

ROUND-TABLE ON 'HOMOGENEOUS COUNTING SYSTEMS'

JACK D. DAVIDSON*

Columbia University, New York, New York

BOTTLES

THREE years ago, the few people engaged in this type of counting were using rather large 85 ml weighing bottles as their sample containers. In our laboratory we tested various smaller and cheaper containers and finally adopted 5 dram medicine vials as our standard containers. Table I shows the counting

TABLE I

	Volume ml	C ¹⁴ Sample counts/min	Blank counts/min
85 ml Weighing bottle*	50	6590	115
	40	6570	86
	30	6450	78
	20	6250	55
	10	5970	36
5 Dram vial**	15	6190	53
	10	6400	46
	5	6480	32

Comparative counts on two types of sample container with and without a fixed amount of C¹⁴ at various volumes of toluene containing 0.3% PPO.

* Kimble #15145, 50 mm diameter × 60 mm high.

** Kimble #7600, K-5, Opticlear, 26 mm diameter.

rates of replicate samples made up in various volumes of the same toluene-PPO solvent in the weighing bottle and in the 5 dram vial. It is apparent that these various counts do not differ significantly when corrected for the blank counting rates but that there is an almost fourfold difference in the blank rates accompanying the tenfold span in volumes. This clearly shows the advantage to be gained by using the smallest volume of solvent consistent with good optical performance in a given counter. In the Tri-Carb instrument we believe that this is 5 ml and we exceed this only when greater dilution is necessary to carry the sample. The small bottles are also economical of storage space.

Other considerations regarding the sample bottles are leakage, uniformity

* Present address: *National Institutes of Health, Bethesda, Maryland.*

and clarity of glass, and low inherent radioactivity content. The last is chiefly due to K^{40} inherent in all glass; only quartz is free of it. The Wheaton Co. is currently manufacturing a Crystal Lite vial of No-Sol-Vit glass which is reportedly low in potassium and in our laboratory has indeed shown 30% fewer blank counts than grossly identical vials of ordinary glass. Dr. Agranoff will have more to say on this topic tomorrow. Over and above the composition of the glass it is obvious that it is advantageous to use as small a mass of glass as possible. This puts a premium on small counting bottles with thin walls.

The cap for bottles should be leak-proof and insoluble in the solvents. Polyethylene fulfills these requirements. The stopper type caps tend to pop out under the pressure of the volatile solvents so that the snap-on caps are preferable. If molded screw caps are used, attention must be paid to their liners. Commonly these are made of paper coated with a water-proof lacquer that is generally soluble in aromatic solvents and to be avoided. Tin foil is satisfactory for most purposes but may be attacked by some sample components. Polyethylene liners should be ideal but in our experience a large percentage of such commercially supplied vials leaked due to the incompressibility of the polyethylene liners.

Lastly on the subject of bottles, mention must be made of the phenomenon of photoactivation of glass. All types of glass fluoresce to some degree and fluorescence, or more properly 'phosphorescence', can be excited by exposure to light. The most effective wavelengths are in the ultraviolet and blue end of the visible. In practice a 5 dram counting vial exposed to a 254 m μ Mileralite for 1 min will record 20,000–100,000 c/m when counted under conditions appropriate for C^{14} , even without any solvent or scintillation solute. The decay of this phosphorescence is complex and appears to have at least a fast component with a half-time of 1–3 min and a slow component with a half-time of an hour or two. This phenomenon constitutes a practical hazard in sample counting since the difference between low *bone fide* C^{14} activity and some residual, slowly-decaying photo-activation is not readily apparent. Such photo-activation can arise from inadvertent exposure of the sample bottles to window daylight or strong fluorescent laboratory illumination. It can be completely avoided by restricting illumination to the incandescent type, of moderate intensity. The presence of such spurious 'activity' can be determined by its decay in contrast to the stability of C^{14} , or by observing the counting rates in single channels. The energy of the glass phosphorescence is so low that each event commonly excites only the most proximate of the two phototubes and it is only their high frequency with consequent significant chance coincidence that yields 'counts'. Thus, the single channel rates in the presence of phosphorescent glass are several times their basal value rather than merely increased by the observed coincidence counting rate.

