

Chapter 1

Some Problems in Sample Preparation for Liquid Scintillation Counting and their Solutions

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INTRODUCTION

Our august predecessors who have been given this subject to discuss in the twice-yearly meetings at Brighton or Bath have tended to discuss one specific technique of sample preparation or have developed arguments for one admonition or another. Although one of us has authored more than his share of 'Warnings' and 'Pitfalls' we find it difficult to choose between admonitions, much as a fundamentalist preacher finds it hard to decide which of the many preoccupying sins committed in his parish should be the topic of next Sunday's sermon. Suffice it to say that we find that many of our colleagues in medical research (who are cautious, careful, and even cynical in other respects) are content to take a sample, drop it into a scintillation vial, add a commercial scintillator-solvent combination with a catchy name, press the start button on a liquid scintillation (LS) counter and treat the resultant numbers as items of immutable truth. The radioactivity of a sample is regarded as not at all subject to the same vagaries that beset the investigations which have produced the sample.

Thus, although a discussion of sample preparation at a meeting organized by the Radiochemical Methods Group should probably not be a recitation of problems and their solutions (or vice versa), such a recitation is the purpose of this paper. Although some new information is worth reviewing (e.g. for counting α particles and Cerenkov radiation), we still find ourselves preoccupied with the common sources of errors in LS counting. We therefore intend to remind our readers of the existence of these problems and when possible how to discover and avoid them.

We assume at the outset that a 'sample' encompasses the scintillation vial and anything put into it. That the composition of the total sample is important has been amply documented by Kalbhen and Rezvanie¹ who showed that using the same preparation of β -emitting nuclide, different problems were encountered with each of 17 scintillation cocktails, three emulsifiers, and two sample oxidation methods.¹ Little and Neary² have shown in addition that for each type of radioactive sample and solvent there are different optimal concentrations of scintillator.

The possibility that using commercial mixtures of primary and secondary solvents will result in less than ideal counting conditions should be an important consideration for anyone doing LS counting. Unfortunately such thoughts and the consequent studies of methodology are strange to most laboratories. In the pious hope that knowledge of the problems of sample preparation is power, we discuss:

- (a) disparity between impurity (chemical) and color quenching;
- (b) the peculiarities of dioxane as a scintillation solvent;

- (c) difficulties with alkaline samples;
- (d) the effect of vials on counting efficiency;
- (e) difficulties with heterogeneous samples (solid supports, emulsions).

Once the investigator is aware that he is confronted with a problem, it is often possible for him to solve it.

We have also elected to review, albeit briefly, significant recent advances in the use of LSC for the measurement of Cerenkov radiation, α emitters, and nuclides which are 'novel' as far as LSC is concerned: those which decay primarily by electron capture or by γ -ray emission.

COLOR VS IMPURITY ('CHEMICAL') QUENCHING

Even with sample homogeneity established, the question of whether acceptable quench-correction curves can be constructed from series of standards of different composition, remains. Neary and Budd³ originally pointed out that while there was no divergence of color and impurity quench-correction curves for tritiated samples, the effects of significant color quenching on ^{14}C efficiency could not be predicted accurately from plots of sample channels ratios (SCR) or external standard channels ratios (ESCR) obtained with a series of colorless quenched standards.⁴ The remedial strategy of making up suitable color-quenched standards is complicated by the necessity of closely matching the absorbance spectra of the unknown samples (which may be quite complex) in the sensitive wavelength band of the⁵ photomultiplier tubes of the specific liquid scintillation counter being used.

Decolorizing each sample will eliminate this problem of adequate standardization⁴ which of course applies to counting any nuclide with emission energy above the tritium range, and to Cerenkov counting as well as standard LS counting. In the special case of the ferric ion which is known to be a strong color quencher, Blanus⁶ has shown that adding fluoride ion (as NaF) would eliminate most of the effect of the ion on counting efficiency. Chemical decolorization will, however, by adding impurities to the sample, increase quenching and if peroxides are used with ^{14}C -labelled samples, radioactivity may be lost as $^{14}\text{CO}_2$. Combustion of the sample will yield colorless counting mixtures of known composition, but has not⁷ yet been shown to be a reliable methodology except for ^3H and ^{14}C and perhaps ^{35}S .⁷ It requires, moreover, considerably more time and expense spent in sample preparation and the acquisition of automated sample oxidizers if many samples are to be prepared.

Several advances in LS counter design should eliminate or at least minimize the overestimation of the counting efficiency of colored samples by impurity quench-correction plots from instruments in which pulse height spectra are summed. One is a technique introduced by Laney into Searle Analytic⁸⁻¹¹ (Nuclear Chicago) LS counters a few years ago: lesser pulse height analysis. The other has been introduced even more recently to Beckman LS counters by Horrocks: the H number (H#) a measurement of the inflexion point of the Compton electron spectrum generated by a single γ -emitting external standard.¹² Either of these new electronic features, in addition, makes it possible to determine the absolute radioactivity of an unknown sample.

Painter⁵ has recommended that the quenching in colored particulate samples be assessed by internal standardization. When samples are truly homogeneous and differences between the partition coefficients of added labelled standards and unknowns are not a problem, this is an excellent strategy. When LS counters which do not have lesser pulse height analysis are used to count colored samples, quenched series of internally standardized unknown samples should always be prepared to discover whether quench-correction by sample or external standard (Compton electron) channels ratio techniques will give acceptable counting accuracy. If samples are severely color-quenched, however, spectra are so distorted^{13,14} that there is no algorithm which can be used to predict efficiency.

