

Chapter 7

Recent Advances in Sample Preparation for Liquid Scintillation Counting

B. W. FOX

Paterson Laboratories, Christie Hospital, Manchester, England

There are three basic questions to be considered when sample preparation for liquid scintillation counting is to be undertaken:

- (i) Has the elaborate nature of the technique, apparently necessary, destroyed accuracy?
- (ii) Has the simplicity of the technique to be used destroyed sensitivity?
- (iii) Is the intended counting system homogeneous or heterogeneous in character?

HOMOGENEITY AND HETEROGENEITY IN SCINTILLATION COUNTING

It is important to recognise whether the scintillation system and the β -emitter are totally within a single phase (homogeneous) or whether they are distributed unevenly between two phases, either solid-liquid or liquid-liquid (heterogeneous). For correction of quenching it would appear that in the majority of cases *sample channels ratio* may be used. The use of internal standard (other than the β -emitter itself as standard) or external standards should, however, be used with considerable care in heterogeneous systems.

Ideally, one should strive to obtain an homogeneous counting system in which the β -emitter is in direct thermodynamic contact with and within the same phase as the scintillant system itself. This is, however, ideal, and many biochemical experiments, due either to the nature of the final sample to be measured or sheer numbers, cannot be undertaken without the use of heterogeneous procedures. Mueller¹ has described an *operational definition* of a true solution from the point of view of scintillation counting as a mixture of components in which any one of those components is counted with the same efficiency, if tagged with a given radionuclide. This definition would allow certain regions of colloidal compositions to be treated as though they were effectively homogeneous. However, where absolute levels of radioactivity are required, or where mixed isotope counting is to be undertaken, homogeneous systems should be made the prime aim.

An apparently elaborate procedure may often be used effectively to concentrate β -emitters, prior to assay. Bulk impurities are first cleared away to reduce impurity quenching. An example of such an elaborate preparation is the formation of osazone derivatives of sugars from biological fluids, which due to their high insolubility will effectively concentrate radionuclide-tagged sugars. However, the yellow and orange colours of the osazone create colour quenching. A colourless derivative was thus described by Steel *et al.*,² the glucotriazole into which the osazone may be converted quantitatively. However, although colourless, this derivative is very insoluble, but is readily solubilised in a boric acid mixture described by Jones and Henschke.³ This mixture consists of naphthalene 60 g, PPO 4 g, POPOP 0.2 g, methyl alcohol 100 ml, ethylene glycol 20 ml, *p*-dioxane 880 ml and boric acid 25 g. Up to 100 mg of the triazole may be dissolved in 5 ml of this mixture. However, at this stage of elaboration one would begin to question again the exact requirements of the experimenter. Would thin layer or column chromatography be more appropriate, quicker and perhaps more quantitative?

The final choice of sample preparation must be a judgement arrived at as a result of a large number of individual decisions. One can reduce these decisions to a few important questions:

- (i) is the isotope insoluble in hydrocarbon solvents?
- (ii) is the sample to be measured of low specific activity (i.e. say less than 1000 counts $\text{min}^{-1}/0.2$ ml or 40 mg) or of higher specific activity (i.e. greater than this value)?
- (iii) are there *few* (less than 20) or *many* samples to be measured?
- (iv) does one need absolute activity *or* relative radioactivity assays between samples?
- (v) is there likely to be several millilitres available of each sample or only a very small volume (1.0 ml or less)?

The answers to these kind of questions can critically determine the sample preparation techniques appropriate to the sample being measured.

HOMOGENEOUS SYSTEMS

If the isotope is soluble in toluene, there is no great problem, and it is worth remembering that many lipids, long chain fatty acid salts of metals, noble gases, hydrocarbons, both liquid and gas, certain alcohols, especially the longer chain series, and ethers are soluble in toluene and are best assayed in a simple solution of PPO in toluene. A recent example of the employment of this fact has been described by Vikari⁴ for the estimation of labelled cholesterol in plasma, where steroid was partitioned directly into a 15 ml mixture of a toluene-based scintillation mixture containing 37.5% ethylene glycol monomethyl ether and 1 ml of water. The upper scintillant phase contains all the cholesterol and the lower most of the impurities. No bleaching procedures are necessary and chemiluminescence is negligible. Being an homogeneous system, external standardisation can also be freely applied.

