

Part XI
Liquid Scintillation Counting (LSC)
Outline

- I. Principles of LSC
 - A. General Aspects of LSC
 - B. Factors Influencing Resolution
 - C. Liquid Scintillation Cocktails
 - D. Counting Vials
 - E. Sample Preparation
 - F. Chemiluminescence
 - G. Quenching
 - 1. Chemical Quenching
 - 2. Color Quenching
 - 3. Quench Corrections
 - a. Internal Standard Method
 - b. External Standard Method
 - c. Channels Ratio Method
 - H. Dual Labelling
- II. LSC for Alpha Emitting Nuclides
- III. Cerenkov Radiation and Counting
- IV. Problems
- V. References

SECTION I

Principles of LSC

A. General Aspects of Liquid Scintillation Counting (LSC)

Radionuclide is placed in solution or in a colloidal suspension in intimate contact with scintillation material

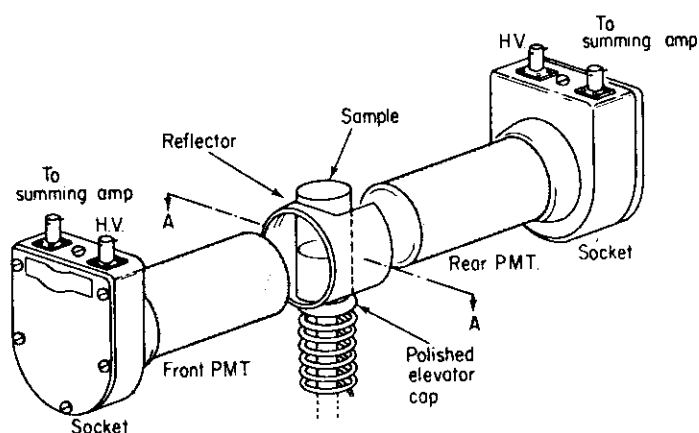


FIG. 1. Diagram of a typical scintillation counter.

Scintillation cocktail - solution of solvent + scintillator solutes

Advantages of LSC:

1. No self-absorption, thus high counting efficiency
2. LSC used to count weak β^- (e.g. ^{14}C , ^3H) but also higher energy β^- and EC. nuclides as well as α emitters
3. Possible to count two isotopes at the same time such as ^{14}C and ^3H - dual labelling

4. Easily automated analysis; often preset time or preset count option
5. Technology of sample preparation and counting highly developed

B. Factors Influencing Resolution in LSC

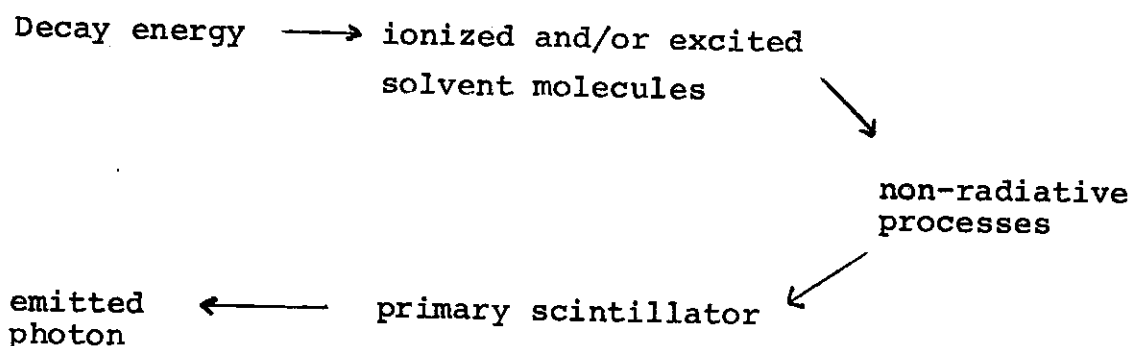
1. Energy of radiation: high detection threshold requires the background to be minimized for signal detection of low energy β
2. Type of radiation: α emitting nuclides are only 10% as efficient as β nuclides in converting the energy into photons
3. Instrumental: geometrical arrangement of detectors, photomultiplier tubes and associated electronic circuitry and the temperature of the scintillator are important
4. Scintillation cocktail: the composition of the basic scintillation solution and distribution of sample in the cocktail affect efficiency and resolution
5. Quenching: results in a decrease in the counting efficiency, and a shift in the pulse height spectrum

