

SAMPLE PREPARATION IN LIQUID SCINTILLATION COUNTING

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ABSTRACT

Recent literature on sample preparation, combustion, efficiency determination, liquid scintillators, applications, isotope characterization, and multichannel counting is reviewed. Disposal of liquid scintillation waste is also discussed.

This paper is comprised of three parts.

I. SAMPLE PREPARATION

Liquid scintillation counting is a versatile radioassay technique for α , β , and γ emitters. The sensitivity and efficiency in detection has made it especially indispensable for measuring soft β emitters. Biological and biochemical samples containing ^3H , ^{14}C , etc. are assayed by mixing an aliquot of the sample with a scintillant followed by liquid scintillation counting (LSC). This simple act is accompanied by a number of pitfalls which must be avoided if a correct result is to be obtained. To produce a compatible sample for counting, it is necessary to understand the nature of the specimen to be assayed, the solution capacity of the scintillant for the sample, the quenching property of the scintillation system, and the counting efficiency by which the sample CPM can be corrected to sample DPM.

A number of review articles¹⁻³, monographs⁴, and conference proceedings⁵⁻⁸ on liquid scintillation and sample preparation have appeared which the interested reader should consult. This paper will only mention some of the more recent advances in this area.

Samples for radioassay may be gases, polar and nonpolar liquids, or solids in the form of silica gels, biological specimens, etc. Each of these must be introduced into a scintillant to produce a compatible sample for counting.

1. Radioactive Gases

Radioactive gases may be either flow-counted, or solubilized or trapped for batch counting. Using a non-volatile liquid such as silicone oil as scintillant base, Hatagami⁹ was able to measure tritiated water vapor in contaminated air by bubbling it through the non-volatile scintillant. He obtained high efficiencies for ^3H and ^{14}C of about 34% and 91%, respectively. For gas flow counting, liquid scintillants based on toluene, xylene or pseudocumene (with a high vapor pressure) are unsuitable. Kato¹⁰ used ethylene glycol to trap ^3H vapors in the air followed by incorporating the radioactive ethylene glycol into scintillant (Bray's solution) for counting. His technique gives the detectable concentration of ^3H in the air as 10^{-7} $\mu\text{Ci/mL}$ or less. Ryman et al.¹¹ measured $^{14}\text{CO}_2$ respired by plants with a plastic scintillation flow cell; the cell has a sensitivity of 24 nCi/L and a counting efficiency of $34 \pm 2\%$. The small scintillation flow cell is less sensitive than a large volume ionization chamber but is more responsive to rapid changes in $^{14}\text{CO}_2$ concentration. Sugisaki et al.¹² accurately determined the small amount of ^3H in metals by liquid scintillation counting. Their technique involves extracting the ^3H from the metal by heating at high temperature followed by oxidation of the extracted ^3H in a vacuum-tight system with CuO powder to water which was collected for counting. This method analyzes all the ^3H in the metal while the conventional method of dissolution of the metal and aliquoting the solution for LSC causes contamination and measures only a fraction of the ^3H present. Prichard and Gesell¹³ measured the ^{222}Rn concentrations in water by extracting it with a scintillant and counting the sample without separating the two phases. As ^{222}Rn is about 50 times more soluble in toluene than in water, this method can detect ^{222}Rn at a sensitivity of 10 pCi/L in a 40 min. count of a 10 mL sample. It is less sensitive than the "isopiestic" method described by Parks and Tsuboi¹⁴ but the method has the capacity for handling a large number of potable water samples with acceptable sensitivity of detection, which justifies its use¹⁵. Coursey et al.¹⁶ reported the measurement of other radioactive noble gases.

2. Radioactive Liquids

Radioactive liquid samples are non-polar or polar, tractable or intractable with respect to solubilization, and colorless or colored. Non-polar liquid samples are more readily incorporated into liquid scintillators than polar ones and present little problem in sample preparation. Samples that are successfully incorporated into liquid solgel or emulsifier scintillators without resorting to additives or solubilizing aids have high counting efficiencies. Intractable and highly colored samples are converted by solubilization, extraction, oxidation or combustion to more easily soluble radioactive materials for incorporation into the scintillant.

Radioactive liquids such as column effluents may be monitored by liquid flow counting or counted batchwise in collected fractions. Liquid flow counting is especially useful in high performance liquid chromatography (HPLC) to purify labelled compounds or to determine the purity of radiochemicals. For the latter purpose, Cazer and Kessler¹⁷ selected a flow counting system in which the radioactive column effluent is mixed with scintillant internally and counted. The mixing system is considered to be superior to systems using scintillation flow cells. Since scintillation flow cells are usually made of scintillation plastics or packed with glass scintillation beads, they tend to be either affected by organic solvents or contaminated by strong adsorption of the label. The solid scintillator europium-activated calcium fluoride in the 75 to 150 micron size range has a counting efficiency of 5% for ^3H and 75-85% for ^{14}C ¹⁸ but contact with polar solvents raises the background and increases the contamination. The flow cell permits the effluent to be collected unadulterated, which is ideal for purifying labelled compounds, but the cell itself is readily contaminated. In comparison, the scintillator mixing system is practically contamination-free and potentially useful for double isotope studies; its main disadvantage is the loss of sample for further testing.