Another example of the use of partitioning into the toluene phase of the labelled material to be measured has been described by Sankaran and Pogell,⁵ in the determination of methyl transferase enzyme activity, in which the labelled product is extracted directly into the toluene-based scintillant within the vial, which is also used for the reaction.

A β -emitter which partitions into the aqueous phase, however, such as a polar organic substance, biological macromolecule or as a polar salt, provides a solution in which the sample preparation will be largely determined by the specific activity of the solution as a whole. Since many scintillant mixtures have been devised to accept water and aqueous solutions in low proportion (i.e. up to 10% by volume) and still retain homogeneous counting characteristics, then clearly for high specific activity solutions these are the methods of choice.

A useful summary of the assessment of the optimal conditions for a few commonly employed blending solvents was given by White.⁶ He concluded that 2-ethoxyethanol was superior to many other alcohols tried for their ability to blend without introducing excessive quenching. A useful mixture suggested by White consisted of a mixture of 100 g naphthalene, 7 g PPO, 0.6 g POPOP in a mixture of 1000 ml toluene and 300 ml 2-ethoxyethanol. This mixture will accommodate up to 1% of aqueous solutions. For higher proportions of watery solutions (i.e. up to 4-5%), the naphthalene must be omitted, but for higher proportions still, a dioxane-based scintillant, such as that of Bray's,⁷ using methyl alcohol and ethylene glycol as combined anti-freeze and blender must be used.

In many modern counters, the use of ambient temperature conditions would appear to do away with the necessity for the use of anti-freeze in Bray's mixture. We investigated whether the ethylene glycol played a significant role in the counting efficiency, and more particularly the blending properties, of this scintillant, and different volumes of salt solution of different concentrations were examined in Bray's mixture with and without this component. The results are shown in Fig. 1.

A surprising feature was the fact that a low proportion of salt solution to scintillant caused precipitation (of salt?) to occur, even at low concentrations, whereas at higher proportions of salt solution, more could be incorporated, without precipitation occurring. By about 34% aqueous solution, precipitation of naphthalene occurred, even by water alone. The ethylene glycol did allow a higher concentration of salt to be incorporated between 0 and 10%, but made very little difference above that level.

For relatively small volumes of aqueous solution of higher specific activity, this type of scintillant would therefore appear to be the method of choice, since the strict homogeneity of the final scintillant mixture will allow for confidence in the use of external and internal standardisation techniques, as well as the sample channels ratio technique.

It is worth noting at this point that the accuracy of quench correction procedures properly applied have approximate coefficients of variation ranging from internal standard ratio 1.3%, sample channels ratio 2.5% to external standard ratio 3.5%. Automatic dispensing of internal standards at the 10 μ l level allows for variations of between 1.5 and 2.0% between samples.

An alternative sample preparation technique for solid material such as tissue, or biological fluids like plasma and blood containing ^3H , ^{14}C or ^{35}S , is of course combustion. Although eminently suitable for the assay of these isotopes, the method is only really suitable where the number of counts obtained from the sample is sufficient to be statistically reliable after combustion. The automation and convenience of the procedure has much to commend it since the final counting mixture is in the best possible form for absolute determinations. Yet another simple train combustion device has recently been described by Baba *et al.*⁸ A bomb combustion method for up to 10 g of biological and environmental samples in cases where the specific activity is low has also been described by Moghissi *et al.*⁹

