

Chapter 22

The Performance of Liquid Scintillation Counters Using Large and Small Vials

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INTRODUCTION

With the steady increase in the cost of chemicals and supplies used in liquid scintillation counting, the idea of counting samples in small vials with small volumes of scintillation cocktail is becoming more attractive. Savings are apparent in a number of areas: the smaller vial is less expensive and the volume of scintillation solution used in small vials is typically one-third that used in standard vials. This is a clear saving of 67% in the cost of the scintillation cocktail. Another consideration that is frequently overlooked is radioactive waste. With one-third of the volume of liquid in each sample the cost of radioactive waste management and disposal is greatly reduced. These three items alone add up to a significant reduction in the cost per sample and can readily be calculated. Other considerations such as savings in space both for sample storage and for supplies also help reduce costs. One possible item of increased expenditure should be mentioned. Systems designed for large vials require adapters to accommodate the small vials. Since these adapters accompany the vial into the counting chamber and become part of the optical system, contamination and wear require that they be replaced periodically to ensure optimum performance of the system.

After cost, the next consideration should be performance. How do measurements made using small vials, with or without adapters, compare with similar measurements using large vials? The study reported here looks at several important aspects of this question, and presents data obtained using both large and small vials. The intent is to aid in the choice of the vial size best suited to a particular situation.

EXPERIMENTAL DETAILS

Vials suitable for liquid scintillation counting are available in a number of sizes and materials. Glass was chosen in preference to the various plastics because glass vials could be flame-sealed to form samples that were stable over a long period of time. The Packard 20 ml glass ampoule was chosen as typical of the standard or large liquid scintillation vial. After flame-sealing the glass, a white plastic cap was cemented on top. The Packard 7 ml Pico-ampoule was chosen as the small vial. Figure 1 shows the relative size of the two types of vial and the level of liquid in each.

The scintillation solution used for both large and small samples consisted of 4 g of PPO* and 0.08 g of dimethylPOPOP[†] dissolved in 1 l of toluene. Activity was added to each vial as a carefully measured 2 ml aliquot from a master pool of scintillation solution containing either tritiated toluene or ¹⁴C-toluene. Additional non-radioactive scintillation solution was added to make up a total volume of 5 ml in the small vials and 15 ml in the large vials. The master pools of activity were assayed by comparison with NBS standards. The activity at the time of the study was approximately 200 000 dpm for all ³H samples and 69 600 dpm for all ¹⁴C samples. For ³H, the measurement

* PPO = 2,5-diphenyloxazole

† dimethylPOPOP = 1,4-bis-2-(4-methyl-5 phenyloxazoly1)-benzene

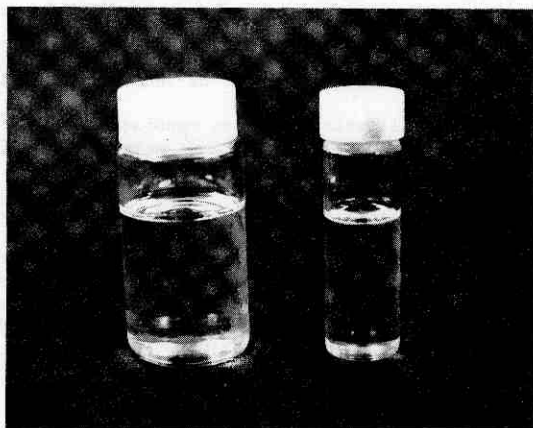


Fig. 1 Large and small vials used in study.

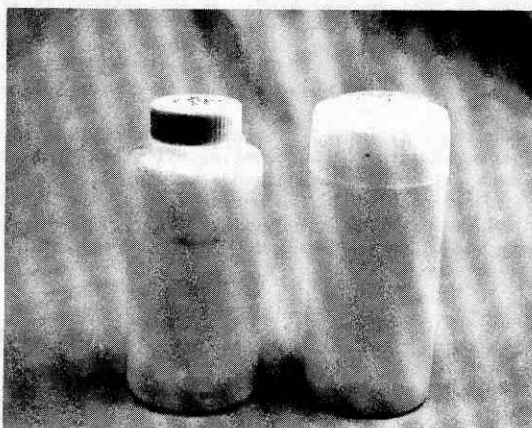


Fig. 2 Adapters used to hold small vials in systems designed for large vials.

was corrected for decay to the day of each measurement. By making both large and small samples from a common pool of activity and scintillation cocktail, direct comparisons can be made independent of the absolute assay of the activity.

