the likely cause if counts from an individual sample vary unpredictably from one measurement to the next. Static can be minimized by wiping the vials with a wet paper towel (water dissipates the static) or by wiping with an antistatic laundry dryer sheet.

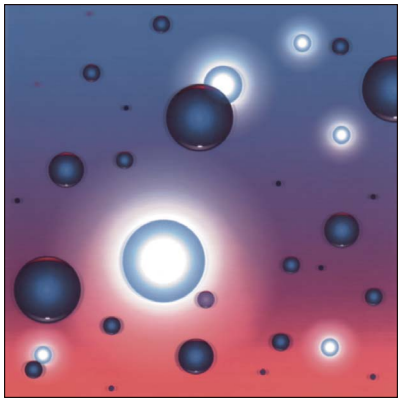
1.7 Waste Disposal Issues

An aspect of LSC which must be considered in experimental design, is waste disposal. Unlike solid scintillation, LSC adds components to the sample increasing the volume of radioactive material by up to 1000 fold. The components of the LSC cocktail may represent a hazard or a disposal problem in addition to the radioactivity. For many experiments, only a small percentage of the samples counted will have significant radioactivity, so disposal of the LSC is the predominating issue. Fortunately, biodegradable LSC cocktails have been developed, such as National Diagnostics' Ecoscint fluids and Uniscint BD, which substantially reduce the difficulty of disposing of LSC waste.

2 Applications of Liquid Scintillation Counting

LSC Applications...Make the Prize Light! - William Shakespeare, *The Tempest*

Like solid scintillation, liquid scintillation counting was originally applied only to discrete samples, either aqueous or nonaqueous, and protocols were developed for each type. The introduction of flow counting apparatus made it possible to use LSC to monitor the effluent from a chromatography column for radioactive peaks. Again, the samples in flow LSC experiments are divided between aqueous and nonaqueous compositions.



Cocktails have been developed for discrete samples and for flow applications, answering the specific needs of each type of experiment. In addition, cocktails are available for a variety of specific applications for the counting of discrete samples with unique characteristics. Most of these cocktails are designed to simplify sample preparation in such applications as counting samples in electrophoresis gels, samples on filters, whole tissue samples or combusted materials.

2.1 Counting Discrete Samples

Liquid scintillation counting of discrete samples is conceptually straightforward. A sample is mixed with an appropriate volume of scintillation cocktail, and the mixture is placed in an LSC vial and counted. For some samples, no additional steps are required, but in many situations, samples must be processed to avoid artifacts. The most common causes of artifacts are static electricity counts, chemiluminescent counts and color quenching. Protocols for sample preparation to maximize the efficiency of counting and minimize background are given below. These protocols can be readily adapted to a variety of samples. Following this section, the preparation and counting of several unique sample types are presented, as "special applications".

The best counting efficiencies are achieved when samples uniformly disperse into the cocktail to produce a clear, colorless, pH neutral emulsion. Uniform dispersion of the sample is achieved by selecting the appropriate cocktail formulation. Organic samples present no problem. With organic samples, the highest efficiency can be achieved in cocktails which contain no emulsifiers and which are only suitable for organics. Organic samples, however, can be successfully counted in emulsifying cocktails, and often the convenience of using one cocktail for all applications outweighs any loss in efficiency. Aqueous samples present more of a challenge. The choice of cocktail will depend upon the balancing of sample holding and efficiency. It is a good idea to choose a cocktail which can hold at least 10% more sample than you intend to add, as sample capacity may be strongly affected by temperature or sample components.

2.1.1 Sample Neutralization (Elimination of Chemiluminescence)

The neutralization of strongly alkaline samples is necessary to avoid chemiluminescence (Section 1.6). Neutralization can be accomplished by the addition of acetic acid. If the sample contains a high concentration of alkali, the addition of acetic acid may increase the overall salt content beyond the capacity of the scintillation cocktail. In such situations, the sample will need to be diluted prior to the addition of cocktail. If neutralization is not practical, samples may be left to stand 1-3 hours, or in some cases, overnight, before counting. This allows time for the chemiluminescent reaction to run its course and die out. If chemiluminescence is suspected, samples should be counted repeatedly at intervals of greater than 1 hour until a stable reading is obtained.