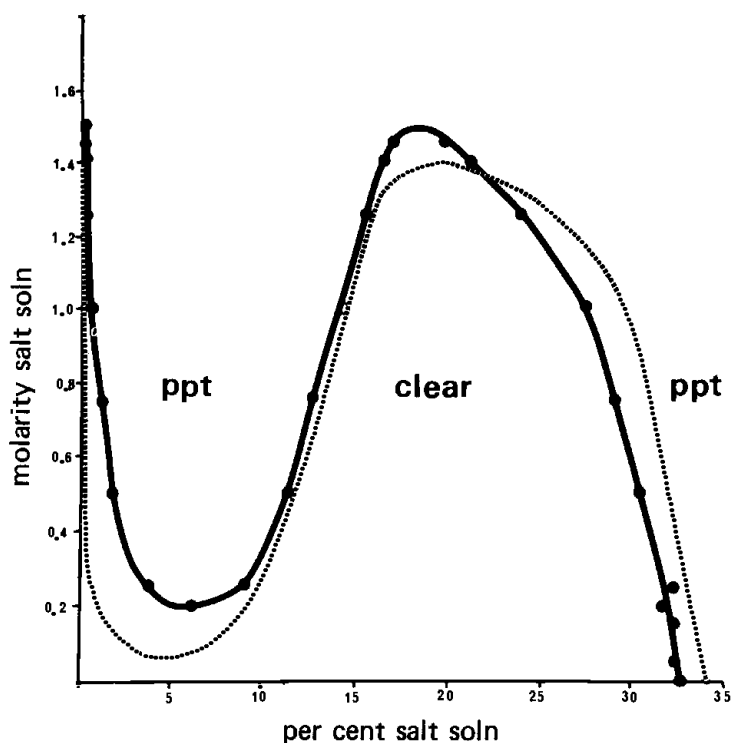


Fig. 1. The effect of adding increasing volumes of sodium chloride solution of different concentrations to Bray's scintillant. The solid line indicates the boundary above which precipitation occurs. The dotted line is a similar plot with Bray's scintillant in which the ethylene glycol has been left out.

SOLUBILISATION METHODS

Solubilisation methods involving only quaternary ammonium salts have continued to provide a suitable procedure for the assay of tissue. The chemiluminescence associated with many of the quaternary ammonium solubilisation procedures has been traced to the lipid component of the tissue being dissolved. Laine-Boszormenyi and Fallot¹⁰ suggested that a lipid extraction prior to assay would reduce the level of chemiluminescence considerably, provided, of course, the label itself is not in this fraction. The incompatibility between certain primary solutes such as PBD and its derivatives observed by Dunn¹¹ has been confirmed by Painter and Gezing,¹² but not by Wetter and Dyck.¹³ Clearly there appear to be other parameters in the local use of these agents which may modify the results.

HETEROGENEOUS SYSTEMS

In many situations in biochemical research, large numbers of samples, often of low specific activity, require to be assayed. The experiment often only becomes possible if counting systems and sample preparation procedures are used which are designed to provide reproducible comparative data between samples. The use of solid supports,

such as paper, thin layer materials, glass fibre or membranes, has been used extensively and it is often desirable to solubilise material from the filter for more accurate assays in homogeneous counting conditions. Jonsonbaugh *et al.*¹⁴ examined this problem with membrane filters and showed that the cellulose acetate membranes (EHWG type) did not produce colour quenching, whereas the mixed ester (HAWG type) did with solubilisers.

Assay of substances on solid supports is a common technique, but quantitation is unreliable unless the β -emitter is completely eluted from the disc into the scintillant mixture before measurement. Care should be taken that complete and not just partial solubilisation has taken place, since the latter will introduce considerable variability in the results. An example of this is in the measurement of monomeric and polymeric carbohydrates on paper chromatograms, described by Sandford and Watson,¹⁵ who also gave optimal conditions for the elution and assay in blended xylene-based scintillation mixtures.

A further example of the use of differential elution in assaying enzyme activity has been described by McKenzie and Gholson¹⁶ for methionine adenosyl transferase on phospho cellulose paper. The enzyme reaction is stopped with 10% perchloric acid and is neutralised by the addition of a fixed volume of 3M potassium carbonate, 0.5M triethanolamine mixture and after washing is assayed in an eluting scintillant mixture.

Counting of energetic β -emitters, such as ^{32}P , on discs should be avoided,¹⁷ since losses occur through the vial base due to the shorter path length of the scintillant available compared with the range of the β -particle.