SAMPLES IN DIOXANE-BASED SCINTILLATION MIXTURES

Since Bray's article¹⁵ of 1960 showed that aqueous samples were miscible with a solution of scintillator in dioxane, we have been concerned with either how much

water dioxane-based cocktails could tolerate, or with occasional chemiluminescence which is attributed to peroxide impurities. Wombacher and Reuter-Smerdka¹⁶ have recently pointed out, however, that unexpected losses in efficiency may occur in dioxane mixtures with only small amounts of water.¹⁶ The presence of metal ions (Ca^{2+} , Mg^{2+} , Cu^{2+}) and labelled compounds with which they may combine (e.g. nucleotides) may lead to the formation of heterogeneous samples: self-absorption thus may occur with no visible evidence of precipitation or of phase change. In such a situation decreased counting efficiency cannot be detected by comparing external standard and sample channels ratios or via internal standardization with $^3\text{H}_2\text{O}$ or ^3H -toluene, one of the 'tests' of sample homogeneity suggested by Bush¹⁷ and Mueller.¹⁸ This problem of 'photon' quenching was not encountered when identical samples were mixed with emulsions or thixotropic gels.¹⁶

As a method of precipitating plasma proteins, adding a portion (1:1) of 1,4-dioxane has recently been proposed¹⁹ rather than ethanol which tends to be a significant quenching agent. The supernatant is then miscible with dioxane-based scintillation media.

ALKALINE SAMPLES

The hydrolysis of labelled biological samples with strong base leads to problems if standard LSC solutions are employed: development of color quenching as a result of reactions of organic scintillators with alkali chemiluminescence,²⁰ and (if organic acids are added to neutralize the base) severe impurity quenching²¹ and phase separation.¹ Under certain circumstances addition of acid may even produce chemiluminescence.¹

The use of a solubilizer tolerant to alkali (Biosolv BBS-2) has been reported^{22,23} as a method which is successful in eliminating chemiluminescence (which presumably results from the oxidation of lipids in the sample)²⁴ or as unsuccessful in doing so.²⁵ Several years ago Neame reported some success with strong acidification,²⁵ addition of water, and then of secondary solvent (Triton X-100).²⁰ Flindt-Eegbak has recommended that Instagel TM (Packard Instrument Co.) be used to dissolve neutralized alkali hydrolyzates inasmuch as no significant chemiluminescence occurs. He has not provided data which would allow us to assess the accuracy of sample channels ratios for the calculation of efficiency, nor has he shown that chemiluminescence is totally eliminated.

In most circumstances, a sample of unknown composition (usually of biological origin) is hydrolyzed. The efficiency is compromised by impurity quenching and color quenching from complex absorption spectra. If the nuclide of interest is ^3H or ^{14}C , combustion of the sample is highly recommended.

PROBLEMS WITH VIALS

Permeation

The loss of scintillators and solvents from soft polyethylene vials over a few days or weeks may be appreciable.^{26,27} Mueller²⁸ and others^{29,30} have reported a continuous drift of external standard channels ratios over several days which is a consequence of progressive migration of scintillator into the vial walls. The extent of this migration is greater at ambient temperature than in refrigerated LS counters; Compton cpm accumulate in the lower energy range. As a result of this phenomenon (which is not observed with glass and nylon vials) small amounts of labelled samples will also be lost. Aqueous samples in emulsions may also be lost to a significant extent over a period of days³¹ resulting in lowered counting efficiency. The conclusions to be reached from these observations are obvious:

1. Soft polyethylene vials should not be used to count samples dissolved in organic solvents.
2. If other plastic vials are used, some idea of the extent of permeation of the vial wall by organic scintillators may be obtained by determining the external standard channels ratio at several intervals over 24 h as suggested by Johanson and Lundqvist.²⁹ If the ESCR changes, then quench correction should be carried out by other approaches.
3. Whether the solvent is organic or aqueous, the vials glass or plastic, it is

likely that significant radioactivity will be lost from the counting solution if it is stored for several weeks. If there are (uncommon) circumstances that require such delays, the vial should be made of glass and securely flame sealed.

Sample adsorption to vial walls

A number of investigators³² have reported a loss in the counting efficiency of labelled samples because of their adsorption to the walls of glass scintillation vials. Wigfield's laboratory in Canada has been active in the past few years in studying this phenomenon and have developed an algorithm (A) the 'Adsorption Shift'^{33,34} which takes advantage of the pulse height shift (to lower photoelectron energy ranges) caused by the backscattering of nuclides adsorbed in 2π rather than 4π configuration. Sample channels ratios (SCR) are compared to external standard channels ratios (ESCR), the former being affected by adsorption and the latter not. Bush has observed previously that quench correction plots of the two ratios vs counting efficiency diverged when heterogeneous samples were examined.

$$\underline{A} = \underline{a} (\log \text{ESCR} + \underline{b} - \log \text{SCR})$$

The constants a and b were obtained by determining the log ESCR and log SCR for a non-adsorbed labelled sample (e.g. hexadecane or toluene), knowing that an equation exists for the relationship: $\log \text{SCR} = \underline{a} (\log \text{ESCR} + \underline{b})$. With samples which are not adsorbed, A = 0.00; with maximal adsorption A is about 0.40.

Wigfield claims to be able to evaluate adsorption losses of ^{14}C and more energetic nuclides,³⁵ but no 'Adsorption Shift' is observed with the decreased counting efficiency of weak β emitters such as tritium,³⁶ and up to 20% of the ^{14}C radioactivity in a vial may be adsorbed before an 'adsorption shift' is detected.³⁷ With energetic isotopes such as ^{32}P , adsorption shifts may be encountered³⁸ which are not necessarily due to simple adsorption to vial walls but to Cerenkov radiation and to other as yet unknown phenomena.³⁹

Two other tests may be performed: emptying the vial and counting it again with fresh scintillation medium is a simple and obvious tactic. Dilution with unlabelled carrier³² is possible only if the specific carrier is available and if precipitation of the sample will not occur as a result. The chemicals, which tend to be adsorbed significantly are high in oxygen content^{38,40,41} and in specific activity. The critical concentration to saturate binding sites was found by Wigfield to be 2×10^{-6} M or 3×10^{-8} mol per 15 ml of counting solution. Solutions to this problem include:

- (a) Using plastic vials. (As already noted, this may result in permeation of the sample through the vial wall.)
- (b) Adding carrier. (This may not be practical.)
- (c) Emulsion counting.

The latter³⁸ has been suggested as a strategy for eliminating ^{32}P orthophosphate adsorption³⁸ but has not to our knowledge been tested with other labelled compounds liable to adsorb to vial walls.