Many inorganic radiochemicals are measured by liquid scintillation techniques. Coursey et al.¹⁶ listed 31 elements, the radionuclides of which have been assayed either by direct activity measurements in which the activity for each vial is determined without the use of

other standards for comparison or by comparative activity measurements in which vials are compared with other vials containing known activity. Solutions of metal, transition metal, and non-metal radionuclides are incorporated into liquid scintillators of various composition with a counting efficiency in the range of 92 to 100%. A different scintillator is used for each of these radionuclides. From the relationship in the periodic chart, these scintillators may also be of use to count radioisotopes of elements that belong to the same group of subgroups. McQuarrie et al.¹⁹ measured positron emitters ^{18}F , ^{67}Ga , and ^{68}Ga by LSC. The efficiency for all 3 radioisotopes was essentially 100%. In comparison, the counting efficiency for ^{68}Ga by 3"x3" NaI(Tl) well detector was in the range of 67 to 71%. The LS system gives well resolved spectra of ^{67}Ga and ^{68}Ga when both are present in a mixture and also has lower limit of detection than NaI(Tl) counting. Coursey et al.²⁰ standardized ^{129}I solutions by LSC and obtained identical results as those by NaI(Tl) counting. Radwan et al.²¹ determined the diffusion of nickel in steel using ^{63}Ni as tracer and measured the low-level Ni activity as dimethylglyoxime complex with an efficiency of about 80% by LSC. Pacer²² determined the activity of ^{99}Tc as aqueous NH_4TcO_4 by LSC over a concentration range of 10^{-4} to 10^{-7} M with an efficiency greater than 94% and a response factor of 3.52 CPM/pmol ^{99}Tc . In the concentration range studied, no loss of ^{99}Tc by sorption on glass or plastic containers was observed.

Peng and Yang²³ measured ^{14}C -labelled methyl and ethyl iodides containing free iodine in Insta-Gel (Packard) and restored the count loss of quenching due to free iodine by addition of powdered crystals of sodium metabisulfite, sulfite, or thiosulfate or 10% ascorbic acid solution of a 2N sodium hydroxide solution. These reducing agents reduce quenching by converting free iodine to iodide. Sodium metabisulfite when added to iodine-quenched samples can restore the counting efficiency within 1 hr but the color returns with time, while sulfite and thiosulfate react very slowly in the absence of water but the restored counts are stable. Sodium hydroxide alters the sample appearance (making it opaque) and generates chemiluminescence. Ascorbic acid gives a clear sample but adding large amounts of the

solution causes an increase in sample opacity.

In the presence of large amounts of interfering substances, radioactive materials may be prepared for counting by extraction directly into the scintillant. Extraction serves as a means of concentrating the radioactive sample into a small volume for better detection. McDowell et al.²⁴ used a liquid scintillator containing trioctylphosphine oxide (TOPO) to extract ^{230}Th and $^{234-238}\text{U}$ from an acid solution of phosphatic materials. The detection threshold is about 1.1 parts/ 10^8 for ^{238}U , 6.9 parts/ 10^8 for ^{232}Th , and 4.0 parts/ 10^{13} for ^{230}Th in a 1.0 g sample. Huskisson and Ward²⁵ employed a liquid extraction method to accurately determine low levels of ^{14}C in blood and fecal samples. Owing to the color and bulk these two specimens are among the most difficult type of biological samples to count. Recovery with the extraction method is complete as determined with n-[^{14}C]hexadecane, D-[^{14}C] glucose, [^{14}C] stearic acid, [^{14}C] methionine or $\text{Na}_2^{14}\text{CO}_3$. The method has the sensitivity of measuring an activity of 33 pCi/g of feces.

3. Radioactive Solids

Biological tissue specimens, samples on silica gels or on filter papers and other solid materials can be counted by liquid scintillation techniques. Biological tissues may be solubilized with commercial solubilizers such as NCS, Soluene, etc. and counted in toluene based scintillant or solgel scintillators. Radioactive materials adsorbed on silica gel are counted after desorption or elution or as suspensions in the scintillant. These are heterogeneous samples. The counting efficiency of heterogeneous systems is not readily determined.