C. Liquid Scintillation Cocktail

1. Processes in LSC:

β (or α) radiation excites solvent molecules;
excitation transferred by collision to other molecules

and eventually to scintillator. This emits photons which have energy distribution related to fluorescence spectrum of scintillator.



2. Primary scintillator (solute) dissolved in appropriate solvent.
 - a. requirements - high degree of solubility of primary solute in solvent and considerable overlap of emission spectrum of solvent and the absorption spectrum of solute.
3. Secondary scintillator - added to cocktail as a wavelength shifter. Absorption spectrum of secondary scintillator must overlap with emission spectrum of primary scintillator.
4. Solvent:
 - 1) must efficiently transfer energy to scintillator
 - 2) must dissolve scintillator and sample in sufficient concentration

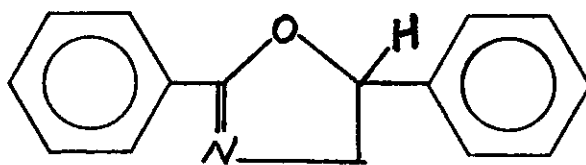
- 3) must not freeze at operating temperature
- 4) cost and purity also important

Common solvents usually aromatic hydrocarbons due to their higher efficiency for 1 .

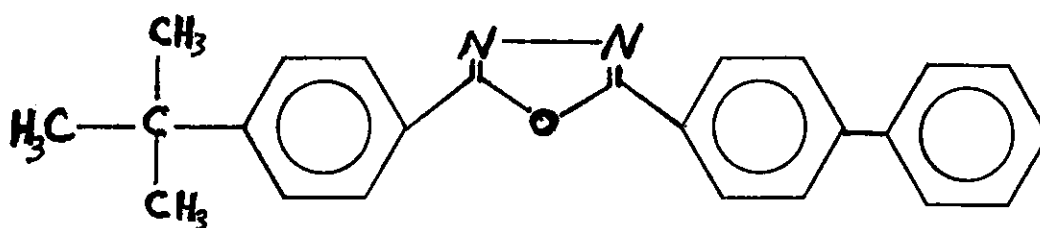
- a. Xylene - greatest pulse-height for a particular solute
- b. Toluene - cheaper, smaller absorption of fluorescent light thus recommended for larger volumes
- c. For aqueous samples - dioxane added to increase solubility
- d. For aqueous samples - quenching is a problem, naphthalene can be added to solvent to damp quenching

5. Some primary scintillators:

- a. PPO: 2-5-diphenyloxole - very commonly used.
4-6 g/l for optimum results
- b. butyl - PBD: [2-(4-t-butylphenyl)-5-(4-biphenyl)-1,3,4, oxadiazole]. Excellent fluorescence properties and higher counting efficiency than PPO.
Not used in biological systems due to color quenching.