2.1.2 Decolorizing

Achieving a colorless solution of sample in cocktail is generally not problematic. Many samples are colorless, or contain so little color that dilution into the scintillation cocktail gives an essentially colorless solution. In those cases where samples are deeply colored, particularly when the sample absorbs in the region of 300-400 nm, where scintillation phosphors emit, several decolorizing protocols are available. As visible color often depends upon long conjugated polyene systems, strong oxidants are used to "bleach" the samples. Samples can be treated quite harshly prior to counting, because chemical changes to the labeled compounds will not alter the number of DPM emitted.

Protocol 2.1.2a

Decolorizing LSC Samples with Ultraviolet Light

Ultraviolet irradiation is often effective in bleaching visibly colored samples. The optimal wavelength, intensity and time must of course be determined for each sample. In many cases, exposing samples to sunlight for 1-2 hours is sufficient. Bleaching by UV has a great advantage over other methods - nothing is added to the sample, avoiding the potential quenching or chemiluminescent effects of other bleaching agents.

Protocol 2.1.2b

Decolorizing LSC Samples with Hydrogen Peroxide

H_2O_2 is a strong oxidant and a very effective bleaching agent. It is inexpensive, easy to work with, and miscible with aqueous samples. The only disadvantage to using H_2O_2 is that it decomposes to produce molecular oxygen, which is an effective quenching agent. Samples must be heated to drive off the O_2 following H_2O_2 bleaching, to ensure reproducible results.

1. Mix: 0.1 - 0.3 ml of 30% H_2O_2 with 1ml sample
2. Incubate 1 hour at 50°C, shake occasionally.
3. Cool to room temperature, add scintillation cocktail and count.

Protocol 2.1.2c

Decolorizing LSC Samples with Benzoyl Peroxide

Samples which are not soluble in water or tissues which have been dissolved in organic solubilizers, can be bleached with Benzoyl Peroxide.

1. Dissolve 1g Benzoyl Peroxide in 5 ml Toluene - heating to 60°C may be required. Filter solution if cloudy. **(Caution: Toluene has a flash point of 7°C. Heating must be carried out in a spark-free fume hood to avoid an explosion hazard.)**
2. Add 2 ml Benzoyl Peroxide/Toluene solution to 1 ml sample.
3. Incubate for 30 minutes at 50°C.
4. Cool to room temperature, add scintillation cocktail and count.

2.2 Special Sample Preparation

The preceding sections have outlined LSC procedures for samples which require only minimal preparation prior to counting. Often it is necessary to count materials which are not well suited to LSC. The problem is usually one of counting geometry, which is related to sample dispersion. Figure 2.2a (pg. 157) shows the difference between the counting geometry of a well-dispersed sample vs. one adhering to filter paper. Counts are lost to absorption because they are emitted in a direction which does not take them into the cocktail. Additionally, in a sample

which rests on the bottom of the tube fully 50% of the counts may be lost, as any downward emissions will either fail to scintillate or will generate photons with no available path to the PMT. Examples of samples which present dispersion problems are silica particles from TLC plates, precipitates collected on filters, tissue samples, and polyacrylamide gels. Protocols are provided here for these samples.

2.2.1 TLC Plates

In a typical TLC experiment, the radioactivity is detected at two points: after TLC it is analyzed by autoradiography (Electrophoresis Theory, Section 4.1.3), to locate radioactive spots. These spots are then scraped off of the plate and counted to provide quantitative information. Each of these steps can be enhanced using the following protocols.

Protocol 2.2.1a

Autoradiography and LSC with TLC Plates

A. Autoradiography (Fluorography)

After the plate has been developed, spray twice with National Diagnostics' Autofluor and allow the plate to dry. This impregnates the plate with phosphors, which will convert the β emissions to more readily detectable photons. Autofluor enhances the speed and sensitivity of detection when the plate is placed on film. For details on autoradiography procedures, see Electrophoresis Theory, Section 4.1.3.