Sets of quenched samples were made by adding to each sample different amounts of nitromethane, a chemical quencher, or the red dye, Sudan III, an optical quencher. Some oxygen quenching was present in all samples since none was eliminated dissolved air. Independent tests, however, showed that nitromethane-quenched samples prepared in air followed the same efficiency vs. ESR relation as argon-purged samples.

A number of different liquid scintillation counters were used in the study. The same samples were counted on each system to a preset time of one minute. The spectrometer settings were either those preset by the manufacturer, or the settings recommended by the manufacturer for general purpose counting of single-labelled ^3H or ^{14}C .

In order to count the small vials on systems designed for large vials, adapters were used. These serve the purpose of holding the small vial in the sample changing mechanism and keeping the vial centered and upright when it is in the optical chamber in front of the photomultiplier tubes. Figure 2 shows two types of adapters used in the study. Both are commercially available and both were made by modifying a

conventional polyethylene vial. After starting the tests with the shorter adapter, which allows easy removal of the vials, it was found that some instruments required a special adapter to trigger the empty position sensor. Thus, the taller adapters were required to accommodate these instruments. Both adapters operate in the same way except that the taller adapter covers more of the vial cap.

Various models of liquid scintillation counters manufactured by Beckman, Intertechnique, Packard and Searle were used in this study. Eight were designed for standard vials and one, the Prias LSC, was designed specifically for small vials. Since the intent of this paper is to compare the performance of large and small vials, the different counting systems are used to determine whether the results observed on one instrument are specific to that model, or are generally true for a number of systems. It is not our intention to discuss here the performance of specific instruments. Anonymous examples will be used solely to illustrate a point. In most cases, only the average performance is presented with an indication of spread around the average. The relatively close agreement in the results from the different instruments tested suggests that the conclusions presented here are most likely common to all commercial liquid scintillation instruments.

RESULTS

Percent counting efficiency is defined as 100 times the ratio of cpm to dpm. In a LS counter this value depends on a number of parameters including radionuclide, scintillation solution, type and degree of quenching, volume, temperature and spectrometer settings, as well as vial type. In the present study, samples in both large and small vials were prepared from the same materials, and counted on the same instruments under the same conditions. Thus, as many conditions as possible have been held constant. The differences observed between large and small vials in adapters counted on the same instrument will be attributed to the vial size and adapters.

The difference in counting efficiency is obtained by the ratio of counting efficiency for the small vial in an adapter to the counting efficiency for the large vial. This comparison is made using only unquenched samples. For ^3H , the average ratio of all systems tested is 0.968 ± 0.017 . The uncertainty is the standard deviation of the values obtained from the different instruments. This ratio indicates that counting a relatively unquenched ^3H specimen using 5 ml of scintillation cocktail in a small vial with an adapter gives approximately 3.2% less counting efficiency than