(a) PPO

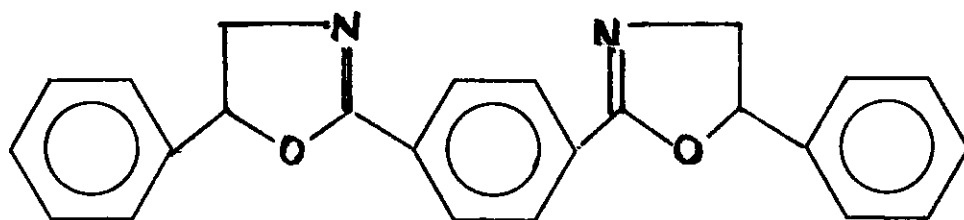


(b) butyl-PBD

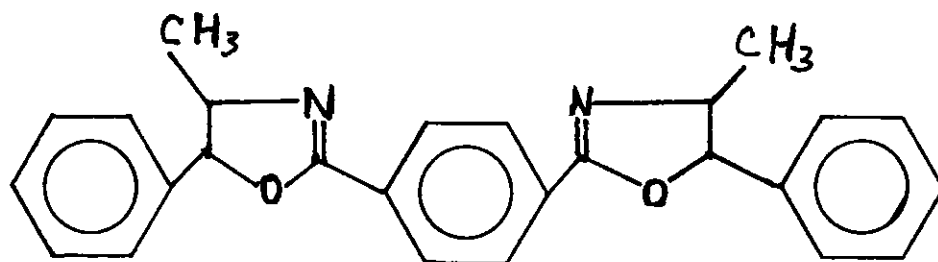
FIG. 2

6. Some secondary scintillators

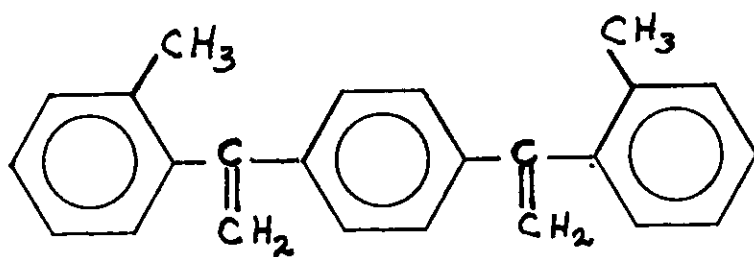
- a. POPOP: 1,4-bis-2(5-phenyloxaxolyl)-benzene
most common, usually used with PPO, efficient
but poor solubility. 0.05-0.8 g/l concentration
range.
- b. Dimethyl-POPOP: 1,4-bis-2(4-methyl-5-phenyloxaxolyl)-
benzene 0.25-1.2 g/l concentration range.
- c. bis-MSB: p-bis-(O-methylstyryl)-benzene more
soluble than POPOP or dimethyl POPOP.



(a) POPOP



(b) Dimethyl - POPOP



(c) bis-MSB

FIG. 3

TABLE 1

NAME	MOL. WT.	CONC. (M)	Toluene	
			λ_1 (Å)	λ_2 (Å)
PPO	221.25	10^{-2}	3650	3800
butyl-PBD	354.47	10^{-2}	3670	3820
bis-MSB	310.44	10^{-3}	4230	
POPOP	364.39	10^{-3}	4200	4410
DM-POPOP	392.44	10^{-3}	4300	

G. Counting Vials - usually 20 ml capacity

1. Glass-high background due to ^{40}K
2. Quartz-lower background but more expensive
3. Plastic-usually polyethylene, inexpensive; cannot be used for sample storage
4. Nylon-much like polyethylene but not permeable to solvents.

H. Sample Preparation

1. Biological Systems

- a. Dissolve directly in aromatic solvents
- b. Solubilization using quarternary ammonium bases-
avoid color quenching
- c. Wet combustion technique
60% HClO_4 ; 30% H_2O_2
- d. The alkalized emulsion cocktail

1. Insta-Gel (Packard Co.)

QUENCH RESISTANCE

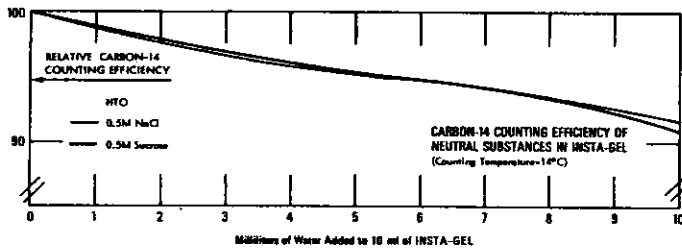


FIG. 4.