SOLVENTS

Turning to the solvents, these have already been discussed by Dr. Hayes and by way of confirming him in part, Table 2 shows our findings for a group of solvents. These were tested for their efficiency in yielding counts from a standardized, dissolved C^{14} sample with an arbitrary 0.3% PPO as scintillator solute. The amplification was increased, however, by increasing the high voltage as much as necessary or practicable to give the highest counting rate.

TABLE 2
Counting Efficiencies of Various Solvents with 0.3% Diphenyloxazole

Compound	Freezes*	H.V.†	Efficiency‡
	°C		%
Toluene	-95	6	100
Methoxybenzene (anisole)	-37	7	100
Xylene (reagent, mixed isomers)	< -20	7	97
1,3-Dimethoxybenzene	-52	8	81
<i>n</i> -Heptane	-90	9	70
1,4-Dioxane	+12	9	70
1,2-Dimethoxyethane (ethylene glycol dimethyl ether)	-71	9	60
Benzyl alcohol	-15	9	38
Diethyleneglycol diethyl ether (diethyl carbitol)	-44	10	32
Acetone	-94	10	12
Tetrahydropyran	-81	10	6
Ethyl ether	-116	10	4
1,1-Diethoxyethane	-100	10	3
Tetrahydrofuran	-65	10	2
1,3-Dioxolane	-95	10	0
Ethyl alcohol	-114	10	0
Diethylene glycol monoethyl ether	-76	10	0
Ethylene glycol monomethyl ether	-85	10	0
Diethylene glycol	-8	10	0
Ethylene glycol	-13	10	0
2,5-Diethoxytetrahydrofuran	-27	10	0
<i>n,n</i> -Dimethylformamide	-61	10	0
Diethyl amine	-49	10	0
<i>n</i> -Methyl morpholine	-66	10	0
2-Ethylhexanoic acid	-117	10	0
Tri- <i>n</i> -Butyl phosphate	< -80	10	0

* Approximate freezing point.

† Relative high voltage (in steps of 70 V) on photomultipliers necessary to give highest counting rate. 10 represents 1250 V which was the maximum practicable at the 2°C counter temperature.

‡ Efficiency, on basis of counts/min, relative to toluene equaling 100%.

Our data agree with Dr. Hayes' findings that the alkyl benzenes are the only highly efficient solvents. Dioxane is very useful and a few other di- and triethers may have specific applications. The specificity of the phenomenon is shown by the activity of dioxane in contrast with the inertness of its homologue, dioxolane; similarly 1,2-dimethoxyethane is active while the isomeric

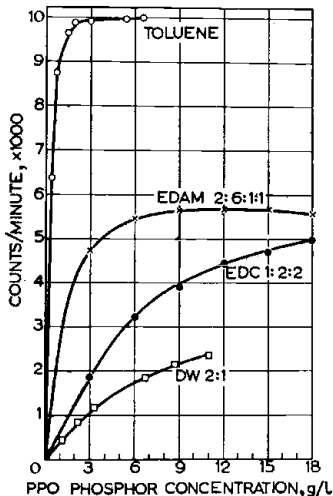


Fig. 1. The influence of diphenyloxazole (PPO) concentration upon counting rate in different solvents. A=anisole, C=diethyl carbitol, D=dioxane, E=ethylene glycol, M=1,2-dimethoxyethane and W=water. The numbers following solvent symbols refer to their relative proportions by volume.

1,1-dimethoxyethane of Hayes series and 1,1-diethoxyethane (diethylacetal) here were inactive. Activity of 1,2-dimethoxyethane persists upon lengthening the molecule as in diethylene glycol diethyl ether but is destroyed if the ether has a free hydroxyl group on it as in the glycol monoethers (Table 2). general the prospects for finding active solvents of a polar type look very dim.

This has led various investigators quite successfully to attack the problem of devising readily prepared derivatives of various classes of organic compounds that are soluble in toluene. Thus, you will hear from Dr. Radin that a diverse group of acidic samples can form toluene-soluble salts with the quaternary amine, Hyamine 10-X. Dr. Steinberg will report tomorrow on the same Hyamine use to dissolve proteins. In a somewhat analogous fashion, most metallic cations and a number of the more basic organic compounds including adenine and guanine become soluble in toluene as salts of 2-ethylhexanoic acid. In a more complicated fashion, Steele has counted glucose in toluene by converting it to the osatriazole and complexing this with boric acid in ethanol and xylene.