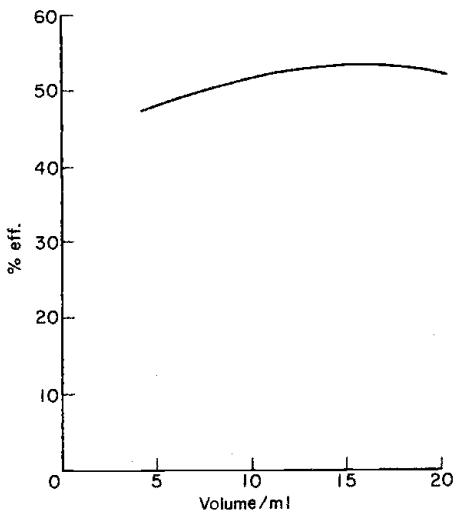


Fig. 3

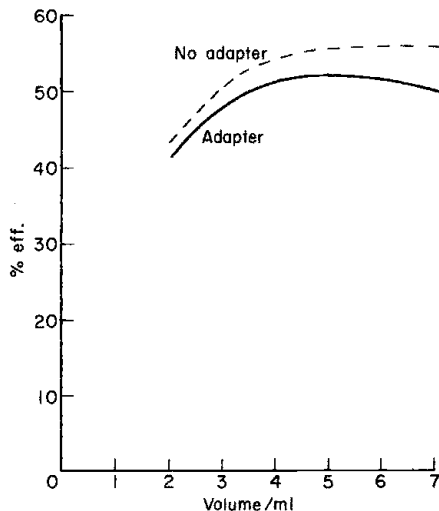


Fig. 4

Fig. 3 An example of ^3H -counting efficiency vs. volume in large vials.

Fig. 4 An example of ^3H -counting efficiency vs. volume in small vials. — with adapters; ---systems not requiring adapters.

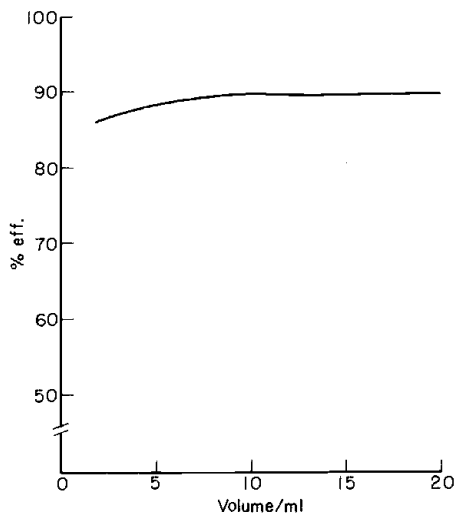


Fig. 5

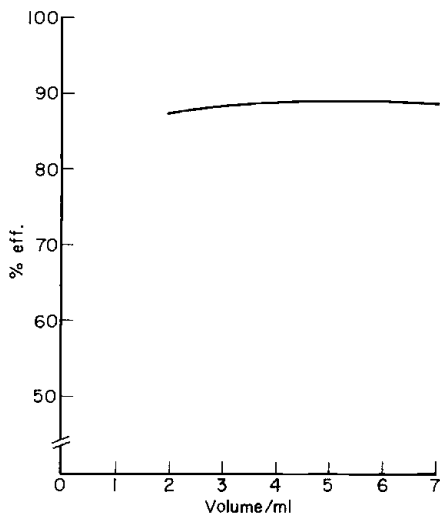


Fig. 6

Fig. 5 An example of ^{14}C -counting efficiency vs. volume in large vials.

Fig. 6 An example of ^{14}C -counting efficiency vs. volume in small vials with adapters.

counting the same specimen with 15 ml of cocktail in a large vial. Most likely, the major portion of this difference is due to the adapter since the system designed to count small vials without adapters gives an efficiency higher than the average large vial counting efficiency.

For ^{14}C , the average ratio of small vial efficiency to large vial efficiency is 0.992 ± 0.007 . Thus, ^{14}C samples can be counted in small vials using adapters with nearly the same counting efficiency as in large vials.

The variation of counting efficiency with sample volume is another important consideration in liquid scintillation counting. An interesting finding of this study is that most systems show a similar pattern of variation in counting efficiency with sample volume in a particular vial size. Figure 3 shows a typical pattern for ^3H in large vials. Here the efficiency is relatively constant from 20 ml to 10 ml, but decreases steadily below 10 ml.

The solid curve in Fig. 4 shows the volume dependence obtained counting ^3H in small vials with adapters on the same counter used for Fig. 3. The curve is typical of the large vial systems studied. Here the working range extends from approximately 3-7 ml. Below 3 ml efficiency drops quite steadily. These two figures indicate that both vial sizes are relatively volume independent from just under the full volume to about half-volume. Since dispensing errors are usually a percentage of the pipette volume, variations incurred during sample preparation should be roughly the same percentage of full volume for the two vial sizes. If vials are always filled more than halfway, variations in efficiency due to dispensing errors should be comparable for the two sizes. The main consideration, therefore, is the original specimen volume. Obviously, the total volume cannot exceed the vial capacity. However, a large number of tests start with only small amounts of specimen and the larger capacity is not necessary. The dashed curve in Fig. 4 indicates the type of ^3H -counting efficiency vs. volume obtained when the small vials were counted on the system not requiring adapters.