Samples on solid supports are frequently the products of thin-layer chromatography. Rodriguez et al.²⁵ found that counting efficiency and recovery of added ^3H - and ^{14}C -labelled lipids including triglycerides, diglycerides, phospholipids, phosphatidic acid, phosphatidyl-inositol, -serine, -ethanolamine, and -choline in a toluene-based scintillator decreased in the presence of silica gel. Silica gel adsorbed as much as 80 to 98% of the radioactivity associated with neutral and polar lipids as determined by measuring

the activity in the supernatant. The decrease in counting efficiency or the recovery of the added radioactivity depends on the amount of silica gel, sample activity and chemical nature of the lipid. It cannot be overemphasized that when molecules labelled with soft β -emitters are adsorbed on a solid substrate, their DPM cannot be accurately analyzed by liquid scintillation counting. To minimize the decrease in counting efficiency and improve the recovery only a minimum amount of silica gel should be used and it should be deactivated with water to decrease its adsorptive power before addition of the scintillant.

The problem of adsorption on silica gel in liquid scintillation counting of ^{14}C -labelled compounds has been studied by Kubik^{27,28}. Using 1-methyl-[2- ^{14}C]imidazolidinone-2 as a tracer, Kubik compared its counting efficiency in the adsorbed state with that determined by homogeneous counting, using scintillants containing PPO, PT, and butyl-PBD as fluors. The study also included the counting of $\text{Ba}^{14}\text{CO}_3$ for comparative purposes. The specific surface area, porosity, and surface structure of silica gel in relation to the number and type of OH groups, can affect the adsorption of labelled compounds as well as the adsorption of the organic scintillator. In this respect, the silica gel has more chemical structure than BaCO_3 . Consequently, counting radioactive compounds supported on silica gel in liquid scintillation medium involves many more parameters affecting the counting efficiency than counting $\text{Ba}^{14}\text{CO}_3$. The counting efficiency and sample channels ratio for counting $\text{Ba}^{14}\text{CO}_3$ parallel those for counting a homogeneous sample but differ from those for counting a sample on silica gel. Silica gel adsorbs the scintillator PPO but not PT on butyl-PBD. The decrease in counting efficiency and the erratic variation of channels ratio encountered in counting samples on silica gel may be caused by sample heterogeneity. In addition, the labelled molecules in an adsorbed state can transfer energy directly to the adsorbed scintillator molecules without the mediation of the solvent molecules. Such a process is inefficient for high photon yield, since adsorbed PPO at high concentrations on the surface of silica gel can undergo self-quenching.

Stocklinski²⁹ reported that the fluorescent dye indicator in

thin-layer chromatographic plate can be a source of photoluminescence in LSC. Takiue and Ishikawa³⁰ treated samples of ^3H , ^{14}C , ^{32}P and ^{55}Fe on cellulose support with concentrated sulfuric acid and hydrogen peroxide, thus converting a heterogeneous sample to a homogeneously dispersed one. The ^3H and ^{14}C could then be measured with 30% and 80% efficiency, respectively, which were much higher than the counting efficiencies in the filter paper disc technique.

4. Combustion

Highly colored and intractable samples including samples on solid supports may be combusted, and the combusted products collected for counting. Gacs et al.³¹⁻³³ reported an automatic combustion method for assaying samples containing ^3H and ^{14}C and a semi-automatic method for those containing ^3H and ^{35}S . Samples containing either ^3H or ^{14}C or both are prepared for combustion by mixing with tungstic oxide (WO_3) and cerium dioxide (CeO_2) to promote combustion. Samples are weighed in cellophane or tin capsules. The sample is combusted in a stream of oxygen at 1000°C ; complete oxidation is ensured by further passage of the vapor through a bed of cerium dioxide and tricobalt tetraoxide. Tritium is collected as tritiated water in a small amount of diethyleneglycol monoethyl ether while $^{14}\text{CO}_2$ is absorbed in 3-methoxypropylamine. The combustion is controlled by a programmer and the entire operation requires about 3 minutes per sample. If the sample exceeds 85 mg, it is put into 2-3 capsules. The recovery of label is better than 99% and is highly reproducible. For samples containing ^3H and ^{35}S , the operation requires about 12 minutes per sample. The sample is mixed with tungstic oxide to combine with phosphorous and to convert stable sulfur compounds into SO_2 . The combustion products THO and $^{35}\text{SO}_2$ are trapped on quartz wool wetted with H_2O_2 which converts $^{35}\text{SO}_2$ to labelled sulfuric acid. The temperature of the quartz wool is maintained at 100°C to facilitate ^3H exchange with fresh H_2O_2 and to vaporize the tritium oxide or tritium peroxide for separate condensation downstream. The condensed tritiated water and $\text{H}_2^{35}\text{SO}_4$ are separately analyzed for ^3H and ^{35}S activity. The recovery of the label is complete and up to 20 mg of sample can be combusted in the apparatus described. It may also be

possible to combine the two methods to analyze samples containing all three isotopes.