Suspension counting of BaCO_3 - ^{14}C has usually been employed for $^{14}\text{CO}_2$ radioassay. Larsen¹⁸ has pointed out that up to 6 mg of BaCO_3 can be dissolved in 1 ml of 0.03M EDTA tetra-sodium salt, which can be assayed in a colloid counting system.

The heterogeneity and loss of 4π counting by adsorption of materials on to the vial walls have been studied by Wigfield¹⁹ and Wigfield and Srinivasan.²⁰ The measurement of adsorption shift, which varies from 0.0 with non-adsorbed components to 0.4 for fully adsorbed compounds, is determined from an equation containing two constants, one based on external standard ratios and the other on the sample channels ratio. Using this concept, a satisfactory calibration curve can be obtained which takes into account both homogeneous and heterogeneous aspects of the scintillation counting process. This is basically similar to the problems encountered in many heterogeneous counting conditions and there appears to be no reason why the principle involved here should not be more widely applicable in these systems.

An interesting observation associated with wall adsorption effects was made recently by Crouthamel and Van Dyke,²¹ who showed that the adsorption of ^{14}C -labelled polyethylene glycol on the walls of a glass vial could be completely prevented by the use of detergent-based scintillant mixtures.

POLYETHYLENE VIALS

There have been a number of reports on the problems arising from the use of polyethylene vials in liquid scintillation equipment having different types of γ -emitting isotopes as external standards. The observation of discrepancies was reported by Rauschenbach and Simon,²² who attributed them to the use of low-energy (^{137}Cs) sources in some instruments and high-energy (^{226}Ra) sources in others. This effect coupled with absorption of the fluor into the walls produced abnormal quenching values. This observation has essentially been confirmed by Gogan and Gogan.²³ Horrocks²⁴ has recommended that when sample counts are high enough the *sample channels ratio* should be used, but *external standard ratios* of either type may be used provided a careful choice of the channels used for the external standardisation is ensured.

COLLOID SCINTILLATION COUNTING

The use of colloidal systems in scintillation counting has predictably increased enormously over the last two or three years. The two-phase nature of the system has not only increased the possible uses to which it has been put, but has increased the possibilities of reporting 'improved' or 'preferred' recipes. It is not surprising, therefore, that lack of easy verification of the claims has resulted in many publications that have not added significantly to either our knowledge of the system or to its efficient use.

I would like, therefore, to summarise some of the aspects of this powerful counting system, and to endeavour to understand some of the ways in which the problems of counting instability and the partial heterogeneity exhibited may be modified and possibly controlled.

The prime value of the colloid scintillation counting system is to incorporate a larger volume of salt or macromolecule-loaded aqueous solution into an heterogeneous association with an efficient scintillator system. Its main advantage does not lie in its absolute efficiency, but in its ability to get maximum counting rates out of a low activity sample that could otherwise be immeasurable in an homogeneous system demanding lower volumes of sample in the mixture. This is an important criterion in determining whether or not to use the system. For solutions of high specific activity, such that 0.2-0.4 ml of solution would give statistically adequate counts, there is no advantage to be gained in using a colloid system at all. Indeed, low sample volumes in colloid systems (i.e. 5-10%) could result in considerable instability in counting.

In an attempt to establish some criteria for a proper comparison of these systems, there are certain conditions that should be met before an advantage can be claimed for one colloidal system over another:

- (i) does the modification allow increased water acceptance without excessive loss of efficiency?
- (ii) does the modification increase the stability of the counts obtained between repeated assays over a 48-h period?
- (iii) does the modification increase the variety of solutions capable of assay?

Between any detergent, hydrocarbon solvent and aqueous solution there are clearly an infinite number of combinations. Unless it is possible to describe the properties of a combination uniquely in these terms, then clearly we shall continue to be fed with an increasing number of new, so-called improved combinations.

The word solubilisation was coined by McBain *et al.*²⁵ in the Forties to describe the way in which certain dyes may be introduced or suspended in water by long chain amphiphilic salts or detergents. A similar phenomenon concerning liquids in liquids, such as aniline by short chain amphiphilic salts, was described by Neuberg²⁶ earlier and referred to as 'hydrotrophy'. Although the latter would appear to be a much more descriptive term for the events which occur in colloidal scintillation systems, the term solubilisation has gained wide usage.