Figure 5 shows the small variation of ^{14}C -counting efficiency with volume observed on most instruments. The efficiency is nearly constant from 20 ml down to 5 ml. Below this level, the efficiency decreases slightly.

As seen in Fig. 6, small vials in adapters show a nearly constant response from full

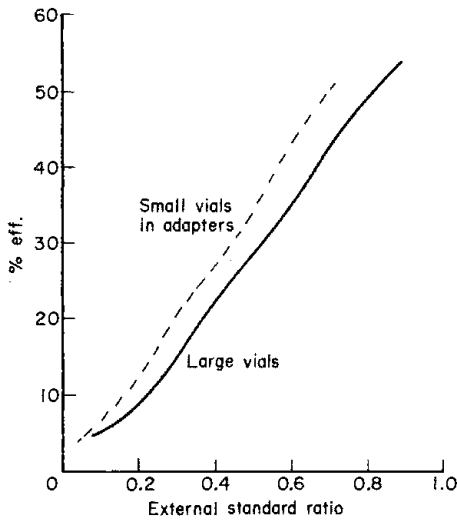


Fig. 7

Fig. 7 An example of ^3H -counting efficiency vs. external standard ratio. — large vials; --- small vials with adapters. Quenching agent: nitromethane.

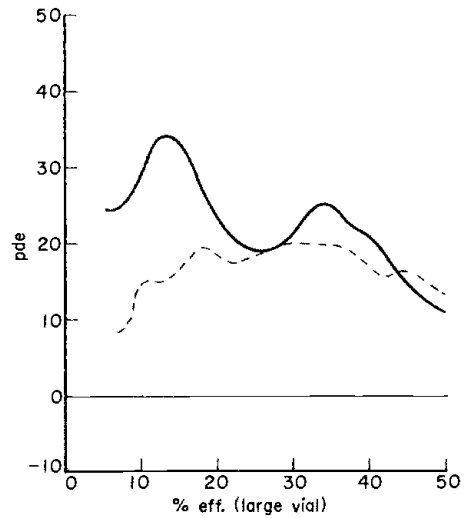


Fig. 8

Fig. 8 Examples of percent difference in ^3H -counting efficiency between large vials and small vials with adapters vs. efficiency in large vials. — same data as used in Fig. 7.

volume down to 2 ml, the smallest volume tested. The small vials were counted in the system designed for small vials and produced a similar curve.

To measure the performance of a liquid scintillation counter using unquenched standards only is unrealistic. Actual samples consist of scintillation cocktail plus specimen. The presence of the specimen affects the production of light in the solution with the result that the counting efficiency is reduced by an unknown amount. This quenching can vary significantly from sample to sample, and is a major concern in liquid scintillation counting. A number of different schemes have been developed to measure and compensate for this effect. In the present study, all of the instruments tested use a quench indicating parameter based on some form of external standard measurement: external standard ratio, external standard pulse or H number. In practice, a relationship is established between sample counting efficiency and the quench indicating parameter by measuring a set of standards of known activity and different amounts of an appropriate quenching agent. This relationship is then used to convert the measured cpm to sample dpm using the quench indicating parameter. The success of the various schemes depends in part on the procedure for establishing and using this relationship, and on the nature of the quench indicating parameter. To avoid unnecessary complications associated with the method of establishing efficiency correlation, the comparison of results from different systems will not be based on computed dpm. Instead, the measured cpm and efficiency will be used directly. Figure 7 contains examples of the observed relation between ^3H efficiency and external standard ratio for nitromethane-quenched samples. The solid curve is for large vials and the dashed curve is for small vials in adapters. All instruments tested exhibit a distinct curve for each vial size. It is very apparent that using standards in large vials to correct the measurements of samples in small vials would lead to considerable errors in computed dpm. The same is true for standards in small vials used with samples in large vials. Both standard and sample must be in the same size vial.