DeFillipis and Everett³⁴ described a method for analyzing samples containing ^{51}Cr , ^{14}C , and ^3H , using Packard 306 Sample Oxidizer. The sample oxidizer can quantify samples containing ^3H and ^{14}C by separately collecting the combusted products HTO and $^{14}\text{CO}_2$ into individual vials for LSC but its design is incompatible for combusting samples containing metal salts. In a controlled experiment of combusting ^{51}Cr in a quartz cup approximately 1-2% of the ^{51}Cr activity was volatilized and condensed in the conduit tubing downstream, but most of the ^{51}Cr activity was with the quartz crucible from which a major fraction of the activity was leached out with hot dilute acid solution. No ^{51}Cr activity appeared with either ^3H or ^{14}C samples but the recovery was incomplete. In view of these findings, the authors recommended that the triply-labelled sample be analyzed by splitting into two portions, with one portion assayed for total activity and the other portion combusted to determine ^3H and ^{14}C activity in the sample. The difference in activity between these two portions represents the ^{51}Cr activity in the sample.

5. Efficiency Determination

Efficiency determination is a critical procedure for obtaining DPM from CPM, and the accuracy of the method is frequently scrutinized by the user. An external γ -ray standard is used to generate Compton electrons in the liquid scintillation medium and to provide a radioactive source of reproducible strength against which quenching is measured. Methods for quench correction by the use of an external standard include ESCR (external standard channels ratio), ESP (external standard pulse), the H number, and SIE (spectral index external standard); and those based on a shift of the pulse height spectrum with quenching are SCR (sample channels ratio) and SIS (spectral index sample). Older LS counters use ESCR and SCR for automatic quench correction but modern spectrometers use newer techniques such as the H number or SIS. The H number depends on the shift of the inflexion point of the Compton edge induced by quenching. When the counter has fast, stable electronics and multichannel

registering capability, the H number can be found quickly and precisely. The H number is unique and single-valued for ^{137}Cs due to its sole γ -ray energy at 662 keV. In contrast, the SIS is based on averaging the sample pulse height spectrum as it shifts with quench.

The high cost of scintillation chemicals plus the inconvenience and high cost of their subsequent disposal as low level radioactive waste have compelled the users to use smaller volumes of liquid scintillants for radioactive measurements. Ring et al.^{34,36} reported no variation of the correlation curve between SIS and the counting efficiency for ^3H and ^{14}C when the sample volume is varied from 2 mL to 20 mL. Horrocks³⁷ compared the use of the H number and the ESCR with ^{137}Cs and ^{226}Ra as external standard for volume corrected DPM. The results showed that the H number is inherently more accurate than ESCR for quench correction.

Patterson et al.³⁸ compared the performance with respect to the change in counting efficiency with ESCR of six LS counters as sample volume was varied from 1 mL to 20 mL and found more variation in some counters and less in others. External standard channels ratio depends on the pulse height spectrum of the Compton scattered electrons generated in the sample. The number of scattered electrons, depending upon the mass of the scattering medium is a function of γ -ray energy. Liquid scintillation counters from different manufacturers have different γ -ray sources as external standards. Consequently, the pulse height spectra of Compton electrons differ, which may contribute to the changing counter response to changing sample volumes.

Counting efficiency may also be affected by the ratio of the amount of scintillation cocktail to the amount of aqueous solution sample to be counted. Tschurlovits and Niesner³⁹ have shown that for counting ^3H or ^{14}C in urine, decolorized urine, or water, there is an optimized amount of sample that can be added to an amount of scintillator to obtain high efficiency. This optimizing method is useful only if ample amount of sample is available.

Neame⁴⁰ compared the percentage errors in determining counting efficiency using quench curves constructed from SCR or from ESCR. The quench correction curves derived from counting homogeneous samples cannot be used to determine the efficiency of gel samples, cloudy

samples, two-phase samples, samples in which the radioactive material is precipitating, and samples on solid supports. In general, the quench correction curve from SCR showed less dependence on sample volume variations and on different vial sizes than that from ESCR. The statistical error for the channel ratios within the range of 0.3 to 0.8 showed a relatively small change.

Dahlberg⁴¹ combined the internal standard (IS) method for quench correction with the sample channels ratio method and named the combined method, the IS-SCR method. It involves counting a sample to determine its counting efficiency by the SCR method, followed by addition of IS and recounting. The SCR method is inaccurate at low sample count rate, and the IS method depends on accurate pipetting. The combined method has none of these disadvantages.

Knoche et al.⁴¹ studied the effect of sample volume and quenching on the estimation of counting efficiency of ¹⁴C-labelled samples. The unquenched samples showed a decrease in counting efficiency when the sample volume was below 6 mL or above 18 mL. When small volume samples (2 mL) with different levels of quenching were assayed, the magnitude of error was greater with ESCR than with SCR in predicting the counting efficiency.

Malonda^{43,44} calculated the correlation curves between counting efficiency and ESCR for pure beta emitters ¹⁴C, ⁹⁹Tc, ¹⁸⁵W, ³⁶Cl, ⁸⁹Sr, and ³²P, and the electron-capture-decay nuclide ⁵⁵Fe, using an experimental quench curve of ³H. Gibson⁴⁵ discussed the theoretical limitation of counting radionuclides emitting low energy radiations with accuracy. Kato⁴⁵ derived an exponential quenching equation which is applicable to a wider range of quencher concentration than the Stern-Vomer equation. The equation is identical to an equation obtained (earlier) experimentally for quench correction⁴⁷. The same author also gave a mathematical expression for transforming a linear liquid scintillation pulse height spectrum to a logarithmic pulse height spectrum⁴⁸.