B. Liquid scintillation counting of scraped TLC silica

Option 1: Suspend the silica powder in 10ml of a cocktail which has a gel phase with water such as National Diagnostics' Hydrofluor. Shake well, and add 3ml H_2O. Shake until gel forms, and count.

Option 2: Suspend the silica in 10ml of an organic based (non-emulsion) cocktail such as National Diagnostics' Ecoscint O. Add 0.5-1g finely divided silica thixotrophic agent to form a clear gel in the solution, keeping the TLC particles suspended. Count as usual.

Use in Autoradiography and Scintillation Counting with TLC Plates

Autofluor **LS-315**
National Diagnostics' autoradiographic image intensifier, Autofluor, is a water based phosphor yielding superior results to PPO-DMSO.

Hydrofluor **LS-111**
High performance scintillation fluid with a traditional solvent base, Hydrofluor offers a gel phase option for counting particulate samples.

Ecoscint O **LS-274**
Ecoscint O is a biodegradable scintillation fluid designed to count non-aqueous (organic soluble) samples.

2.2.2 Counting Samples on Cellulose-Ester Filters (Millipore™ filters)

A common radiotracer technique is to precipitate macromolecules (protein & DNA) with TCA or some other strong denaturant, collect the precipitate on a filter and count it. Often such procedures give variable results, depending upon the degree to which the sample disperses from the filter into the cocktail. A typical artifact is counts which rise over time as more material dissolves off of the filter. It is possible to avoid these artifacts by using a cocktail which dissolves the filter, reproducibly releasing all of the sample for counting.

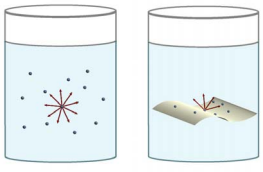


Figure 2.2a *The well-dispersed sample on the right achieves 4π counting geometry, while half of the counts with the sample on the right are lost due to absorption and attenuation of emissions by the filter paper.*

Protocol 2.2.2a

Counting Samples on Cellulose-Ester Filters

Do not dry the filters, because this will slow the dispersion process. If a filter has dried, dampen it with 1-2 drops of distilled water.

1. Place damp filter with sample into 10ml of National Diagnostics' Filtron X.
2. Allow to stand at room temperature for 15 minutes. Shake well and count.

Counting Samples on Cellulose Ester Filters

Filtron-X **LS-201**
A complete scintillation fluid Filtron-X solubilizes cellulose acetate, cellulose nitrate and mixed ester filter disks assuring homogeneous counting.

2.2.3 Counting Tissue Samples

Samples of animal or plant tissue are rarely thin or small enough to allow for full counting efficiency. Homogenization of such samples will allow them to be dispersed into a cocktail, but processing large numbers of radioactive samples by homogenization is not practical. To allow for efficient and consistent counting of tissue samples, tissue solubilizers have been developed. These products contain strong denaturants and other agents, which can dissolve tissues at moderately elevated temperatures.

Protocol 2.2.3a

Counting Tissue Samples

BIOSOL/BIOSCINT

1. Place up to 200 mg of tissue, or 1 ml of blood, in a glass scintillation vial. Ground or minced tissue will dissolve more rapidly. Avoid adhesion of the sample to the bottom of the vial, as this will extend the digestion time.
2. Add 1 ml of Biosol. Agitate gently (do not vortex).
3. Incubate in shaking water bath at 50°C for 1-4 hours, until clear.
4. If necessary (for blood or other pigmented samples) decolorize with 0.2 ml of 30% H_2O_2. Cap loosely and incubate at 50°C 1 hour.
5. Cool to room temperature, add 10 ml of Bioscint and count.

SOLUSOL

1. Place up to 100 mg of tissue, or 0.5 ml of blood, in a glass scintillation vial. Ground or minced tissue will dissolve more rapidly. Avoid adhesion of the sample to the bottom of the vial, as this will extend the digestion time.
2. Add 0.2-0.4 ml of Solusol. Agitate gently (do not vortex).
3. Incubate at 50°C for 1-2 hours, or at room temperature for 3-5 hours, until clear.
4. If necessary (for blood or other pigmented samples) decolorize with 2 volumes of 20% benzoyl peroxide in toluene. Cap loosely and incubate at 50°C 30 minutes. Lightly colored samples may be bleached by UV or sunlight exposure.
5. Cool to room temperature, add 10 ml of Soluscint O and count.