To study this further, the various counters were compared to see if there is a common trend. Graphs which use the quench indicating parameter along one axis (see, for example, Fig. 7) are not suited for comparing systems using different quench indicating parameters. Each different quench indicating parameter expands or contracts different quenching regions, and there are no simple relations converting one to the other. A graph is needed that does not use the quench indicating parameter explicitly. One

approach is a graph on which the abscissa is the percent counting efficiency in large vials, and the ordinate is the percent difference in counting efficiency (PDE) between large and small vials relative to the counting efficiency in large vials. Expressed mathematically,

$$PDE = \frac{[E_S(QIP) - E_L(QIP)]}{E_L(QIP)} \times 100$$

where E_S and E_L represent the small vial in adapter counting efficiency and large vial counting efficiency, respectively, at the same value of the quench indicating parameter (QIP). Of course, both large and small vials in adapters must be counted under identical conditions to avoid complications.

The solid curve in Fig. 8 is an example of a PDE vs. efficiency relationship for 3H comparing large vials and small vials in adapters. It is based on the data shown in Fig. 7. Note that the values of quench indicating parameter needed to generate the curve are not shown explicitly. Thus, curves for systems using different quench indicating parameters can be compared directly using this type of graph.

Another useful feature of the PDE vs. efficiency method comes from the relation between efficiency, cpm and dpm. The equation used to compute dpm is

$$dpm = \frac{cpm}{\text{Efficiency}}$$

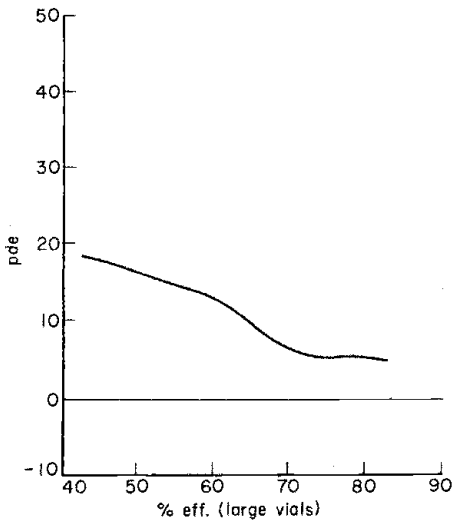


Fig. 9

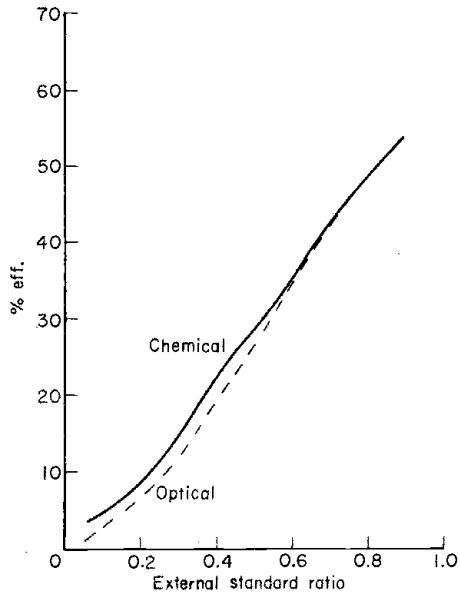


Fig. 10

Fig. 9 An example of percent difference in ^{14}C -counting efficiency between large vials and small vials with adapters vs. counting efficiency in large vials.

Fig. 10 An example of 3H -counting efficiency vs. external standard ratio. — chemical quenching; --- optical quenching.

For a constant cpm, the percent change in dpm is equal to the percent change in efficiency. Thus, the value of PDE is also the percent difference in dpm due to the difference in efficiency. The solid curve in Fig. 8 can be used as an indication of the error expected for ^3H if large vial standards are used for computation of dpm of samples in small vials with adapters.

Having devised a method of comparing instruments, it was found that there was little recognizable similarity in the PDE vs. efficiency curves for different instruments. For most, the magnitude of PDE decreased with increasing counting efficiency, but the shape of the curve was markedly different for each instrument. The dashed curve in Fig. 8 is an example of another instrument for which the PDE between large and small vials varied in a different manner.