SAMPLES ON SOLID SUPPORTS

Our laboratory has already been heard from in warnings of the inaccuracies encountered when β emitting samples are counted on paper,⁴² cellulose ester filter discs, or glass fiber paper.^{43,44} Recently Long et al.⁴² have reiterated the message that 'it is necessary to dissolve the filter in order to obtain the true activity of the sample and to give the magnitude of error encountered...' Despite all this, many investigators assume that losses in counting efficiency are constant or can be calculated through the use of standard quench-correction procedures. Drying the filters first frequently results in the translucency of filters in scintillator solutions — an effect which improves counting efficiency but not to the same extent as dissolution of the filters. Samples are still heterogeneous unless the filter is dissolved or the sample is completely taken up from the filter into the scintillator medium. If the dissolution is incomplete the sample will be counted in two phases.

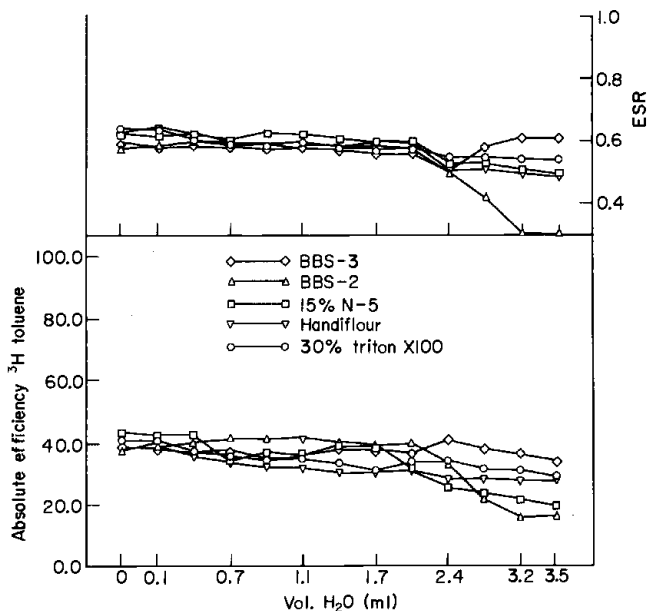


Fig. 1. The effect of added water and surfactants on the efficiency of counting ^3H -toluene which was obtained from the New England Nuclear Corporation, Boston, Massachusetts, U.S.A. 22 000 dpm were added to each vial. Radiolabelled $50\ \mu\text{l}$ samples were added to a 10 ml counting solution consisting of toluene and $5.5\ \text{g l}^{-1}$ Packard Instrument Co. Permablend (91% PPO, 9% POPOP) (except when Handifluor-MallinckrodtTM was involved). The surfactants indicated in the figures were added to the volume. Each ml of water is thus representative of the percent volume if multiplied by 10 (e.g. 3 ml = 30% H_2O). Counting was carried out at ambient temperature with a Beckman LS-230 counter using an optimal wide channel for the isotope of interest. External standard channels ratios were calculated from the Compton electron spectra of a ^{137}Cs source using fixed channels. Biosolv BBS-2 and BBS-3 were obtained from Beckman Inc., Triton X-100TM from the Rohm and Haas Co., N-5 HandifluorTM from Mallinckrodt Inc. N-5 is a mixture of 94% nonylphenoxy ethanol and 6% sodiundihexylsulfosuccinate prepared in our laboratory.

The use of tissue solubilizers to dissolve filters is inadvisable, inasmuch as considerable color quenching usually ensues.⁴⁵ Beckman Inc. have developed an emulsion system - Filter SolvTM, which will dissolve cellulose acetate, cellulose nitrate or mixed ester filters.^{43,44} It is worthwhile remembering that the sample may remain precipitated and may therefore be heterogeneous despite the dissolution of the filter. We thus find ourselves in disagreement with the statement of Apelgot and Duquesne⁴⁶ that 'it suffices simply to have a contact between the β particles and the liquid scintillator'.

Neither automatic quench-correction techniques nor internal standardization should be employed. External standard channels ratios are completely insensitive to losses of efficiency because of the 2π configuration of the sample. Sample channels ratios are unreliable inasmuch as the SCR may drop when samples are dissolved from filters while the count rate increases.⁴⁴ With a weak β emitter such as tritium no SCR vs efficiency plot should be employed to correct for absorption of β s; with more energetic nuclides a relationship between SCR and lost efficiency may be plotted but almost always with unacceptably high variance. With energetic nuclides such as ^{36}Cl and ^{32}P , on the other hand, there may be significant losses of efficiency because the sample is positioned less than 1 cm (the approximate maximal range of ^{32}P in toluene-based scintillation fluid) from the vial wall.^{47,48}

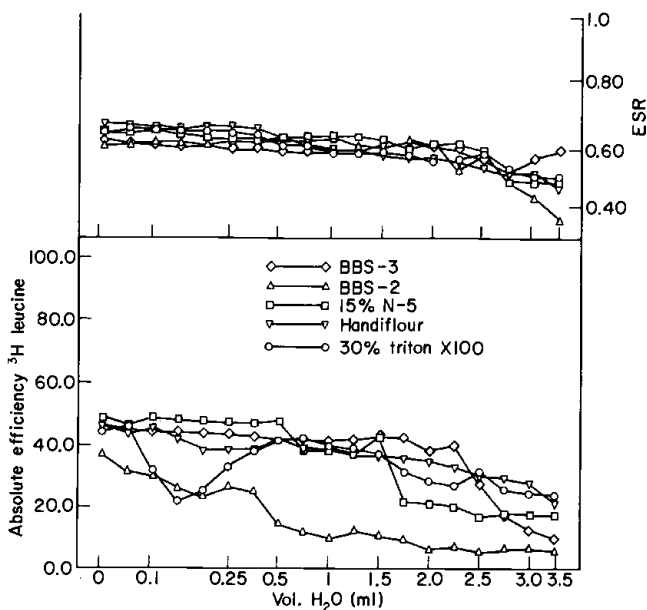


Fig. 2. The effect of added water and surfactants on the efficiency of counting 4,5-³H-L-leucine (New England Nuclear, 62 Ci mol⁻¹). 36 666 dpm were added to each vial. See the legend to Fig. 1 for further details.