For certain applications it is useful to employ the ethers as solvents despite

their lower efficiency. This was done in our laboratory to permit counting various purines. Dioxane appears to be the best ether but presents the handicap of a $+12^{\circ}\text{C}$ freezing point. Since purines are appreciably soluble only in water or ethylene glycol, we explored various combinations of these two sample-carrying solvents with several ethers to find the most efficient mixture with an adequately low freezing point and which would not separate into two phases at a temperature of about 0°C . Two things soon became apparent. The first was that very little aromatic hydrocarbon solvent could be incorporated into such aliphatic ether mixtures and that for this purpose anisole (monomethoxybenzene) was distinctly more miscible than toluene or xylene, with 1,3-dimethoxybenzene no better. Secondly, in such mixtures the traditional 3 or 4 g of PPO per l. was a very suboptimal concentration of scintillation solute. Figure 1 shows the effect of 'phosphor' concentration upon counting rate for a few of these mixtures in comparison with toluene. All of the points plotted represent counts on samples containing the same amount of C^{14} , recorded for a 10–100 V window but employing whatever high voltage gave the maximum counting rate. Of these ether mixtures, only the EDAM ('glycol-polyether') shows a plateau with respect to PPO concentration. The curve for water-polyether is identical with that shown for EDAM. In the case of glycol-polyether it is possible to fortify the system further with 0.04% POPOP and 60 g of naphthalene per liter so that it reaches the same 70% absolute counting efficiency as toluene but requires 70 V more on the high voltage. The water-polyether solvent is not miscible with any significant amount of naphthalene.

The efficiency of water-polyether for tritium is about 6% where the tritium water can constitute 20% of the solvent volume. This appears to offer greater sensitivity for counting tritium water than the other mixtures of water and alcohol in toluene or dioxane that have been described.

In practice it is convenient to make large batches of the polyether solvents containing the phosphors and naphthalene. The ethers all have to be rigorously purified since this has been found to increase their efficiency two to four times over that of the best commercially available grades.

THE COUNTING

Once the sample bottle and scintillation solvent system have been selected there remains the practical point of selection of optimal instrument settings for counting. Figure 2 shows the frequency distribution of pulse heights given by a C^{14} sample at various levels of high voltage on the photomultipliers. The inset bar diagram gives the integrated frequency from 10 to 100 V pulse heights for each of the curves. It is apparent that this sample yielded a maximum counting rate at high voltage tap 8. This voltage, however, may not be optimal for the greatest stability in counting nor for the most advantageous ratio of sample counts to background counts.

As plotted for this example, the background counting rate appears negligible above a pulse height of 10. This 10 would therefore represent a good value for the lower discriminator. If, however, similar data were collected for a sample only 1% as active as this one and were plotted with the same background on an expanded scale, it would be more apparent that the background

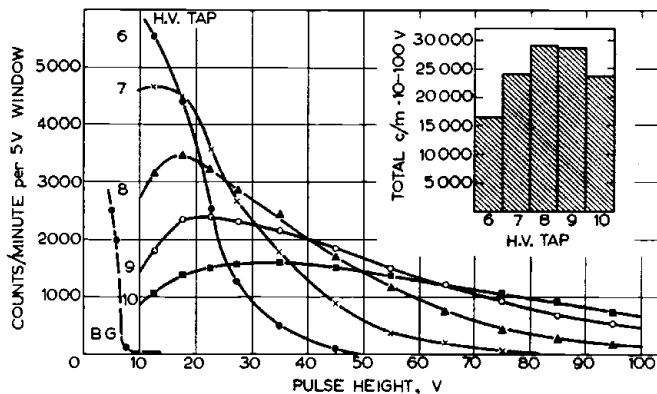


Fig. 2. Frequency distribution of C^{14} pulse heights in glycol-polyether (EDAM 2 : 1 : 1 : 1) solvent at various levels of photomultiplier voltage. The counting rates of the 44,400 disintegrations/min sample were recorded through successive 5 V windows throughout the 0-100 V range of the discriminators for voltages of 1040-1320 in 70 V steps designated taps 6-10 respectively. The dotted line marked B.G. represents the counts from a corresponding blank sample at h.v. tap 8.

is not insignificant above 10 V and that every 10 V increment in pulse height adds a few counts to the background. This means that a better ratio of sample rate to background rate might be gained by employing a lower value of high voltage, e.g. tap 7, and concomitantly reducing the upper discriminator from 100 to 70.