Products for Counting Tissue Samples

Biosol **LS-310**
Bioscint **LS-309**
Together, Biosol and Bioscint form a nonhazardous, biodegradable tissue solubilizer and scintillation system. The combination eliminates chemiluminescence and renders the mixture nonhazardous.

Solusol **LS-311**
Soluscint O **LS-312**
Soluscint A **LS-313**
Solusol is National Diagnostics' traditionally formulated solubilizer. Soluscint O is the scintillant designed for use with most routine samples solubilized by Solusol. Use Soluscint A with large aqueous samples.

2.2.4 Counting $^{14}CO_2$

Prior to the introduction of Liquid Scintillation counting, a primary route of radiotracer analysis was to combust the organic material and detect the $^{14}CO_2$ so generated in a gas phase proportional counter. Many protocols still call for combustion and $^{14}CO_2$ counting, and many metabolic studies require quantitation of $^{14}CO_2$ exhaled by tracer-fed animals. $^{14}CO_2$ is assayed by trapping it in a liquid phase as a complex with a strong base (carbamate) and then counting the liquid phase.

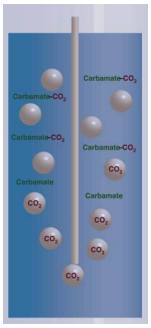


Figure 2.2.4a *Bubbling $^{14}CO_2$ through carbamate traps the gas in the liquid phase, yielding a suitable sample for liquid scintillation counting.*

Protocol 2.2.4a

Counting $^{14}CO_2$

OXOSOL C14

This is a single solution containing both carbamate and scintillators. Gas containing $^{14}CO_2$ is shaken with the cocktail or bubbled through with a sparger. It is advisable to extract the gas with a second volume of solution to ensure capture of >90% of the $^{14}CO_2$. Cap the vials and count.

CARBAMATE-1 + OXOSOL 306

In this system, the carbamate is provided as a separate solution, which may enhance capture efficiencies. Shake or bubble the gas with 1ml of carbamate. Add 10ml of Oxosol 306 and count.

Products for Counting $^{14}CO_2$

Oxosol C14 **LS-211**
Oxosol C 14 is a complete scintillator designed to absorb and count $^{14}CO_2$ produced by sample combustion.

Oxosol 306 **LS-231**
Complete scintillation counting solution specifically formulated to count $^{14}CO_2$ samples trapped in Carbamate.

Carbamate-1 **LS-241**
Carbamate-1 is a high-capacity CO_2 absorber intended to be used in conjunction with Oxosol 306. One (1) ml absorbs 5.8mM CO_2 at saturation.

2.2.5 Samples in Polyacrylamide Gels

Complex radioactive samples are often fractionated on polyacrylamide gels. Analysis of radiolabeled samples in electrophoretic gels follow the same pattern as that on TLC plates. The gel is analyzed as a whole for radioactive bands, which are then excised and counted to obtain quantitative results. Autofluor, described in Section 2.2.1 for TLC plates, is also excellent for enhancing autoradiography of PAGE Gels (Electrophoresis Theory, Section 4.1.3). Once bands are located and excised, they can be dissolved using hydrogen peroxide and then counted efficiently.

Protocol 2.2.5a

Counting Samples in Polyacrylamide Gels

AUTOFLUOR FLUOROGRAPHY OF ELECTROPHORESIS GELS

1. Stain and fix gel as usual.
2. Rinse gel for 15 minutes in deionized water to remove fixative.
3. Immerse the gel in Autofluor. Agitate gently for 30 minutes per mm of gel thickness. Pour off the Autofluor and retain for future use. LABEL AS RADIOACTIVE MATERIAL!

continued

4. Place gel directly onto filter paper and dry under heat (80°C) and vacuum.
5. Expose at -80°C. 24 hours is generally sufficient for ^{14}C or ^{3}H samples, although up to 72 hours may be required for maximum detection of ^{3}H.