SAMPLES IN EMULSIONS

Surfactants combined with the usual scintillators and organic solvents (toluene or xylene) have become extremely popular for counting β s in aqueous samples. Such solutions are able to hold water in micelles; the physical character of the micelles is affected by the amount of water added, the solutes present, and the temperature.⁴⁹ The use of Triton X-100 (a non-ionic surfactant) was first recommended 15 years ago⁴⁹ and still receives considerable attention (despite some variability between lots as to capacity for water and potential for chemiluminescence).^{50,51} Most commercial surfactants are, however, combinations of non-ionic surfactants and lesser amounts of anionic surfactants.¹⁸ There are a number of excellent reviews, including those by Fox⁵²⁻⁵⁹ and by Mueller¹⁸ in previous volumes of this series. We have already pointed out that surfactants may act as scintillators and that counting standards should thus contain the surfactant, solvent and scintillator employed for the preparation of practical samples.^{60,61}

In the present review, we seek to emphasize that there may be considerable inaccuracies in applying automatic (ESCR and SCR) quench-correction techniques if the samples are functionally homogeneous (i.e. if the nuclides are counted in '2 π ' rather than '4 π ' configuration, virtually cutting counting efficiency in half). The greatest problem is that phase separation may occur before a visible change in the sample (e.g. see Ref. 52). If emulsions do break into two distinct phases, each has its own character as a scintillation medium, the partition of the radionuclide(s) being measured in either phase being a function of the solubility of the labelled sample in water in the organic solvent. Emulsions are also sensitive to varying amounts of other solutes and to the specific surfactants employed; their physical behaviour is thus difficult to predict unless the nature and amount of labelled unknown samples are extremely reproducible.

The experiments summarized in Figs 1-4 showed (in confirmation of many other studies) that the ESCRs could not predict counting efficiencies and that organic (³H and ¹⁴C-labelled toluene) standards could not predict the efficiencies observed when aqueous samples were counted. That different surfactants behave differently is also evident; what is not immediately evident is that degradation in counting

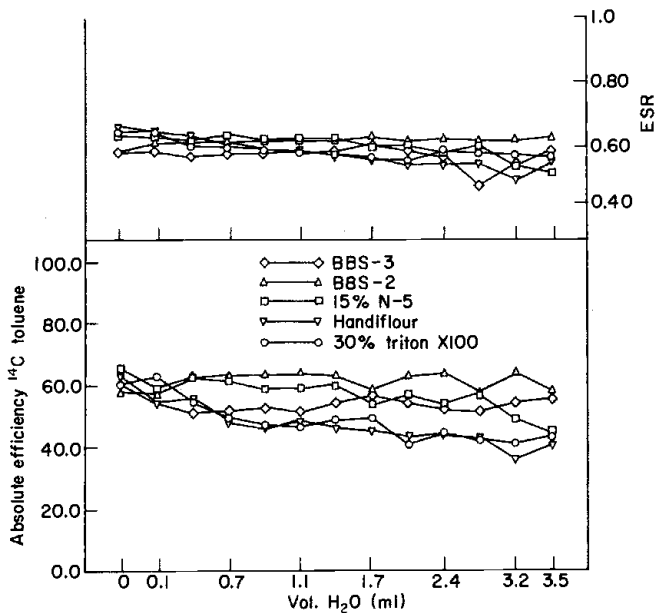


Fig. 3. The effect of added water and surfactants on the efficiency of counting ¹⁴C-toluene (New England Nuclear). 20 600 dpm were added to each vial. See the legend to Fig. 1 for further details.

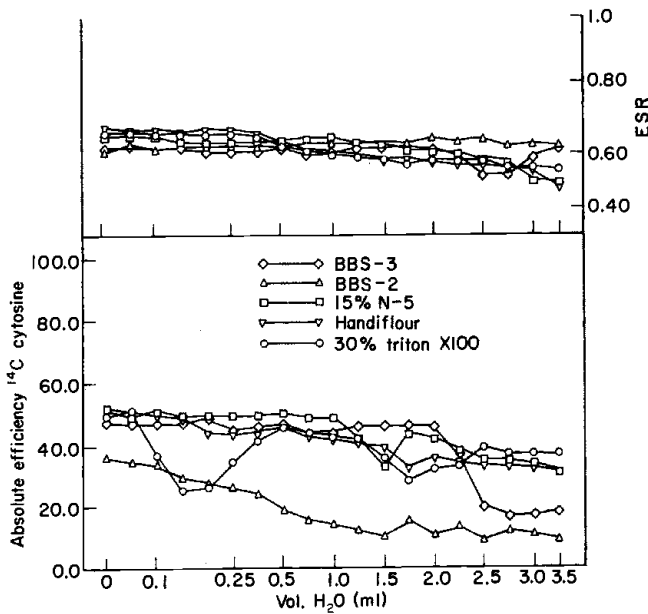


Fig. 4. The effect of added water and surfactants on the efficiency of counting 2-¹⁴C-cytosine (New England Nuclear, 5 Ci mol⁻¹). 36 666 dpm were added to each vial. See the legend to Fig. 1 for further details.

efficiency usually could be detected with a lesser amount of added water than it took to achieve a visible phase separation and that, as Wagstaff and Ware⁵⁶ have pointed out, such drops in counting efficiency predict phase separation. In most of the emulsions (especially with Triton X-100) a certain volume of water is necessary to achieve optimal efficiency.^{51,62}

Comparison of ESCR vs efficiency and SCR vs efficiency plots is usually (but not always) a sufficient practical test of sample homogeneity if ^{14}C or more energetic β s are to be counted. How reliable this is depends on the LS counter being used. Lesser pulse-height analysis, an electronic feature of some LS counters, has, as has been noted above, been helpful in eliminating differences between color and impurity quench-correction curves. The accuracy of comparing ESCR and SCR quench-correction curves to assess sample heterogeneity is, however, much less certain than with summed pulse-height analysis. For tritiated samples the 'double ratio' plot is insensitive to heterogeneity:cpm are simply lost. Internal standardization with the same radiolabelled substance as that in the unknown samples is one remedy, but addition of more solute may then significantly change the physical character of the emulsion. Making up standards with nuclides which are restricted to the aqueous phase on one hand and to the organic phase on the other (e.g. $^3\text{H}_2\text{O}$ and ^3H -toluene) and comparing efficiencies as suggested by Mueller¹⁸ is a useful and fairly stringent test. It is also worthwhile remembering that the surfactants have their individual quenching properties. Laney, for example, has pointed out that Triton X-100 exhibits more color quenching than other surfactants despite visual similarity of samples containing either secondary solvent.