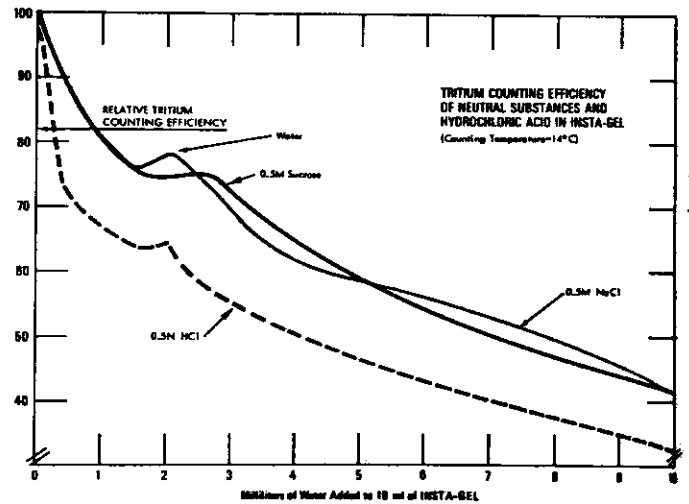


FIG. 5.

e. Milipore filters

1. Filters are either dissolved or suspended in gel.

F. Chemiluminescence

In LSC, arises from spurious luminescence events which results in an increase in the backcount.

Procedures for minimizing chemiluminescence:

Avoid preparation of samples under fluorescent lighting or direct sunlight.

Be sure digested sample is cooled before adding scintillation cocktail.

Add the maximum amount of water that will stay in solution when the sample is diluted with scintillation solution.

Neutralize digested sample to a pH of 6-7 with glacial acetic acid.

Dark adapt samples for 24 hours at 0°C before counting.

Adjust the liquid scintillation counter to operate at lowest possible temperature.

Increase lower window (discriminator) on the liquid scintillation counter to eliminate the spurious low-energy emissions. (This will not apply to tritium samples, but works nicely for high energy beta emitters such as ^{32}P .)

As a last resort: add small increments of methanol until acceptable background levels are obtained.

Note: Some of the above techniques, while reducing chemiluminescence, also reduce counting efficiency. Should experiment to ascertain conditions for the highest counting efficiency and lowest background.