DISSOLUTION AND COUNTING OF GEL SLICES

1. Using the autoradiography film as a template, cut out the band(s) of interest.
2. To every 100mg of gel, add 0.5ml 30% H_2O_2.
3. Incubate in a LSC vial at 50°C until digested (1-4 hours).
4. Heat at 37°C for one additional hour to drive off residual O_2.
5. Cool, add 10ml of a scintillation cocktail capable of holding 0.6ml of aqueous material (such as Ecoscint H) and count.

2.3 Flow Liquid Scintillation

Radiolabeled materials are often analyzed by chromatography. The original application of liquid scintillation counting to chromatographic techniques was to collect and count discrete fractions. This manner of counting is extremely laborious, and resolution is limited by the size of the fractions collected. Flow detectors were introduced to allow continuous LSC monitoring of column effluents. This gives extremely high resolution results that are simultaneous with the separation. Modern flow detectors also have a switchable outflow, allowing radioactive peaks in the chromatogram to be collected for further analysis, or simply to sequester radioactive from nonradioactive waste.

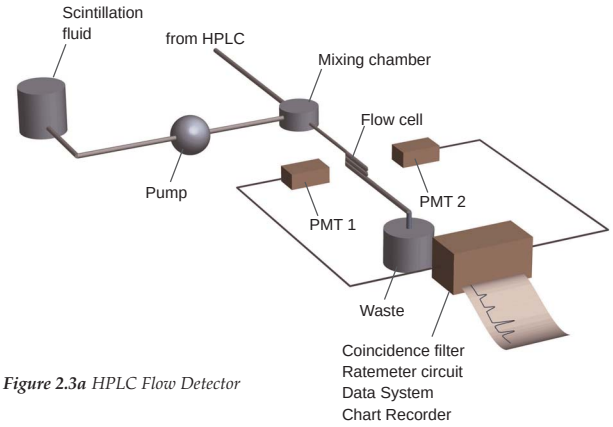


Figure 2.3a *HPLC Flow Detector*

Flow detectors operate by passing a mixture of column effluent and scintillation cocktail through a transparent or translucent channel, which is monitored by a photomultiplier tube. The flow rates of effluent and cocktail are metered to provide a constant ratio, and the channel (generally a plastic tube) is coiled to cover the entire window area of the PMT, (see figure 2.3a). Cocktails for flow LSC must provide for high efficiency, high sample capacity, and low viscosity. High efficiency allows for more sensitive detection, and minimizes any decrease in signal due to sample composition or detector geometry.
The sample capacity is important because flow LSC tends to generate surprising amounts of waste materials. A typical HPLC flow rate is 1ml/min. Over an 8 hour day, continuous use of such a system will generate 1 X 60 X 8=480ml of spent solvent. If a low capacity cocktail is used (one which requires 10 volumes of cocktail per volume of sample), up to 5 liters of waste will be produced. Use of a high capacity cocktail (3:1 or 2:1) will reduce this amount by up to 70% and keep disposal costs under control. Because of the large amounts of cocktail used, switching of labeled peaks to a separate waste collection and using a biodegradable cocktail are recommended.

Applications of National Diagnostics Flow Scintillation Cocktails			
Cocktail	**Sample Capacity (ml /10ml cocktail)**	**Biode-gradable**	**Applications**
Ecoscint Flow **Order # LS-288**	10	☒	All purpose scintillation fluid for a wide range of sample types. Ultra-high sample hold.
Monoflow 1 **Order # LS-281**	N/A	☐	Organic effluents. Lipid and steroid separations.
Monoflow 2 **Order # LS-282**	3	☐	Routine low salt aqueous effluents (<200mM salt)
Monoflow 3 **Order # LS-283**	5	☐	Routine low salt aqueous effluents. Higher sample holding capacity than Monoflow 2
Monoflow 4 **Order # LS-284**	3	☐	High salt aqueous samples. Can accommodate 2M salt gradients.
Monoflow 5 **Order # LS-285**	3	☒	Biodegradable cocktail for routine low salt aqueous samples (<200mM salt)
Uniscint BD **Order # LS-276**	3	☒	Biodegradable cocktail for high salt aqueous samples. Can accommodate up to 2M salt gradients.