CERENKOV COUNTING

Cerenkov radiation is emitted when a charged particle exceeds the speed of light in a transparent medium. It is almost completely independent of the chemical nature of the medium, and is thus unaffected by 'impurity' quenching. Samples are, however, subject to color quenching and should be quench-corrected by internal standardization if possible.

There have been two very recent excellent reviews of the measurement of Cerenkov radiation in liquid scintillation counters.^{63,64} The threshold energy for β s in water is 264 keV but can be increased by addition to the solution of a compound with a high effective atomic number. Methyl salicylate, for example, has been shown by the Radiopharmacy Group at the University of Alberta to increase counting efficiency dramatically, partly as a result of its high refractive index and partly because of its properties as a wavelength shifter.⁶⁵

If a wavelength shifter with the properties of a scintillator (e.g. dimethyl POPOP, 4 methyl-umbelliferone) is added to the sample, then the independence from impurity quenching is mitigated. Ross has recently introduced a quartz counting vial with an isolated sealed external compartment containing a wavelength shifter, which matches the spectrum of emitted light to higher wavelengths necessary for good photomultiplier response and thus increases counting efficiency.⁶⁴

Gamma-emitting isotopes may be counted by the Cerenkov effect of the Compton electrons they generate at low (1.5-10%) efficiencies. Despite some suggestions in recent journals that samples adsorbed to filters of charcoal can be reliably counted by measurement of Cerenkov radiation, samples not in solution will suffer self-absorptive losses which cannot be monitored. Samples for Cerenkov counting should, therefore, always be in solution.

ALPHA COUNTING

That α particles should be counted with liquid scintillation equipment was evident more than 20 years ago.⁶⁶ Although the energy of α emissions is high (generally between 4 and 6 MeV) compared to that of β emitters, the range of α s is short, and the fluorescence quantum yield is much less than for β s - about 10%. Standard LS counting techniques can be employed if the α -emitting nuclide is in the same phase as the organic scintillator, if the α activity is at least several hundred cpm, and if no β - or γ -emitters contaminate the sample. Since the presence of impurities in the sample will quench and shift α -energy spectra, a peaks are in

practice sometimes located with a multichannel analyzer interfaced to the LS counter prior to counting; otherwise contamination by β - and γ -activity in the sample will add to an already high background. This approach does not permit pulse-height discrimination of α s.

With plutonium isotopes and some other α -emitting nuclides, it is possible to lower background counts, decrease the shift of α peaks due to quenching, and decrease β and γ interference by using an extractive scintillator. HDEHP (di-(2-ethyl-hexyl)-phosphoric acid) is frequently used to extract plutonium from aqueous samples.⁶⁷ Using an extractive scintillator it is possible to discriminate and count different α s if there is an energy differential of at least 1 MeV. Backgrounds are still fairly high (15-20 cpm) and contamination by β - and γ -emitting nuclides cannot be dealt with adequately.

The development of liquid scintillation equipment designed for α spectrometry in the last few years has resulted in the ability completely to resolve α s with energy differences of 200-300 keV, with backgrounds of less than 1 cpm. Special circuitry for pulse timing has permitted pulse-shape discrimination⁶⁸⁻⁷⁰ lowering backgrounds to 0.01 cpm. When pulse-shape discrimination is used to eliminate β s and γ s it has been considered very important that oxygen be removed from the samples by sparging them with an inert gas. Under these circumstances the detection limit for α s is approximately 0.03 cpm (0.02 pCi). Cross and McBeth⁷¹ in a recent note have shown that the scintillator does not need to be de-oxygenated for accurate α counting, but that the α source need only be incorporated into a dioxane-based scintillator. No specific scintillator or scintillation solvent has emerged as the one to use for α counting. Organic solvents, dioxane-containing samples,⁷² emulsions and gels have all been used: a recent critical review by McKlveen⁷³ is recommended.

We have not been able to find much advice on quench correction in the recent literature. The effects of quenching on the shape as well as the height of peaks are dramatic and are difficult to calibrate with external standard channels ratios. McKlveen has shown that the effects of color quenching on the shape and width of α spectra may be quite different from that observed with impurities.⁷² Suffice it to say that quench-correction curves should be constructed with standards as close as possible in composition to practical samples and should be checked by internal standardization if possible.

COUNTING 'NOVEL' RADIONUCLIDES

For electrons of energies up to 100 keV and slightly above, the usual scintillation solution volume of 15 ml is enough to permit interaction of β with scintillator molecules. As noted above, highly energetic β s (e.g. ^{32}P and ^{131}I) tend to escape from the vial and may thus be counted with efficiencies of considerably less than 100%. For X- and γ -rays there is, however, a high probability of escape.

Isotopes which decay by electron capture release either fluorescent X-rays or Auger electrons. The interactions are complex, but have recently been described for ^{125}I ⁷⁴ and for ^{55}Fe .⁷⁴ Quench-correction procedures are similar to those used for β s. Other EC isotopes which may be counted by liquid scintillation are listed in Table 1.