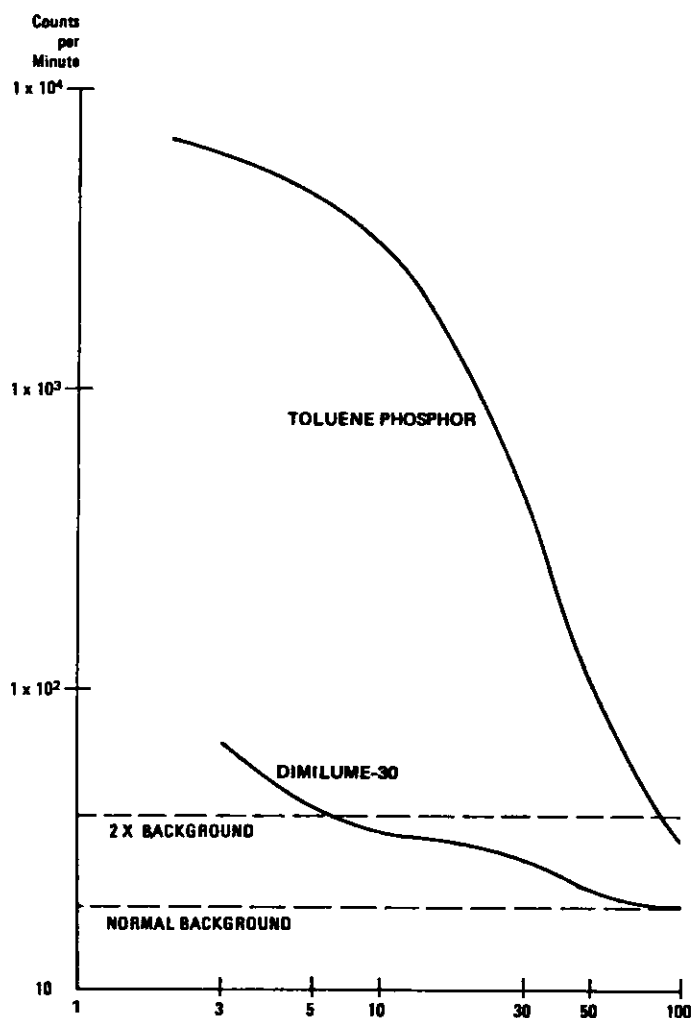


FIG. 6. TIME (MINUTES)
Reduction of Chemiluminescence at 24°C.
Sample: 0.1 ml blood plasma, 1ml SOLUENE-100,
and 10 ml phosphor.

G. Quenching - most probable source of error in LSC

1. Chemical Quenching - decrease in counting efficiency due to removal of excitation energy from scintillator via collisions before scintillator undergoes radiative de-excitation. Also shifts pulse height spectrum.

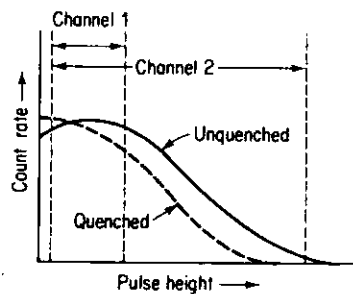


FIG. 7. Typical pulse height spectra for quenched and unquenched samples

- a. Effective chemical quenchers - molecules containing very electronegative atoms such as oxygen (air, water, ketones, aldehydes) or halides (organic halides)

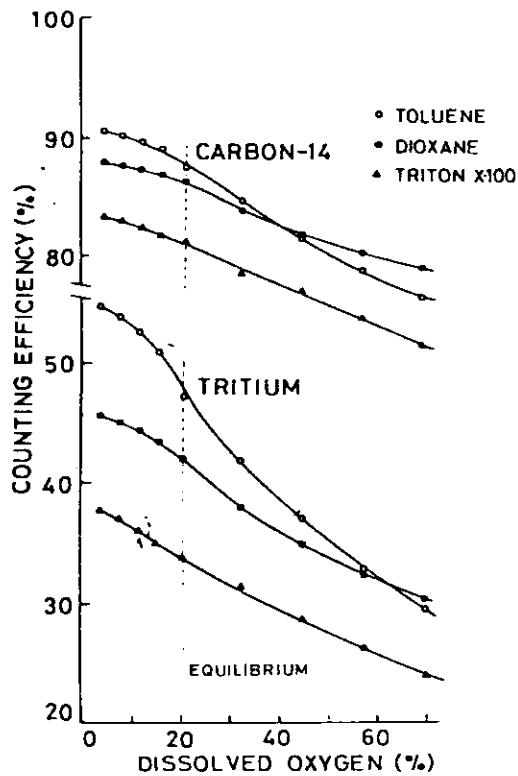


FIG. 8. Variation of counting efficiencies for T and C-14 vs. dissolved oxygen

2. Color Quenching - compounds which absorb photons from scintillator. Usually colored impurities can be removed from sample

3. Quench Corrections

a. Internal Standard Method

Unknown sample is counted

A known quantity of a standard is added, usually 5-10 nci (nanocuries, 10^{-9} curies), of the same nuclide as in the unknown.

Determine counter background by counting a vial containing only the scintillation cocktail

$$\text{Sample Activity (nci)} = \frac{A_s (R_1 - R_b)}{(R_2 - R_1 - R_b)} \quad (1)$$

R_b - background rate

R_1 - count rate of unknown sample

R_2 - count rate of sample + standard

A_s - activity of added standard

$$\text{Detector Efficiency (\%)} = \frac{(R_2 - R_1) \times 100}{A_s} \quad (2)$$

Disadvantages:

Original sample is altered by addition of the standard.