In the solubilisation of water-insoluble liquids (in our case toluene or xylene) in water, or vice versa, by means of detergent, a characteristic and theoretically important liquid crystalline phase can be achieved at certain specific concentrations of the components. The liquid crystalline state can vary from a thick grease to a firm gel or more correctly a *smectic* phase. In this region of composition the molecular components are arranged in layers like packs of extremely thin, highly polished playing cards. Through polaroid filters, the light is characteristically orientated in these regions. Under the microscope, using polaroid optics, one can see the general organisation of the liquid components.

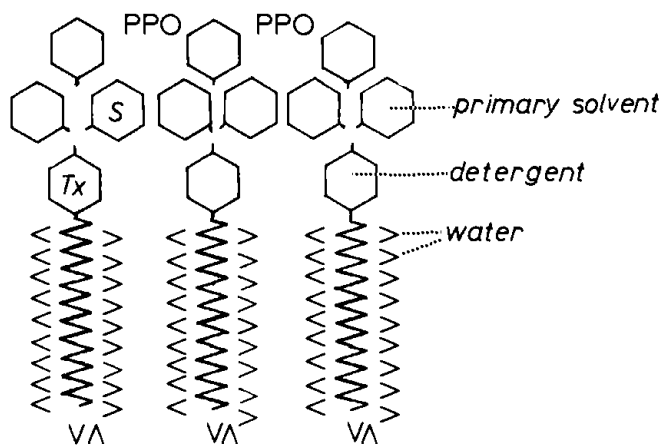
The establishment of this carefully aligned and organised molecular architecture will require time for assembly and this time will presumably vary with the component proportions, some combinations being capable of more rapid assembly than others. This is a technically difficult parameter to observe and to quantitate optically. Its implications are, however, important to liquid scintillation counting in this system and although we do not understand precisely how the energy of a β -particle is conducted from the disintegrating isotope to the hydrocarbon layer, this clearly happens and probably via the detergent molecule itself.

Triton X-100 itself may act as a primary solvent in its own right (Turner²⁷) and it seems likely that for the colloid scintillation process to operate successfully the detergent molecule itself may require some electron-transferring properties. Figure 2 illustrates a possible arrangement of the molecules of a toluene:Triton X-100:water system at its optimal point for counting. The relative numbers of molecular species are correct but the orientation as shown is purely speculative.

The molecular architecture in the vicinity of the β -particle will clearly influence the counting efficiency at that point. In particular, the more intricate and complex the architecture, the longer it may take to achieve its final organised state and this will depend on mixing efficiency and thermal history of the sample. A convenient way of visualising this settling down phenomenon, therefore, would be to observe the changing count rate of selected combinations with time. Such a parameter is the coefficient of variation of the counts taken five or six times during a 48-h period.

We have used this parameter to examine the stability of counts at various points in the phase diagram of any combination. The method of construction has been described previously by the author.²⁸

We have used the xylene:Triton X-100:water system containing PPO as our study system. Xylene appears to result in an equally efficient system to that of its toluene equivalent, but does show a greater degree of instability (Fig. 3).



Schematic representation of the micelle at the optimum point.

Fig. 2. Represents the number-relative of molecules of the scintillant components at the optimum counting region in the toluene:Triton X-100:water system. The primary solute is assumed to be in the non-polar zone and is not shown.

Whether this instability is due to the added complexities of molecular orientation of the solvent layer imposed by the additional methyl group in the molecule is a matter for speculation, but is presumably open to testing experimentally. This instability produced by xylene offered, however, a convenient system to examine the nature of the problem. By adding either a long chain or a short chain alcohol to this system, it would be expected that the former may become associated with the detergent and the hydrocarbon layer, whereas the latter would become associated with the detergent and the aqueous layer. In the experiments to be described here, the effect of octan-1-ol on this system was examined systematically by replacing the detergent molecule in stepped amounts with the alcohol and measuring the efficiency and stability parameters of the resulting four-component systems. The results of the addition of 2% octan-1-ol are shown in Fig. 4. It can be seen from this figure that the region of less than 1% coefficient of variation has spread to cover a greater area of the phase diagram as a whole. Increasing this percentage to 5% increases the area still more (Fig. 5).