Iodine-125 may be counted by liquid scintillation at fairly high efficiencies,^{73,75,80} It is possible, using standard LS mixtures, to obtain a good separation of ^{125}I ⁷⁵ and ^{131}I for double-label counting at creditable efficiencies for both isotopes. It has therefore been difficult to understand why doping the scintillation mixture with heavy metals (e.g. tetrabutyltin which is miscible with organic scintillation solvents⁷⁶) has been suggested for counting ^{125}I by itself. The use moreover of tetrabutyltin or tetramethyltin is impractical because of the toxicity and considerable expense.⁷⁹ Lindqvist et al.⁷⁷ have therefore suggested that water-soluble thallium or lead salts in a Triton X-100 or InstagelTM system be used to achieve separation between ^3H and ^{125}I . With the usual scintillation mixtures, ^3H and ^{125}I photoelectron spectra overlap too closely to permit double-label counting; addition of atoms with large cross-sections shifts the more energetic ^{125}I photopeak to a higher energy range while pre-eminently quenching the photoelectron peak of lesser energy.

Table 1. 'Novel' nuclides measured by liquid scintillation counting.

Mode of Decay	Isotopes	Reference
EC	^{125}I	73-78, 79-83
	^{55}Fe	74, 78, 84
	^{37}Ar , ^{71}Ge , ^{97}Tc	74
	^{131}Cs , ^{179}Ta , ^{205}Pb	
γ	^{65}Zn , ^{109}Cd , $^{115\text{m}}\text{Cd}$	85
	^{203}Hg	
	^{57}Co	86
	^{137}Cs , ^{241}Am , $^{99\text{m}}\text{Tc}$	87
	^{40}K , ^{42}K	
β^+	^{18}F	88

The proposal for ^{125}I -counting in 'gamma vials' in which an ^{125}I sample is in a well surrounded by a sealed scintillator containing heavy atoms, and the suggestion that ^{125}I activity be determined in a well counter and then (after comparison with a standard) be subtracted from the total ^{125}I and ^3H cpm (see, for example, Ref. 79), are both dangerous, since neither strategy can comprehend the effects of sample geometry and self-absorption on ^{125}I detection. Effects of geometry on the self-absorption of ^{125}I samples are frequently observed in Na(Li)I crystal well counting. Iron-55 may be counted alone, or with ^{59}Fe in emulsion systems; (e.g. Triton X-100-toluene or InstagelTM 84) in a similar manner to ^{125}I and ^{131}I .

The counting of γ -emitting isotopes, in most cases by virtue of the detection of Compton electrons, has been recommended⁸⁴⁻⁸⁶ but has not been put to much practical use. It is worthwhile remembering, however, that if a sample is being assayed for β activity by LSC then contamination γ emitters may contribute significantly to the count rate by virtue of low-energy conversion electrons. The one example of a position emitter we have found to have been measured by LSC is ^{18}F .⁸⁸ The isotope is measured almost as well by Cerenkov counting in a solution of methyl salicylate as it is with a standard LS scintillator solution.

CONCLUSIONS

The conclusion of this essay is that the preparation of samples for LSC requires a great deal of care. Unless the investigator can anticipate the problems implicit in each technique available, the counting data he obtains may be unreliable or worthless. We have reviewed these problems and the solutions offered in the literature of the past few years. One principle mentioned but not really emphasized in the paragraphs above is that the standards chosen to allow correction for losses in counting efficiency be as much like the practical (unknown) samples as possible. In situations where the exact nature of the radiolabelled substance being counted is not known, we recommend sample oxidation as a solution to the problem of sample heterogeneity. Procedures for both wet and dry oxidation have been reviewed comprehensively last year by Kisielęski and Buess.⁷ We have little to add except the statement that for other than ^{14}C - and ^3H -labelled samples there

is very little information published on the oxygen flask combustion technique (the best approach in many respects).

REFERENCES

1. D.A. Kalbhen and A. Rezvanie, in Organic Scintillators and Liquid Scintillation Counting (D.L. Horrocks and C.T. Peng, Eds) Academic Press, New York, 1971, p.149.
2. R.L. Little and M.P. Neary, in Organic Scintillators and Liquid Scintillation Counting (D.L. Horrocks and C.T. Peng, Eds) Academic Press, New York, 1971, p.431.
3. M.P. Neary and A.L. Budd, in The Current Status of Liquid Scintillation Counting (E.D. Bransome, Jr., Ed.) Grune and Stratton, New York, 1970, p.273.
4. E.Z. Helman, V. Spiehler and S. Holland, Clin. Chem. 20, 1187 (1974).
5. K. Painter, Clin. Chem.(N.Y.) 22, 928 (1976).
6. M. Blanusa, Anal. Biochem. 69, 120 (1975).
7. H. Kisielewski, in Liquid Scintillation -- Science and Technology (A.A. Noujaim, C. Ediss and L.I. Wiebe, Eds) Academic Press, New York, 1976, p.229.
8. B.H. Laney, in Liquid Scintillation Counting -- Recent Developments (P.E. Stanley and B.A. Scoggins, Eds) Academic Press, New York, 1974, p.455.
9. B.H. Laney, in Liquid Scintillation Counting, Vol.4 (M.A. Crook and P.A. Johnson, Eds) Heyden, London, 1977, p.74.
10. P.J. Malcolm and P.E. Stanley, in Liquid Scintillation Counting, Vol. 4 (M.A. Crook and P. Johnson, Eds) Heyden, London, 1977, p.15.
11. C. Ediss, A.A. Noujaim and L.I. Wiebe, in Liquid Scintillation Counting -- Recent Developments (P.E. Stanley and B.A. Scoggins, Eds) Academic Press, New York, 1974, p.91.
12. D.L. Horrocks, in Liquid Scintillation -- Science and Technology (A.A. Noujaim, C. Ediss and L.I. Wiebe, Eds) Academic Press, New York, 1976, p.185.
13. F.E.L. ten Haaf, in Liquid Scintillation Counting, Vol. 2 (M.A. Crook, P. Johnson and B. Scales, Eds) Heyden, London, 1972, p.39.
14. J.A.B. Gibson, in Liquid Scintillation -- Science and Technology (A.A. Noujaim, C. Ediss and L.I. Wiebe, Eds) Academic Press, New York, 1976, p.153.
15. G.A. Bray, Anal. Biochem. 1, 279 (1960).
16. H. Wombacher and M. Reuter-Smerdka, Anal. Biochem. 74, 526 (1976).
17. E.T. Bush, Int. J. Appl. Radiat. Isot. 19, 447 (1968).
18. E.B. Mueller, in Liquid Scintillation Counting, Vol. 3 (M.A. Crook and P. Johnson, Eds) Heyden, London, 1974, p.47.
19. P.H. Springell and D.E. Wright, Int. J. Appl. Radiat. Isot. 27, 85 (1976).
20. K.D. Neame, Anal. Biochem. 64, 521 (1975).
21. E.D. Bransome Jr. and M.F. Grower, in The Current Status of Liquid Scintillation Counting, Grune and Stratton, New York, 1970, p.342.
22. M. Pollay and F.A. Stevens, in The Current Status of Liquid Scintillation Counting, Grune and Stratton, New York, 1970, p.207.
23. A. Stevens, E. Estrada, M. Pollay and R. Kaplan, Anal. Biochem. 37, 1 (1970).
24. Laine-Boxzormenyi and P. Fallot, Int. J. Appl. Radiat. Isot. 25, 241 (1974).

