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 Rezvanie[1] 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 Neary[2] have shown in addition that for each type of radioactive sample and solvent there are different optimal concentrations of scintillator.[2]

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 Budd[3] 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.[3] The remedial strategy of making up suitable color-quenched standards[4] 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 the photomultiplier tubes of the specific liquid scintillation counter being used.[5]

Decolorizing each sample will eliminate this problem of adequate standardization[4] 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, Blanusa[6] 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]CO_2. Combustion of the sample will yield colorless counting mixtures of known composition, but has not yet been shown to be a reliable methodology except for ^{3}H 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 Analytic (Nuclear Chicago) LS counters a few years ago: lesser pulse height analysis.[8-11] 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 H_2Compton 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.

Painter[5] 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 that there is no algorithm which can be used to predict efficiency.[13,14]

SAMPLES IN DIOXANE-BASED SCINTILLATION MIXTURES

Since Bray's article[15] 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-Smerdka[16] 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 ^3H_2O or ^{3}H-toluene, one of the 'tests' of sample homogeneity suggested by Bush[17] and Mueller.[17,18] 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 proposed[19] 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 quenching[21] and phase separation. 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,[26] addition of water, and then of secondary solvent (Triton X-100).[25] Flindt-Eegbak[25] 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 ^{3}H 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] Mueller[28] 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 days[31] 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 investigators[32] 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[17] has observed previously that quench correction plots of the
two ratios vs counting efficiency diverged when heterogeneous samples were examined.

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

The constants $\underline{a}$ and $\underline{b}$ 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 SCR = $\underline{a}$ (log ESCR + $\underline{b}$). With samples
which are not adsorbed, $\underline{A}$ = 0.00; with maximal adsorption $\underline{A}$ 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 encountered[38] 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
carrier[32] 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 latter has been suggested as a strategy for eliminating ^{32}P orthophosphate
adsorption[38] 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.[42] Recently Long et al.[43,44] 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 in incomplete
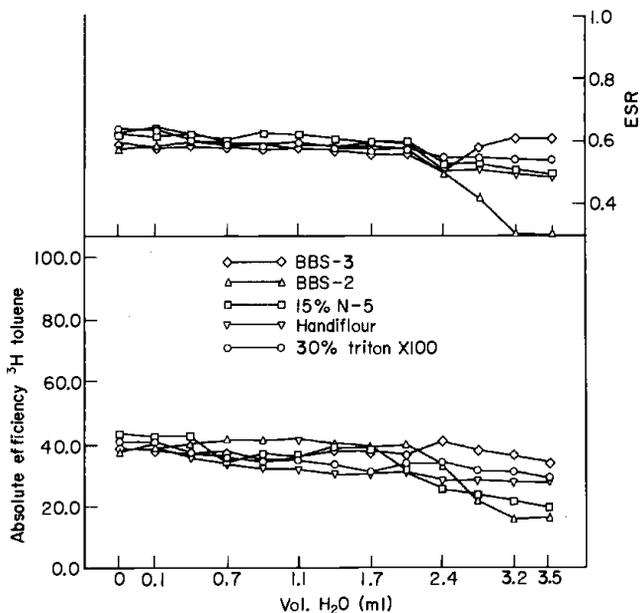the sample will be counted in two phases.

Fig. 1. The effect of added water and surfactants on the efficiency of counting
^{3}H-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 μl
samples were added to a 10 ml counting solution consisting of toluene and 5.5 g l^{-1}
Packard Instrument Co. Permablend (91% PPO, 9% POPOP) (except when Handifluor-
Mallinckrodt^{TM} 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$_2$O). 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-100^{TM} from the Rohm and Haas
Co., N-5 Handifluor^{TM} from Mallinckrodt Inc. N-5 is a mixture of 94% nonylphenoxy
ethanol and 6% sodiumdihexylsulfosuccinate 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 Solv^{TM}, 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 Duquesne[46] 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 βs; 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
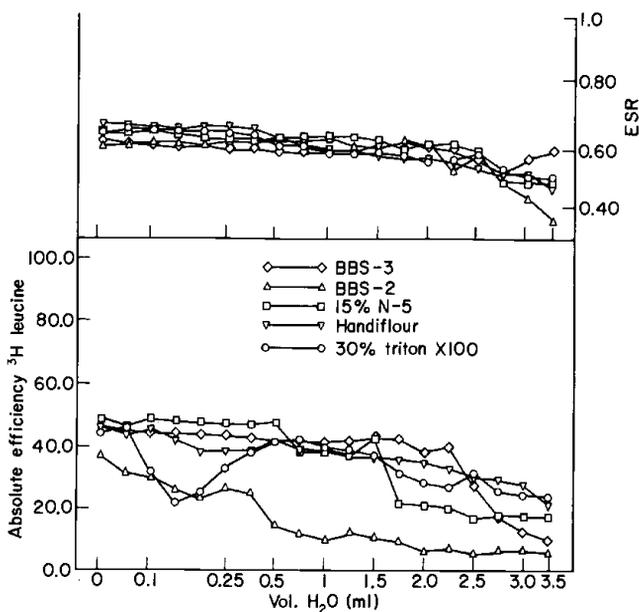the vial wall.[47,48]

Fig. 2. The effect of added water and surfactants on the efficiency of counting 4,5-^{3}H-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 βs 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 ago and still receives considerable attention (despite some variability[50,51] between lots as to capacity for water and potential for chemiluminescence). Most commercial surfactants are, however, combinations of non-ionic surfactants and lesser amounts of anionic surfactants.[52-59] There are a number of excellent reviews, including those by Fox and by Mueller[18] 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[60,61] employed for the preparation of practical samples.