Ediss et al.⁴⁹ used a mixture of dyes (PAQ) to produce a uniform absorption from 375 to 525 nm. PAQ can be dispensed dried on filter paper. The use of dried filter paper carrier disc greatly simplifies the addition to counting vials. PAQ quenches by color and has no

chemical quenching action.

6. Liquid Scintillators

The most widely used scintillators are the solgel scintillators, also known as emulsion or emulsifier scintillators². These scintillators have as a solvent base a mixture of surfactant and aromatic hydrocarbons, such as toluene, xylene, and pseudocumene. The higher aromatic hydrocarbon homologs have higher flash points and lower toxicities⁵⁰. The commercial solgel scintillators used blended surfactants⁵¹ in addition to xylene or pseudocumene. A typical proportion for a solvent base is 1 part Triton X-100 to 2 parts toluene. Triton X-100 is highly soluble in toluene and forms micelles of M.W. 90,000 at concentrations greater than 0.24 mM (critical micellar concentration) in water⁵². In the solgel scintillator, addition of water leads to the formation of inverse water-Triton micelles which contain about 17 molecules of Triton X-100 and about 323 molecules of water. In the region of 1-10% water, the scintillation solution is clear (sol), and the micelles are spherical in shape. When the water concentration is increased to 20-30%, it becomes a translucent gel, and the micelles are laminar in structure. The high water content causes a small tritium β -energy loss due to self-absorption; as a result, the counting efficiency for [³H] water is $93.1 \pm 5\%$ of that for [³H]toluene. When using [³H]toluene as an internal standard for water soluble samples, it should be restricted to samples with about 10% aqueous solution. The solgel scintillators can incorporate a large amount of water and still count with a higher ³H efficiency than other water-miscible liquid scintillators.

Aromatic solvents have high scintillation efficiencies but poor microemulsion-forming properties. The incorporation of water into the solgel scintillator may affect the phase contact and lower the counting efficiency, but the presence of salt may destroy the microemulsion. Reich and Zarbybnicky⁵³ studied the influence of seven cations (Li^+ , Na^+ , K^+ , NH_4^+ , Cs^+ , Mg^{2+} , Ca^{2+}) and three anions (Cl^- , Br^- , SO_4^{2-}) on ³H counting efficiency and stability in samples formed by adding 1 mL or 4 mL of salt solution to 9 mL of the

scintillant. Their results show that LiCl, KCl, NH₄Cl and CsCl solutions up to 2 mol/L and NaCl, MgCl₂, and CaCl₂ up to 1 mol/L have negligible effect on ³H counting efficiency. Among the anions, NH₄Cl is as good as monovalent cations in regard to the counting efficiency and sample stability. Bromide ion causes a slight decrease in counting efficiency but the stability of the sample is comparable to that of NH₄Cl. The SO₄²⁻ compares less favorably to the Br⁻ in both counting efficiency and stability.

Triton X-100 is a necessary ingredient of the solgel scintillators. It is also used as a wavelength shifter in Cerenkov counting. When Triton X-100 is mixed with 1% sodium salicylate or 0.5% PPO, a water-miscible, nonhazardous liquid scintillation cocktail results with a counting efficiency of about 80% for ¹⁴C and about 17% for ³H^{54,55}. It has a high flash point (>300°F) and is non-volatile and inert towards polyethylene vials; these are the desirable characteristics for a scintillant. Homologs of Triton X-100 have been tested for their scintillation properties. Phenoxy ethanol, when containing 0.5% PPO, has a counting efficiency of 59% for ³H, higher than that of the Triton scintillant, but it accepts no more than 10% of water as a miscible solution⁵⁶. Phenoxy tri- and penta-ethyleneglycols have been patented as scintillation solvent⁵⁷.

An inexpensive scintillation mixture based on an emulsifiable oil rather than an aromatic solvent has been employed to count alkaline extracts of blood and feces containing low levels of ¹⁴C activity²⁵. The scintillation mixture has the composition of White Spirit 100 (53.8% v/v), Synperonic NP9 (30.8% v/v), octanol-2-ol (10.8% v/v), benzyl alcohol (4.6% v/v) and PPO (3 g/L). White Spirit 100 is a petroleum fraction also known as mineral turpentine, Stoddard solvent, or varsol. Synperonic NP9 is a nonyl phenolpoly(ethylene oxide) condensate with a hydrophile-lipophile balance (HLB) of 12.8. This scintillation mixture forms a stable micro-emulsion with the alkaline extracts of blood and feces and can incorporate about 30% or more of the total sample volume of the alkaline extract of blood and feces. Counting efficiency may be determined by the ESCR method. The lowest limit of detection for ¹⁴C is about 33 pCi or 75 CPM per g of fecal material. Noble et al.⁵⁸ reported a thermostable cocktail for

heterogeneous liquid scintillation counting and a scintillant was developed to circumvent the variation of ESCR due to temperature variation from 10° to 30°C in counting charred triglyceride on silica gel. The authors reported the thermostability of the modified emulsifier-scintillator but revealed neither scintillator composition nor chemical identity of its component.