Table 2.3a

2.4 Liquid Scintillation and Radiation Safety

Working with radioactive isotopes requires diligent attention to safety measures, in order to avoid hazardous exposure(s). Because radioactivity cannot be detected without instrumentation, spills can easily be spread through and even out of the lab before they are noticed. Safety in radioisotope work requires sufficient attention to both containment and surveillance. Containment measures are designed to prevent the release of isotopes in unprotected areas. Surveillance aims to detect such accidental releases as rapidly as possible to prevent the spread of contamination.

Containment and Monitoring

Containment measures are taught in radiation safety courses. A brief summary is presented here, which is not intended as a substitute for such a course. The primary safeguard against radioactive spills is common sense. Radioactivity should be handled only in designated areas, which should be covered with disposable absorbent pads. Droplets of radioactive solutions will be absorbed and trapped by the pads, which are then disposed of as solid radioactive waste. Users of radioactivity must wear lab coats, gloves and eye protection. Gloves should be monitored with a Geiger counter if the isotope emissions are detectable by this means. In any case, frequent changing of gloves if contamination is detected or suspected will keep exposure to a minimum. Shielding should be used for ^{125}I and ^{32}P work, or for any isotope whose emissions can penetrate skin, but it is important to keep in mind that the primary long term danger from radioactive components is through ingestion or skin absorption. Eating or drinking or even chewing gum must be excluded from radioactive use areas.

Surveillance begins with personal dosimeters and Geiger counters. Dosimeters are most often film badges and film rings. These are worn during radioactive experiments, and the film contained within them is exposed by the emissions which affect the worker. A correlation between film exposure and worker exposure allows the detection of dangerous levels of radiation in the lab. Radiation safety departments also use film badges to ensure that no user exceeds their long term exposure limits in a given year. Short term monitoring of exposure to high-energy emissions can be done with a Geiger counter, which detects the ionization of a gas in a sealed tube. Only those emissions which are energetic enough to penetrate the tube can be detected: ^{32}P and the highest energy emissions from ^{35}S. Geiger counters can be invaluable for checking gloves for contamination during the course of an experiment.

Wipe Testing

Once an experiment is finished, a comprehensive and sensitive check of all work areas is required. This is accomplished by use of "wipe tests". A 4 cm^2 piece of paper or other absorbent material is rubbed vigorously over the work area, placed in scintillation cocktail and counted. If counts above background are detected, the contaminated area is subdivided and the divisions wipe tested. Contaminated areas are cleaned and retested until no contamination can be detected.

The type of filter used in wipe testing has a marked effect on the reliability of the results obtained. Often standard filter paper discs are used. Such discs generally adhere to the side or the bottom of the scintillation vial. If the vial is placed in the counter such that the filter is on the side facing the photomultiplier tube, much of the light emitted by the cocktail will be absorbed by the filter. This will give artificially low numbers of counts, measuring contaminated areas as clean. This hazard is avoided by the use of a wipe which dissolves in the scintillation cocktail such as National Diagnostics' Nuc-Wipes.

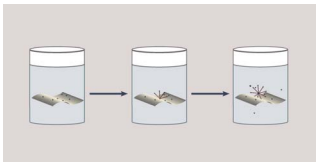


Figure 2.4a *Using intact filters for environmental wipe tests can give inaccurate, erratic results. β particles originating from particles on intact filters are attenuated and absorbed by the filter. Furthermore, depending on the relative affinity of the material for the solution, as material leaves the filter for the solution, counts change over time.*

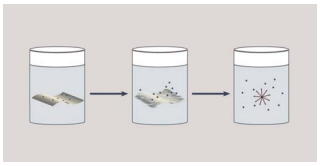


Figure 2.4b *Nuc-Wipes eliminate the dependence of results on the direction of the filter paper and time. Because Nuc-Wipes dissolve in scintillation fluid, there is no intact filter to absorb or attenuate beta emissions. 4π counting efficiency is achieved, giving reproducible results.*

Efficient and effective cleaning of spills requires some knowledge of the chemical nature of the labeled compound. Water soluble materials will come off of nonabsorbent surfaces with detergent solutions. Hydrophobic compounds require the use of higher detergent concentrations. Extremely hydrophobic compounds or absorbent surfaces may require the use of organic solvents. If an unknown sample is spilled (spent culture medium, cellular extracts, etc.), a general purpose radioactive decontamination agent should be tried, such as National Diagnostics' Nuclean, followed by solvents if necessary.