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 '2π' rather than '4π' 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 (^{3}H and ^{14}C-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
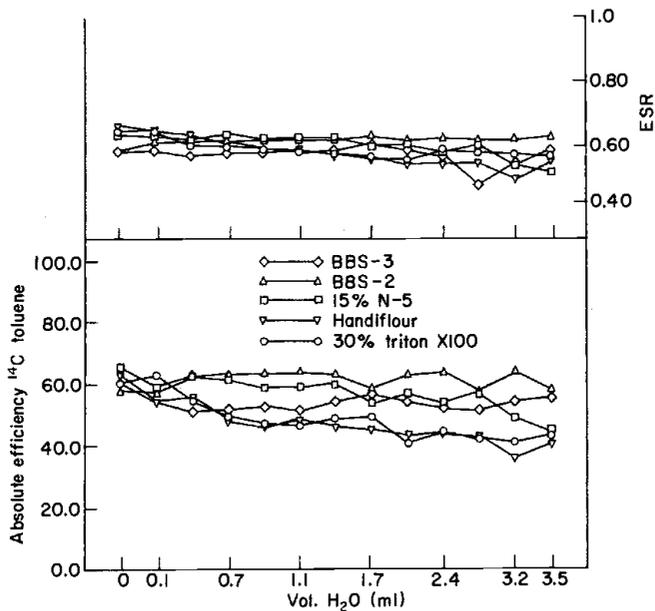
Fig. 3. The effect of added water and surfactants on the efficiency of counting ^{14}C-toluene (New England Nuclear). 20 600 dpm were added to each vial. See the legend to Fig. 1 for further details.
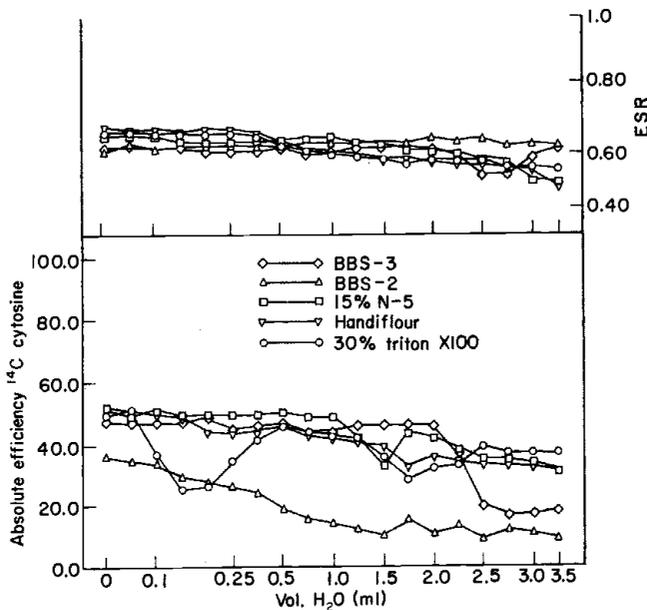

Fig. 4. The effect of added water and surfactants on the efficiency of counting 2-^{14}C-cytosine (New England Nuclear, 5 Ci mol^{-1}). 36 666 dpm were added to each vial. See the legend to Fig. 1 for further details.

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 Ware[56] 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 βs 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.[7] 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}H$_2$O and ^{3}H-toluene) and comparing efficiencies as suggested by
Mueller[18] 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.[9]

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 βs
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 recenty 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.[54]

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 αs is short,
and the fluorescence quantum yield is much less than for βs — 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, α 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 αs.

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 αs 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 αs with
energy differences of 200-300 keV, with backgrounds of less than 1 cpm. Special
circuitry for pulse timing has permitted pulse-shape discrimination[68-70]
lowering backgrounds to 0.01 cpm. When pulse-shape discrimination is used to
eliminate βs and γs 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 αs is approximately 0.03 cpm (0.02 pCi).
Cross and McBeth[71] 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, emulsions and gels have all been used: a
recent critical review by McKlveen[72] 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 α 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 βs (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[73] and for ^{55}Fe.[74] Quench-correction procedures are similar to those used
for βs. 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
and ^{131}I for double-label counting at creditable efficiencies for both isotopes.[75]
It has therefore been difficult to understand why doping the scintillation mixture
with heavy metals (e.g. tetrabutylin which is miscible with organic scintillation
solvents[76]) 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 Instagel™ system be used to
achieve separation between ^{3}H and ^{125}I. With the usual scintillation mixtures,
^{3}H 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, ^{115m}Cd	85
	^{203}Hg	
	^{57}Co	86
	^{137}Cs, ^{241}Am, ^{99m}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,[83] 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.[82] Iron-55 may be counted alone[78] or with ^{59}Fe in emulsion systems (e.g. Triton X-100-toluene[84] or Instagel^{TM}[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 recommended[84-86] 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.[89] 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 Kisieleski 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).

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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 $\pm$ < 3% for the 2 ml set and $\pm$ <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.