Kato⁵⁹ replaced the toluene in toluene scintillator with silicone oil. The resulting silicone scintillator has comparable counting efficiencies for ³H and ¹⁴C as those obtained with a toluene scintillator and exhibits less quenching effect.

7. Applications and other alternative uses of LS counter

Multiplier phototubes with alkali photocathodes plus microprocessor controls for automatic count recording, sample handling, etc. have made LS counters extremely sensitive and sophisticated light measuring instruments. Although the majority of the LS counters are assigned to multiuser use and kept fully engaged, a few are dedicated to individual research projects and can be diverted to analytical measurements other than determination of radioactivity, such as radiometric analysis and chemiluminescence measurement.

a. Radiometric analysis

Nobel et al.⁶⁰ outlines the advantages of using LS counter for quantifying by color, by uv absorption, for particle sedimentation, enzyme assays and other special uses. Temporary diversion of the LS counter from its designed applications for development of analytical techniques in the laboratory makes the operation of the instrument more cost effective. Shand and Noble⁶¹ demonstrated the accuracy with which the lipid mass can be quantified by LS counting the thin-layer chromatogram after charring it with 3% (w/v) cupric acetate and 8% (w/v) orthophosphoric acid followed by heating at 180°C for 15 minutes. Quench correction was based on ESCR, and lipid materials up to 100 µg might be used. Results are comparable to quantification by more established procedures but the procedure using LS counter is both quick and accurate.

Heitzmann and Ford⁶² used as light source a sealed liquid scintillation solution containing ⁹⁹Tc and quantified the color of a test solution by the degree of color quenching it causes. The advantages of this type of photometric measurement over conventional photometry include (1) accuracy and precision which are limited by statistical considerations of radioactive counting; (2) ability to handle a wide range of concentrations with equal reliability; (3) stability of the light source; and (4) ability to feed digital output directly into a computer without analog to digital conversion.

Radin and his co-worker⁶³ assayed the glycolipid synthetase, UDP-glucose glycosyltransferase, by incubating the lipid substrate with the radiosugar-labelled nucleotide in a small plastic scintillator vial and at the end of the incubation, stopped the reaction by adding water and perchloric acid followed by a scintillation cocktail. The radioactivity was measured directly in the two-phase sample without separation of the liquid. Other lipid synthetases can be assayed with a radioactive hydrophilic substrate. Mafart et al.⁶⁴ studied the condition for using [¹⁴C]lysine of high specific activity to determine the lysine uptake by micro-organisms as a quick means of identifying them.

Pacer and Benecke⁶⁵ measured rhenium (VII) over a concentration range of 5×10^{-5} to 1×10^{-2} M by an isotope dilution analysis procedure, using ⁹⁹TcO₄⁻ as a radioisotope tag. This pseudoisotope dilution analysis is not interfered with by a 10-fold excess of the following salt solutions: ZnCl₂, MnCl₂, K₂CrO₄, FeSO₄, NaMnO₄, and NaF, but the presence of KSCN, KI, NaClO₄, KMnO₄, and SnCl₂ in solution lowers the count rates and yields high apparent Re(VII) concentration.

Carmon⁶⁶ used a LS counter to assay hard β emitters ⁸⁹Sr and ⁹⁰Y by Cerenkov counting without interference from the soft β 's of ⁹⁰Sr. Smith⁶⁶ pointed out that the variation in the wall thickness of glass and plastic counting vials may cause variable quenching in Cerenkov counting; this is attributed to the short wavelength and directionality of Cerenkov radiation which is readily attenuated. Quenching in Cerenkov counting may be monitored by sample channels ratio method. Paredes et al.^{68,69} measured the ³²P formed from the

sulfur tablet used in neutron dosimetry by Cerenkov and proportional counting and also performed LSC of ^{32}P after removing the sulfur by burning in the counting vial. The Cerenkov counting efficiency for ^{32}P was 43%, comparable to that for the proportional counting but the efficiency for LSC was over 90%.

b. Chemiluminescence measurement

An LS counter can be used as a photon counter capable of measuring single-photon events, provided that the coincidence gating is inactivated. Pekoe et al.^{70,71} used an LS scintillation counter to measure chemiluminescence (CL) generated in the myeloperoxidase-luminol- H_2O_2 -high Cl^- system. Most of the CL is emitted initially in a burst but some is emitted delayed over an extended period of time. Chemiluminescence from such reaction is inhibited by non-steroidal anti-inflammatory drugs such as Sulindac sulfide, Indomethacin, BW755C. As the CL is emitted in an initial burst, use of an LS counter for the assay will miss this initial burst entirely because lowering the sample to the counting chamber requires several seconds in an LS counter. To avoid this drawback, these authors used a direct feed system to enable the counting to begin as the sample is mixed. This system is later replaced with a commercial flow liquid scintillation system (FLO-One, Model DR, Radiomatic Instruments, Tampa, Florida) and a pumping system to pump and mix the reagents in the flow cell for detection. The study was capable of demonstrating that ascorbate and BW755C were the most inhibitory at doses around 10^{-7}M range, Sulindac and Indomethacin next at doses about 10^{-6}M range and aspirin and salicylate partially inhibitory at doses about 10^{-5}M range.