Sample Overload A

Exceeding the sample holding capacity of a scintillation cocktail will yield unpredictable results. The mixture will become opaque, with most photons being lost to internal reflectance. Phase separation may occur, which can increase or decrease counts depending on how the sample partitions.

1) Sample composition: The salt content, pH, protein content, etc., of a sample will determine the amount of cocktail needed to provide a clear, countable emulsion. Samples above 0.5M salt, or with pH < 4 or pH > 10 will generally require more cocktail.
2) Temperature: Sample/cocktail emulsions have a range of temperature in which they remain clear and countable. This range narrows as the sample volume approaches the cocktail holding capacity. The heat of mixing associated with adding sample to the cocktail can cause sample/cocktail mixtures which are initially clear to cloud on standing, leading to a drop in counting efficiency. Addition of more cocktail will restore a clear solution.
3) Phase separation: The opaque solution generated by sample overload may separate into two phases on standing. If the sample molecules partition into the organic phase, this can increase efficiency. However, because this is a slow process, the effect is unpredictable, and manifests as erratic counting from sample to sample.
4) Standard curves: It is important to plan standard curves so that the sample capacity is not approached, to ensure that the efficiency is the same for all points.

Counting Geometry B

Particulate samples, or samples bound to a solid support, will have some emission events which are absorbed by the solid before reaching the cocktail. This can reduce efficiency by as much as 50%.

1 Particulate samples: Insoluble powders must be suspended in a cocktail which forms a gel, to avoid changes in counts as the particles settle.
2) Samples on solid support: For TLC plate scrapings, see (A) particulate samples, above. Samples bound onto modified cellulose filters, use Filtron-X to dissolve the filter. For glass fiber filters, use a cocktail which will dissolve the sample components, and allow sufficient time and mixing for complete elution. Be sure filter is not in the path of the PMT.

Troubleshooting Liquid Scintillation Experiments

Symptom	Diagnosis
Counts increase with time	Sample not fully separated from solid support (B-2) or solid sample not fully dispersed/dissolved in cocktail (B-1).
Counts decrease with time	Chemiluminescence (C-1) or sample overload (A-2,3).
Erratic counting- recounting gives inconsistent results	Static electricity (C-2), sample overload (A-2,3), or solid sample settling out of cocktail (B-1).
Low efficiency- liquid samples	Color Quenching (D-1), chemical quenching (D-2), or sample overload (A-1).
Low efficiency- solid samples	Sample not fully dispersed or dissolved (B-1).
Low efficiency- sample on filter	Sample not eluted from filter (B-2), filter blocking PMT window (B-2), or quenching (D-1,2).
Standard curve not linear	Sample overload (A-4).

Chemiluminescence and Static Electricity C

Light emission from cocktails can be stimulated by static electricity or chemically excited molecules. Such emissions are relatively short lived.

1) Chemiluminescence: Caused by reactions which generate chemically excited products, its lifetime is limited by the amount of substrate in the sample. Samples should be recounted repeatedly until a stable result is obtained. Alkaline samples are particularly susceptible; neutralization can minimize the problem.
2) Static electricity: Static buildup, particularly on plastic vials, can cause bursts of light emission, giving wildly erratic counts. Run water over the vial, or wipe with an antistatic dryer sheet.

Quenching D

Scintillator light emissions can be absorbed by colored sample components (color quenching). In addition, the energy from radioactive emissions can be trapped by sample components before it reaches the cocktail phosphors (chemical quenching). In both cases, total counts are reduced, and the ratio of high to low energy counts is decreased.