For maximum stability of the counting, the discriminators and the high voltage should be balanced so that there is a small reservoir of pulses above the value of the upper discriminator and that the counting rates in the vicinities of the two discriminators bear the same ratio to one another as the discriminator voltages bear to each other. For example, under such conditions a 5% attenuation of all pulses caused by optical absorption, quenching, amplifier drift or anything else, will not alter the recorded counting rate. This is due to the fact that the shift of the total pulse spectrum on the discriminator window causes, over a small range, the same number of counts to enter the window over the upper discriminator as are lost from the window at the lower discriminator. This type of operation is observed with h.v. tap 9 and a window of 10-50 for the polyether solvent shown in Fig. 2 that we have been considering. Figure 3 illustrates the effect of sample quenching on this system. Here replicate samples of a fixed amount of C^{14} were prepared

containing zero, 1 mg and 2 mg of adenine per 5 ml polyether solvent. The quenching effect with increasing adenine concentration is clearly seen in the decline of the counting rate as recorded for h.v. tap 8 through a 10–100V window, the conditions for greatest counting efficiency. This is less pronounced at tap 9 where there are more pulses over 100 V to serve to com-

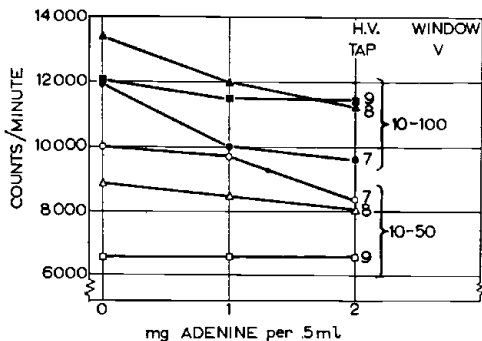


Fig. 3. Quenching effect of adenine upon the counting rate of a fixed amount of C^{14} in glycol-polyether solvent under various conditions of photomultiplier voltage and discriminator window size.

pensate for quenching, but the quenching effect is completely obviated only when the further change is made to a small (10–50 V) window.

This has brought up the last topic that I have time to mention, namely 'quenching'. In internal sample homogeneous counting every sample substance is a potential quencher. Doctors Kerr, Hayes and Ott have recently published their findings on compounds that quench and what types of compounds can be expected to do so. When it occurs it can sometimes be overcome for practical purposes as in the example with adenine that was discussed above. If concentrations are kept very low it may not be a problem and this is one reason for going to large sample volumes. The change from 5 ml to 50 ml dilutes a quencher by a factor of 10 without any loss of inherent counting efficiency.

The bigger problem is in recognizing that quenching is occurring in a given sample. There are three convenient ways to do this. One is by dilution. Either count two different dilutions of the sample or count it, for example, at 5 ml volume and then recount it after the addition of 10 ml more scintillation solvent. This threefold dilution will unmask all but total quenching. The second method affords more information and involves the use of an internal standard. Here the sample is counted, then 'spiked' with a known number of additional counts contained in a relatively insignificant volume of solvent, and recounted. Any shortage in the counting rate increment indicates quenching and quantitates it proportionally to the known value of the 'spike'. The third method is one that we have used routinely because of its

simplicity. In Fig. 2 the pulse height frequency distribution curves are skewed to the left. This makes it possible to detect any shift in this spectrum, such as quenching causes, by the simple expedient of simultaneously counting two different windows and observing the ratio of the two counts. To facilitate doing this ratio by inspection and to minimize the statistical counting error, we experimentally determine a setting for a middle discriminator which in the absence of quenching divides the counting rate of the larger window, e.g. 10–100, in half. This setting is specific for the particular solvent-phosphor-isotope system. Thus, for our instrument, the polyether solvent, and C^{14} , a window of 10–32 gives just half the counting rate of the 10–100 V window. When quenching occurs, more counts appear in the 10–32 window. With two scalers it is possible to do this quenching test in a single counting and without additional manipulation of the sample. This test becomes positive, however, even when the quenching is compensated by the balanced operation discussed earlier. It also becomes positive at very low counting rates, unless calculated on the basis of net counts, due to the difference in the pulse distribution curves for the background as compared with C^{14} .

These remarks have only touched on a few arbitrarily selected aspects of this subject but I hope they may stimulate further questions and discussions.