Robinson and Penny^{72,73} measured phagocyte chemiluminescence by using a standard LS counter with slight modification and connected the output to a computer/plotter so that a complete analysis of the CL profile, such as peak height, slope, and total counts can be provided. In vitro and in vivo evidence indicates that CL and microbial killing reflect similar events. Measuring CL by LSC to determine phagocytic and opsonic defects in cells has the advantages over the use of photometer in speed and automation. The method allows measurement of

multiple samples automatically, with standardized mixing and measurement times and at a constant temperature. The CL measurement enables one to assess the effects of paraprotein on human neutrophils and monocytes. Paraprotein containing sera (classes IgG, IgA and IgM) depresses CL response of monocytes but have no effect on neutrophils. This difference may be due to the interaction of paraprotein with differing membrane receptors in phagocytes. Earlier, Allen⁷⁴ has discussed the CL in the humoral-phagocyte axis in host defense against infection. Chemiluminescence of monocytes and neutrophils were discussed by Jederberg and Krueger⁷⁵ and Horan and his collaborators⁷⁶, respectively.

II. MULTICHANNEL COUNTING

The ultimate goal in analyzing a radioactive sample is to determine the intensity of radioactivity and to characterize the radionuclide. For γ -ray emitting radionuclides, this is achieved by γ -ray spectrometry in connection with measurement of the areas under the spectrum or the photopeak. For β -emitting radionuclides spectral identification was not direct or precise until the introduction of LS spectrometers with fast electronics and spectral capability. In LSC, isotope characterization by spectrum search requires time and manual operation, a condition very impractical for handling a large number of samples. In addition, the downward shift of the pulse height spectrum with quenching complicates the task of isotope characterization.

Peng⁷⁷ introduced the use of the spectrum ratio for isotope characterization. The spectrum ratio is defined as the ratio of counts from two specified channels of a radioactive sample containing ^3H , ^{14}C , ^{32}P or ^{125}I at a known degree of quench. The spectrum ratio differs from the sample channels ratio for efficiency determination in that it requires quenching of the sample to be independently determined. A curve correlating the channels ratio with the H number for an unadulterated isotope at all quench levels is known as the spectrum ratio curve which serves as a reference curve for isotope characterization. To use the spectrum ratio curve, one may count the sample in identical channels to those used for the reference curve, and monitor the sample quenching independently by the H number. One

obtains from the curve a spectrum channels ratio corresponding to the H number representing the ratio for the pure isotope. Any discrepancy between the observed ratio and the reference ratio indicates that the isotope in the sample is of questionable purity. The spectrum ratio is not affected by the composition of liquid scintillators but samples in mini vials yield slightly higher values than those in the maxi vials at the same H numbers.

A simpler approach for isotope characterization is to use 3-channel counting instead of one-channel counting in assaying radioactive samples. The distribution of counts in three separate channels reflects the pattern of the pulse height spectrum of the isotope. Three-channel counting requires no additional sample counting as compared to one-channel counting but offers extra information for spectrum pattern recognition.

Spectrum recognition can detect misuse and abuse of liquid scintillation techniques in radionuclide metrology⁷⁸ and can prevent fake scientific experiments involving the use of radioactive tracers. The spectrum ratio method is more precise than spectrum pattern recognition for ascertaining radioisotope purity in quenched samples.

Aside from isotope characterization, multichannel counting is used for counting dual or triply-labelled samples. DeFilippis and Everett⁷⁹ measured dual labelled samples containing tritium and iodine-125 by two channel counting. Kolb⁸⁰ analyzed the pulse height spectrum of each isotope to set the windows for counting ¹²⁵I and ⁵⁵Fe in a dual-labelled sample. Horrocks⁸¹ determined the DPM, of ³H, ¹²⁵I, and ¹⁴C in triply labelled samples by three-channel-counting using the H number for quenching correction.

III. LIQUID SCINTILLATION WASTE

The wide spread practice of liquid scintillation counting in biochemical research necessitates the handling of a large number of samples and generates a copious amount of low-level combustible radioactive waste. An estimate made in 1981 indicates that research laboratories and hospitals throughout the United States used between 84 and 159 million vials per year, which represent between 200,000 and 400,000 gallons of liquid scintillation media. The liquid

scintillation waste contains mainly ^3H and ^{14}C . Disposal of this waste in radioactive waste burial grounds requires approximately 400,000 cu. ft. of space at a cost of over \$13 million per year for packaging, transport and disposal⁸².