25. P. Flindt-Eegbak, Int. J. Appl. Radiat. Isot. 27, 173 (1976).
26. E. Rapkin and J.A. Gibbs, Int. Appl. Radiat. Isot. 14, 71 (1963).
27. R. Lieberman and A.A. Moghissi, Int. Appl. Radiat. Isot. 21, 319 (1970).
28. E.B. Mueller, in The Current Status of Liquid Scintillation Counting, Grune and Stratton, New York, 1970, p.181.
29. K.J. Johanson and H. Lundqvist, Anal. Biochem. 50, 47 (1972).
30. K.D. Neame, Int. J. Appl. Radiat. Isot. 26, 393 (1975).
31. L.A. Muse and V. Rao, Health Physics 31, 457 (1976).
32. G.J. Litt and H. Carter, in The Current Status of Liquid Scintillation Counting, Grune and Stratton, New York, 1970, p.156.
33. D.C. Wigfield and V. Srinivasan, Int. J. Appl. Radiat. Isot. 21, 613 (1973).
34. D.C. Wigfield, Anal. Biochem. 59, 11 (1974).
35. D.C. Wigfield, in Liquid Scintillation - Science and Technology (A.A. Noujaim, C. Ediss and L.I. Wiebe, Eds) Academic Press, New York, 1976, p.295.
36. E.D. Bransome, Jr., in The Current Status of Liquid Scintillation Counting Grune and Stratton, New York, 1970, p.333.
37. D.C. Wigfield, Anal. Biochem. 63, 286 (1975).
38. R. Tykva and E. Podracka, Int. J. Appl. Radiat. Isot. 26, 495 (1975).
39. D.C. Wigfield, Int. J. Appl. Radiat. Isot. 27, 129 (1976).
40. D.C. Wigfield and V. Srinivasan, Int. J. Appl. Radiat. Isot. 25, 473 (1974).
41. W.G. Crouthame1 and K. Van Dyke, Anal. Biochem. 66, 234 (1975).
42. E.D. Bransome, Jr. and M.F. Grower, Anal. Biochem. 38, 401 (1970).
43. E. Long, V. Kohler and M.J. Kelly, in Liquid Scintillation - Science and Technology (A.A. Noujaim, C. Ediss and L.I. Wiebe, Eds) Academic Press, New York, 1976, p.47.
44. E. Long, in Liquid Scintillation Counting on Filter Supports 916-NUC-76-8T: A publication of Beckman Inc. (1976).
45. R.E. Johnsonbaugh, J.O. Kleiman and J. Sode, Anal. Biochem. 54, 490 (1973).
46. S. Apelgot and M. Duquesne, in Liquid Scintillation - Science and Technology (A.A. Noujaim, C. Ediss and L.I. Wiebe, Eds) Academic Press, New York, 1976, p.33.
47. E. Blasius and J. Spannhake, Int. J. Appl. Radiat. Isot. 24, 301 (1973).
48. F.G. Winder and G.R. Campbell, Anal. Biochem. 57, 477 (1974).
49. R.C. Meade and R.A. Stiglitz, Int. J. Appl. Radiat. Isot. 13, 11 (1962).
50. U. Fricke, Anal. Biochem. 63, 555 (1975).
51. S.V. Pande, Anal. Biochem. 74, 25 (1976).
52. A.A. Noujaim, et al., in Liquid Scintillation - Science and Technology (A.A. Noujaim, C. Ediss and L.I. Wiebe, Eds) Academic Press, New York, 1976, p.199.
53. B.W. Fox in Liquid Scintillation Counting, Vol. 4 (M.A. Crook and P. Johnson, Eds) Heyden, London, 1977, p.103.