1) Color quenching: Samples which absorb light at 350-450nm will attenuate the light emitted from the phosphors. In general, samples which appear yellow or brown will be quenched to some extent. Quenching shifts counts from high to low energy- a shift in the ratio of high and low channels is diagnostic. Samples can be bleached with hydrogen peroxide, taking care to remove the residual oxygen, which is a chemical quencher (see (2), below).
2) Chemical Quenching: Many chemicals are able to intercept the energy from a radioactive emission event before it can be converted to light. The effect is the same as color quenching: lower counts, and proportionally more low energy counts. Water can be a chemical quencher- to ensure consistent efficiency, all samples should have the same amount of water added. Molecular Oxygen is another quenching agent, it can be removed by warming the sample to degas it. Organic compounds containing oxygen (i.e. aldehydes and alcohols), or halogens (e.g. chloroform) are generally strong chemical quenchers. Such samples should be counted in as dilute a solution as possible, to minimize the quenching effect.

Useful Information for Liquid Scintillation Counting

Radioisotopes: Decay Products and Energies

Isotope	Emission	Energy (MeV)	half life ($t_{1/2}$)
Tritium (^{3}H)	β	0.019	12.3 yrs
Carbon (^{14}C)	β	0.156	5730 yrs
Sulfur (^{35}S)	β	0.167	87.2 days
Phosphorus (^{32}P)	β	1.710	14.3 days
Phosphorus (^{33}P)	β	0.249	25.3 days
Iodine (^{125}I)	γ	0.178	59.9 days

Radioisotopes: Range of Emissions

Isotope	Maximum Range in material (cm)		
	Air	Water	Plexiglass
Tritium (^{3}H)	0.6	-	-
Carbon (^{14}C)	24	0.28	0.23
Sulfur (^{35}S)	26	0.3	0.25
Phosphorus (^{32}P)	800	1	0.8
Phosphorus (^{33}P)	50	0.6	0.5

Units of Radioactivity

1 Becquerel(Bq) = 1 disintegration per second (dps)
= 60 disintegrations per minute (dpm)

1 Curie(Ci) = 3.7 X 10^{10} disintegrations per second
= 2.2 X 10^{12} disintegrations per minute

Half-life ($t_{1/2}$) Calculations

$t_{1/2}$(half life) = the time required for 1/2 of the atoms present to undergo decay.

λ (decay constant) = $0.693/t_{1/2}$

$N = N_0 e^{\lambda t}$

where: N= number of atoms remaining at time t
N_0=number of atoms at start (t=0)

Suggested Reading in Liquid Scintillation Counting

Items in orange may be ordered from National Diagnostics (see pp. 205)

General Resources

Schimel, David S. (1993) *Theory and Application of Tracers*. Academic Press.

Knoche, H.W. (1991) *Radioisotopic Methods for Biological and Medical Research* Oxford University Press, NY.

Slater, R.J. (ed) (1990) *Radioisotopes in Biology - A Practical Approach*. Oxford University Press.

Coleman, David C and Fry, Brian, (eds) (1991) *Carbon Isotope Techniques*. Academic Press.

Ehmann, Wm. D. and Vance, Diane E. (1991) *Radiochemistry and Nuclear Methods of Analysis*. J. Wiley & Sons.

Types of Radioactive Emission

Browne, E. and Firestone, R.B. (1986) *Table of Radioactive Isotopes*. V.S. Shirley, ed. Wiley, NY.

Weast, R.C. (1988) *Handbook of Chemistry and Physics* 69th edition. CRC Press, Boca Raton.

Slack, L. and Way, K. (1959) *Radiations from Radioactive Atoms in Frequent Use*. U.S. Atomic Energy Commission Report.

Ionization Detection

Wilkinson, D.H., (1950) *Ionization Chambers and Counters*. Cambridge University Press, Cambridge, UK.

Liquid Scintillation Counting

Horrocks, D.L. (1974) *Applications of Liquid Scintillation Counting*. North Holland, Amsterdam

Birks, J.B. (1964) *The Theory and Practice of Scintillation Counting*. Pergammon Press, Oxford, UK.

Liquid Scintillation Signal Interpretation

Verrezen, V. and Hurtgen, C., (2000) A multiple window deconvolution technique for measuring low-energy beta activity in samples contaminated with high-energy beta impurities using liquid scintillation spectrometry. *Appl. Rad. and Isotopes* 53 (2000) 289-296.