Liquid scintillation media are flammable and chemically toxic, and are suspected of being carcinogenic. They pose a waste hazard unrelated to their radioactive character. Their flammability was the cause of a truck-fire accident which occurred on May 14, 1979 near Beatty, Nevada, one of the three radioactive waste burial sites in the U.S.⁸³. This fire accident has focused attention on the mode of liquid scintillation waste disposal and the propriety of land burial for this type of waste.

Forty-five percent of the radioactive waste shipped to the burial grounds comprises LS waste from radioassays, drug distribution and pharmacokinetic studies and other studies, containing less than 0.005 μCi of ^3H or ^{14}C per vial. This waste belongs to the "de minimus" category. Consignment of this waste to burial sites will not only use up valuable burial capacity unnecessarily but may eventually cause the leaching of other radiochemicals out of burial trenches even though the liquid waste is solidified by absorption with vermiculite. To conserve its burial capacity, the Richland radioactive waste burial site will not accept liquid scintillation media after December 1984.

In 1981, the Nuclear Regulatory Commission, under advisement, revised their present rule, thus allowing licensees to dispose by release into the sanitary sewage systems a quantity up to 5 curies for ^3H and 1 curie for ^{14}C per year in addition to 1 Ci for all radionuclides and also to dispose materials containing 0.05 μCi or less of ^3H or ^{14}C per gram without regard to its radioactivity⁸². Local rules and socio-political considerations may limit such disposal, since liquid scintillation waste may continue its yearly increase and the long-term cumulative effect of its release is not known at this time. Liquid scintillation waste contains toluene, xylene, pseudocumene, Triton X-100 and co-surfactants, and the fate of these components is difficult to predict. The radioactive materials in the waste may concentrate in certain sewage system components; the aromatic solvents may present a toxic and fire hazard; the Triton

X-100 and co-surfactants may affect the sewage treatment process.

The simplest and most obvious technique for LS waste disposal is incineration⁸⁴. Incineration allows the recycling of counting vials which can lead to substantial reduction of the cost of LS counting [85]. For small institutions and laboratories, the next best choice is the separation of solvent from scintillation waste. Published material on the subject is scanty. The available ones are cited below.

The release of LS waste to sewage systems was mentioned by Lee⁸⁶. For a daily discharge of 100 mCi of ^3H and 20 mCi of ^{14}C , a daily flow of 10,000 L. of sewage is required.

Harwood et al.⁸⁷ reduced the waste volume by distillation by treating 16 L. of the LS waste with 1.5 L. of 3.0 N NaOH twice with stirring in the cold and distilling the washed liquid waste after separation in a still with a spinning band column. The aqueous fraction of the distillate showed minimal radioactivity, the toluene fraction some slight radioactivity and the xylene fraction no radioactivity. The operation was carried out a total of 60 times, 10 times with 16-L. batches and 50 times with 1 L. batches, with consistent results. It is necessary to treat the LS waste before distillation to stabilize the ^3H activity to prevent exchange. Multisorb^o and calcium chloride may also be used to reduce the volatile radioactivity. Kreiger⁸⁸ mentioned charcoal as the simplest and more effective adsorbent in reducing the volatile radioactivity in distillation. Distillate from the untreated waste showed higher volatile radioactivity.

Distillation usually can reduce the LS waste by a factor of two or more depending upon the amount of Triton X-100 or co-surfactants present. The water-miscible residue from the still may be discharged by release into the sewage system or solidified for land burial. The toluene may be reused or burnt as a fuel.

Incineration, if properly carried out, would be the preferred means of disposing of radioactive waste containing ^3H and ^{14}C . Futatsukawa et al.⁸⁹ incinerated LS waste of low level radioactivity. Under normal conditions for burning, the combusted products are water and carbon dioxide. These products tend to localize in the vicinity

of the incinerator. The long-term effect of such localization is socio-politically undesirable and may raise local objection to the establishment of such an incinerator. The emission may fail to meet the standards of the local regulatory agency for the device. But if the combustion process could be modified to produce hydrogen and carbon monoxide instead of water and carbon dioxide as the combustion products from the LS waste, it should be very acceptable. Hydrogen has a specific gravity of 0.09 g/L., is a very light gas and will disperse rapidly into the upper strata of the air flow. Carbon monoxide is lighter than CO₂ and will diffuse over a large area. These products will readily be mixed with the global pool which is estimated to be 28 megacuries for ³H and 280 megacuries for ¹⁴C.⁹⁰ In such a case, the radioactivity that can be released from the stack will not be restricted by the air flow over the incinerator. The process of combustion to produce CO and H₂ is known industrially in the manufacturing of synthesis gas for production of methanol and other related industrial solvents.

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