Not practical for large number of samples.

b. External Standard Method

External source used to introduce photoelectrons which simulate internal standards. Must prepare a set of quenched sources of known activity to calibrate system

Determine detector efficiency =

$$\frac{\text{observed count rate}}{\text{real disintegration rate}} \quad (3)$$

for each source

Count calibration sources with and without external source. Difference in count rates called gamma-increment. Calibration curve obtained from plot of detector efficiency vs. gamma increment

Advantage:

Many samples can be handled in a short time

Original sample not altered

Completely instrumental

Disadvantages:

Gamma increment linearly dependent on sample volume

Must be able to prepare samples reproducibly

Often difficult due to volatility of organic solvent of scintillation cocktail

c. Channels Ratio Method

Quenching not only reduces the counting rate but also shifts the pulse height spectrum. This method uses the shift as a measure of quenching and as a means to determine the counter efficiency

Requires at least two independent "windows"

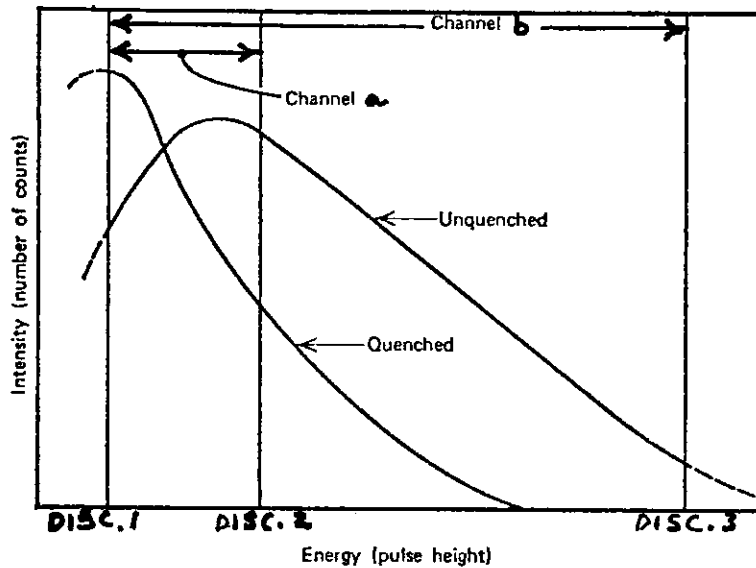


FIG. 9. Pulse height spectrum for an unquenched and quenched sample.

Window A: counts/unit time between discriminator settings 1 and 2.

Window B: counts/unit time between discriminator settings 1 and 3.

Calibrate system by preparing a set of quenched sources.

Determine detector efficiency for each calibration source.

Calibration curve obtained from plot of detector efficiency vs. ratio of counting rates in Windows A and B.

Advantage:

Purely instrumental

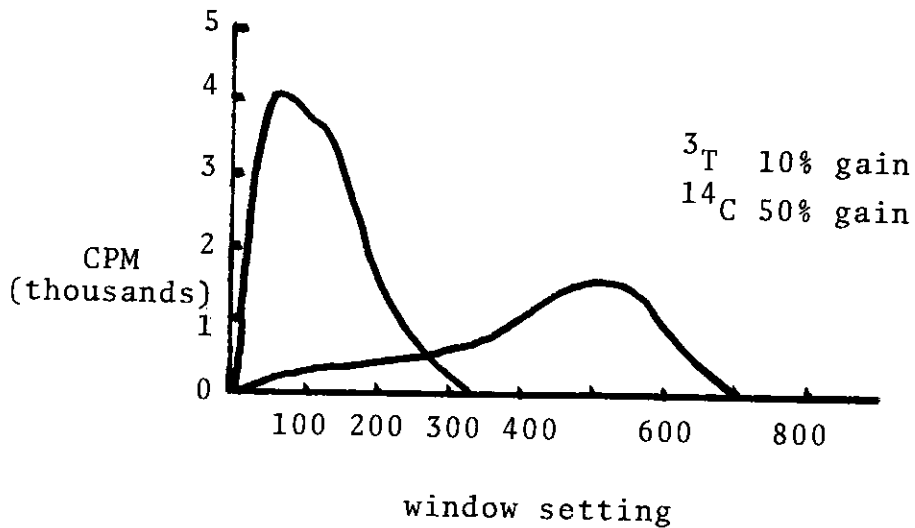
Disadvantage:

When count rate of sample is low counting statistics of monitor window must be considered.