Instrument performance with ^{14}C can be analyzed in the same way. Figure 9 shows a PDE vs. efficiency curve for ^{14}C obtained on the same instrument which produced the solid curve in Fig. 8. In this case, the PDE for ^{14}C exhibits a smaller difference between large and small vials than the PDE for ^3H . The same was true for all instruments tested. However, it is still necessary to count both standards and samples in the same size vial to ensure reliable results in computing dpm.

Another aspect of the comparison which must be considered is the difference between chemical and optical or color quenching. Figure 10 shows two efficiency vs. ESR curves obtained by counting large vials containing different amounts of chemical quencher (solid curve), or red dye (dashed curve). Separate efficiency curves for chemical and optical quenching are observed on all instruments tested. It is convenient to use the same PDE method to compare chemical and optical quenching as used previously to compare large and small vials. The abscissa is the chemically quenched counting efficiency. The PDE used as the ordinate is the percent difference in efficiency between chemical and optical quenching relative to the chemically quenched counting efficiency. The mathematical expression is

$$\text{PDE} = \frac{[E_o(\text{QIP}) - E_c(\text{QIP})]}{E_c(\text{QIP})} \times 100$$

where E_o and E_c are the optically quenched counting efficiency and chemically quenched counting efficiency, respectively, at the same value of QIP.

The solid curve in Fig. 11 shows a PDE vs. efficiency plot of the same data as used in Fig. 10. The true magnitude of the difference at low efficiency becomes apparent.

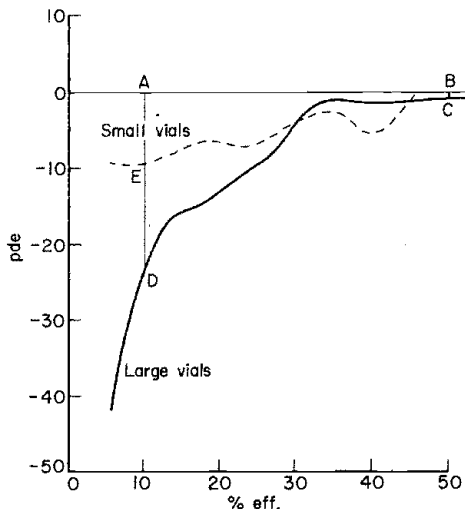


Fig. 11 Examples of percent difference in ^3H -counting efficiency between chemical and optical quenching vs. efficiency. — large vials; --- small vials with adapters.

All systems tested show a similar trend of increasing difference between chemical and optical quenching with decreasing counting efficiency. The PDE values in Fig. 11 also represent the percent difference in computed ^3H dpm if chemically quenched standards are used for computing dpm of colored samples.

The magnitude of the difference varies considerably from instrument to instrument. Apparently the chemical/optical differences of an instrument are very specific to the instrument. This is to be expected since they are known to depend on such things as photomultiplier response, method of processing the signal pulses, window settings, and the nature of the quench indicating parameter. In fact, different instruments of the same model show large differences. While comparison of systems might not be possible, the difference between large and small vials on the same instrument is meaningful.

The dashed curve in Fig. 11 was obtained using small vials in adapters on the same instrument (with the same settings) used to obtain the solid curve. It is quite evident that over most of the efficiency range, the small vials show less difference between chemical and optical quenching than the large vials. The same is generally true of all instruments tested.

In order to quantify the difference between large and small vials, the area between each PDE curve and the zero PDE line is summed between fixed limits along the abscissa. This area is referred to as the integrated percent difference in efficiency or IPDE value, and is a measure of the average difference between chemical and optical quenching over the specified range of counting efficiency. For a perfect system, the PDE curve would be along the zero line, and the IPDE value would be zero. For a system such as shown in Fig. 11, each curve deviates from the zero line and the enclosed area gives an IPDE value that can be compared with the other value made on the same system.

For ^3H , the range of integration along the abscissa is 10-50% efficiency. On Fig. 11, the IPDE value for large vials is represented by the area ABCD, and is equal to 274 units. For small vials, the area ABE has an IPDE value of 170 units. To compare the performance of large and small vials on an instrument, the ratio of the IPDE value for small vials to the IPDE value for large vials is computed. In the present example, this ratio is 0.62. One instrument gives a ratio larger than 1. The average of the other instruments is 0.56 ± 0.14 .