54. B.W. Fox, Int. J. Appl. Radiat. Isot. 25, 209 (1974).
55. J.W. van der Laarse, Appl. Radiat. Isot. 18, 485 (1967).
56. H.S. Wagstaff and A.R. Ware, in Liquid Scintillation Counting, Vol. 4 (M.A. Crook and P. Johnson, Eds) Heyden, London, 1977, p.115.
57. D.L. Horrocks, in Liquid Scintillation - Science and Technology (A.A. Noujaim, C. Ediss and L.I. Wiebe, Eds) Academic Press, New York, 1976, p.117.
58. P. Wood, J. English, J. Chakraborty and R. Hinton, Lab. Pract. 739 (1975).
59. W. Lahmann and A. Hinzpeter, Int. J. Appl. Radiat. Isot. 25, 515 (1974).
60. S.E. Sharpe III and E.D. Bransome, Jr., in Liquid Scintillation Counting - Recent Developments (P. Stanley and B. Scoggins, Eds) Academic Press, New York, 1974, p.113.
61. S.E. Sharpe III and E.D. Bransome, Jr., Anal. Biochem. 56, 313 (1973).
62. R. Tykva, Anal. Biochem. 70, 621 (1976).
63. W.J. Gelmsa, C.L. deLigny, J.B. Luten and F.G.A. Vossenbergh, Int. J. Appl. Radiat. Isot. 26, 443 (1975).
64. H.H. Ross, in Liquid Scintillation - Science and Technology (A.A. Noujaim, C. Ediss and L.I. Wiebe, Eds) Academic Press, New York, 1976, p.79.
65. L.I. Wiebe and C. Ediss, in Liquid Scintillation - Science and Technology (A.A. Noujaim, C. Ediss and L.I. Wiebe, Eds) Academic Press, New York, 1976, p.93.
66. J.K. Basson and J. Steyn, Proc. Phys. Soc. (London) A67, 67 (1954).
67. W.J. McDowell and J.F. Weiss, in Liquid Scintillation - Science and Technology (A.A. Noujaim, C. Ediss and L.I. Wiebe, Eds) Academic Press, New York, 1976, p.17.
68. J.H. Thorngate, W.J. McDowell and D.J. Christian, Health Phys. 27, 123 (1974).
69. K. Buchtela, M. Tschurlovits and E. Unfried, Int. J. Appl. Radiat. Isot. 25, 551 (1974).
70. J.W. McKlveen, Radiat. Res. 66, 199 (1976).
71. P. Cross and G.W. McBeth, Health Phys. 30, 303 (1976).
72. J. W. McKlveen and W.R. Johnson, Health Phys. 28, 5 (1975).
73. D.L. Horrocks, Nucl. Instrum. Methods 133,293 (1976).
74. J.A.B. Gibson, in Liquid Scintillation - Science and Technology (A.A. Noujaim, C. Ediss and L.I. Wiebe, Eds) Academic Press, New York, 1976, p.153.
75. E.D. Bransome, Jr. and S.E. Sharpe III, Anal. Biochem. 49, 343 (1972).
76. J. Ashcroft, Anal. Biochem. 37, 268 (1970).
77. H. Lundqvist, K.J. Johanson and G. Jonsson, Int. J. Appl. Radiat. Isot. 27, 233 (1976).
78. J. Fillet, J. Nucl. Med. 12, 270 (1971).
79. G. Ayrey, L. Evans, D. Exley and B.J. Woodhams, in Liquid Scintillation Counting, Vol. 4 (M.A. Crook and P. Johnson, Eds) Heyden, London, 1977, p.165.
80. P. Tothill, in Liquid Scintillation Counting, Vol. 4 (M.A. Crook and P. Johnson, Eds) Heyden, London, 1977, p.155.

81. J.H. Shand and R.C. Noble, Clin. Sci. Mol. Med. 51, 511 (1976).
82. E.D. Bransome, Jr. and S.E. Sharpe III, in Liquid Scintillation - Science and Technology (A.A. Noujaim, C. Ediss and L.I. Wiebe, Eds) Academic Press, New York, 1976, p.103.
83. J.C. Meunier, Clin. Chim. Acta 66, 141 (1976).
84. A. Wagner, Int. J. Appl. Radiat. Isot. 24, 540 (1972).
85. R.J. Cousins, R.A. Wynveen, K.S. Squibb and M.P. Richards, Anal. Biochem. 65, 412 (1975).
86. S. Gutcho, J. Johnson and H. McCarter, Clin. Chem. (N.Y.) 19, 998 (1970).
87. T. Smith, Int. J. Appl. Radiat. Isot. 27, 555 (1976).
88. D.N. Abrams, S.A. Quarrie, C. Ediss and L.I. Wiebe, in Liquid Scintillation - Science and Technology (A.A. Noujaim, C. Ediss and L.I. Wiebe, Eds) Academic Press, New York, 1976, p. 167.
89. E.D. Bransome, Jr., in Liquid Scintillation - Science and Technology (A.A. Noujaim, C. Ediss and L.I. Wiebe, Eds) Academic Press, New York, 1976, p.291.

DISCUSSION

B.W. FOX: I think you will agree that the data do not compare surfactants but different compositions of different surfactants. For example, had you chosen 50% Triton X100 rather than 30% Triton X100, the curves would have been a very different shape.

E.D. BRANSOME: I agree with your point, but these curves were merely designed to show that the ESR data do not reflect the actual efficiency of the counting observed.

B.E. GORDON: From a practical viewpoint the failure of the ESCR to follow the counting efficiency in detergent systems is simply a matter of care in setting up what the standard cocktail should be, and then staying with it. All four widely varied samples (aqueous) fall into two groups - a small amount of aqueous sample and a large amount. If small (i.e. < 2 ml) it is made up to 2.0 ml by the addition of water and if large it is made up to 6.0 ml with water. To each kind, 15 ml of a commercial diluent cocktail is added, the 2 ml sample being a liquid, and the 6 ml a gel. A calibration curve using sealed toluene-carbon tetrachloride quenched set is generated vs. ESCR. In all cases except coloured samples the efficiencies of the samples are determined to $+ < 3\%$ for the 2 ml set and $+ < 5\%$ for the gelled set. Only in the rarest cases (i.e. high salt concentrations) do we fail to get the correct efficiency (as determined by standards in the same cocktails).

P.E. STANLEY: Would you agree that some of the variation seen in ESR and efficiency for emulsion systems is dependent on the way in which the sample isotope channels are set and also those for the external standard? There is considerable lack of information in the literature concerning the importance of channel settings for isotope and internal standard. These, in my opinion, are important not only when comparing various models of instruments but also units of the same model. Thus we have a problem associated with the variation between spectrometers as well as with the samples.

E.D. BRANSOME: I cannot answer your question with a reply based on thorough or extensive observations. The precise channels chosen for external channels ratios will of course be important under any circumstances. We have not ourselves tried to find out whether different channel settings will make the technique more sensitive to the loss of counting efficiency in emulsions which result from phase separations (and possibly from changes in micelle size).

It may well be that if sufficient care is taken - if the composition of samples is rigorously controlled and the emulsions are homogeneous according to the appropriate tests - ESCRs can be used to construct reliable quench correction curves. Dr Gordon's comments certainly suggest that this is the case.

I must point out that in practice most emulsion counting is carried out without prior methodological experiments being made to reassure the investigator that his samples can indeed be treated as if they were homogeneous.