H. Dual Labelling

Requires at least two independent windows.

Simultaneous determination of two isotopes in the same sample; e.g., ^{14}C and ^3H . Need a known standard for each nuclide. Obtain a pulse height spectrum of each standard as a function of amplifier gain. Choose appropriate gain settings in order to maximize the separation of the spectra.



FIG, 10. Pulse height of ^{14}C at 50% gain and ^3H at 10% gain.

Set the ^3H window discriminators at 1 and 2.

Set the ^{14}C window discriminators at 2 and 3.

Determine the count rate of the ^3H source in both window settings.

Do the same for the ^{14}C source.

Calculate the % of ^3H standard appearing in the ^{14}C window and the % ^{14}C standard in the ^3H window.

Calculate detection efficiency in cpm/nci for each standard in their respective windows.

When a mixture of these two isotopes are counted:

(wd = window)

dpm(³H) =

$$\frac{\text{cpm in } ^3\text{H wd} - \frac{[(\% \text{ } ^{14}\text{C in } ^3\text{H wd})(\text{cpm in } ^{14}\text{C wd})]}{100}}{\text{detector efficiency of } ^3\text{H}} \quad (4)$$

dpm(¹⁴C) =

$$\frac{\text{cpm in } ^{14}\text{C wd} - \frac{[(\% \text{ } ^3\text{H in } ^{14}\text{C wd})(\text{cpm in } ^3\text{H wd})]}{100}}{\text{detector efficiency of } ^{14}\text{C}} \quad (5)$$

SECTION II

II. LSC for Alpha Emitting Nuclides

Use of solvent extraction - radioactivity extracted into scintillation cocktail containing extracting agent.

SECTION III

Cerenkov Radiation and Counting

Cerenkov radiation is electromagnetic radiation produced when a particle is moving faster than the speed of light in the same medium. If the energy of the particle is sufficiently high, no primary scintillation may be needed due to the Cerenkov radiation produced in the solvent. The refractive index of the solvent determines the threshold energy for the emission of Cerenkov radiation. Therefore, lower energy particles may in fact be counted in a high refractive index solvent alone.

SECTION IV

Problems

1. An unknown sample registers 5050 cpm using LSC. A known standard of 8140 dpm is added to the sample and upon re-counting, the sample registers 11240 cpm. The background is already subtracted.
 - a) What is the counting efficiency?
 - b) What is the activity (in dpm) of the unknown?
2. Six aliquots of tritiated water were counted with LSC and the times in minutes required to register 10,000 counts were: 18.2; 17.8; 18.0; 13.1; 18.5 and 18.7. The background gave 1950 counts in 15 min. Calculate the average count rate and its standard deviation.
3. In a dual labeling experiment the efficiencies were determined to be: window 1, ^3H 40%, ^{14}C 40%; window 2, ^3H 3%, ^{14}C 60%. An unknown labeled with ^3H and ^{14}C gave 9000 cpm in window 1 and 5000 cpm in window 2. What was the activity of ^3H and of ^{14}C in the unknown?
4. A Grignard synthesis yielded impure 10g of benzoic acid. To a 100 mg sample, 5 mg of pure benzoic acid was added. The pure acid had a count by LSC of 12,000 cpm per mg. The spiked sample was purified and 8 mg of pure benzoic acid recovered with a count rate of 38400 cpm. What was the amount of benzoic acid in the impure product?