This ratio indicates that, for ^3H , the difference between optical and chemical quenching in small vials produces a potential error only half that obtained with

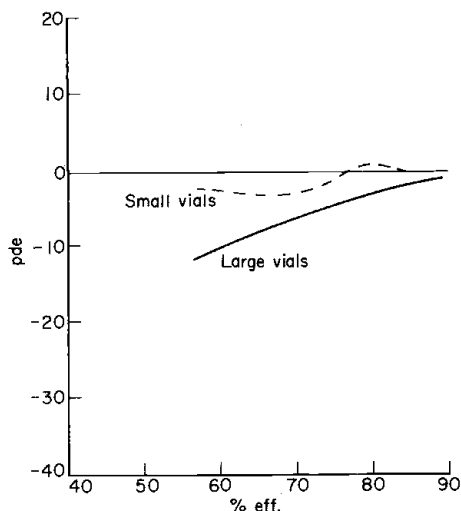


Fig. 12 Examples of percent difference in ^{14}C -counting efficiency between chemical and optical quenching vs. efficiency. — large vials; --- small vials with adapters.

large vials. It is important to recognize this potential error if the efficiency determination is based on chemically quenched standards and the samples contain optical quenching.

The situation appears to be similar for ^{14}C . Unfortunately, complete ^{14}C data were not obtained on all of the instruments. Figure 12 shows examples of PDE vs. efficiency curves for ^{14}C . This instrument shows a relatively small difference between chemical and optical quenching in both large and small vials. Other systems show much larger differences. These particular curves are used here to illustrate the point that even when the chemical/optical differences are small, the small vial in adapter (dashed line) performs measurably better than the large vial (solid line).

Integrated percent difference in efficiency values are computed according to the method described above. For ^{14}C , the integration limits are 60-90% efficiency. The average value of small vial IPDE value to large vial IPDE value is 0.36 ± 0.09 . Even though the number of instruments sampled is smaller, the indication is the same as for ^3H : samples counted in small vials with adapters are subject to less error due to the differences in chemical and optical quenching than samples counted in large vials.

An explanation for this difference might be found in the reduced average optical pathlength for a scintillation photon passing through the cocktail. It is known that reducing the optical pathlength reduces the difference between chemical and optical quenching. The small vial diameter shortens the average pathlength a photon must travel to escape the cocktail and, therefore, the amount of optical quenching is reduced.

CONCLUSIONS

The topics presented here represent some of the more important considerations in comparing instrument performance using large and small vials. Other factors are undoubtedly important to particular experimental situations. Background, for example, is critical where low levels of activity are being measured. Other radionuclides are also counted routinely. These cases can be treated separately. In terms of general liquid scintillation counting, the following conclusions can be drawn from this study:

1. There are definite economic advantages in counting samples in small vials.
2. The ^3H -counting efficiency is reduced when measurements are made in small vials with adapters.
3. The ^{14}C -counting efficiency is nearly the same for large or small vials with or without adapters.
4. The ^3H -counting efficiency exhibits only a small variation from full volume to half volume in either size vial.
5. The ^{14}C -counting efficiency is nearly constant over a wide range of volume in either size vial. However, below 7 ml, the small vial shows less variation than the large vial.
6. It is very important that the same size vial be used for both standards and samples.
7. For both ^3H and ^{14}C , the difference in chemical and optical quenching is smaller when small vials are used.

DISCUSSION

R.L. OTLET: What are the total background count rates (^3T and ^{14}C) for the types of large and small vials you described?

A.R. REICH: There are many factors influencing background count rates, one of which is the ^{40}K content of the liquid scintillation vial. Since there is considerably less glass in the small vials than in the standard vials, the count rate is also less. Time did not permit us to make a comprehensive study of the background contributions of all the systems studied, but on a typical Packard system the background count rate of a small glass vial in adapter is about 2.5 cpm less in the tritium window and approximately 3 cpm less in the ^{14}C window than that of a standard 20 ml vial.