Concomitant with these changes are changes in the Merit Value Contours of the system. The two terms found most useful in working in this system are defined in Fig. 6.

The Instrument Corrected Merit Value (MIV) should always be quoted rather than the merit value itself if comparison with other systems is to be made since the basic efficiency of the instrument itself can lend an apparent advantage to the system where no such advantage exists. The Instrument Stability Corrected Merit Value (MISQ) was devised²⁸ to try to include a term which would also stress the importance of the counting stability in any advantage gained. The MIV values for the system with Triton X-100 alone (i.e. without octanol) are shown in Fig. 7. It can be seen that similar to the coefficient of variation plot (Fig. 3) the disturbed molecular architecture is reflected in a similar pattern of contours, the maximum value observed in this subdivision being 1182, using tritiated water as β -emitter.

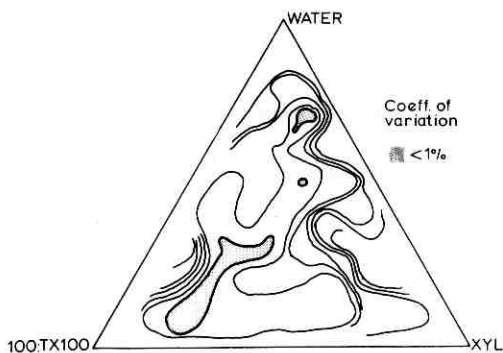


Fig. 3.

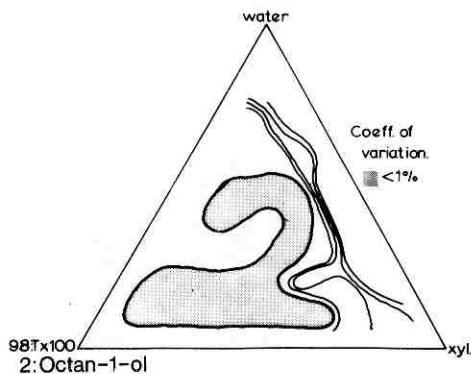


Fig. 4.

Fig. 3. A triangular plot of the percentage coefficient of variation of counts over a 48-h period for the water:Triton X-100:xylene system. The shaded zone represents the region of less than 1%. The remainder of the contours are increasing in 1% increments from the shaded zone.

Fig. 4. As for Fig. 3, except that 2% (v/v) of the Triton X-100 component is replaced by 2-octan-1-ol.

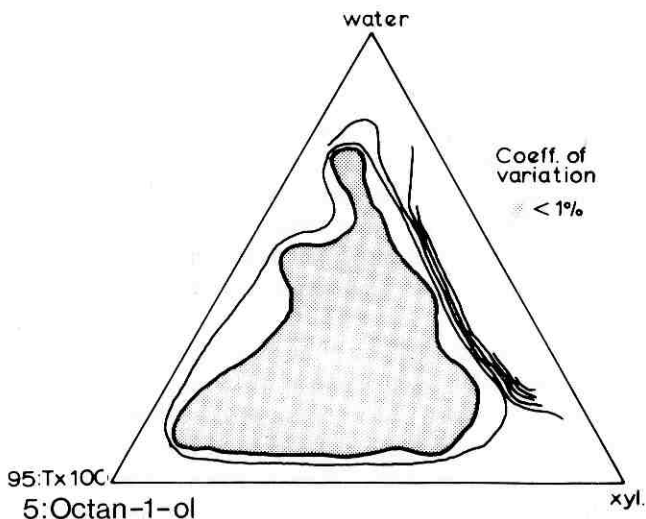


Fig. 5.

Fig. 5. As for Fig. 3, except that 5% (v/v) of the Triton X-100 component is replaced by 2-octan-1-ol.

$$\text{MIV} = \frac{\% \text{Eff.} \times \% \text{Sample} \times 100}{\text{Ref. Eff.}}$$

$$\text{MISQ} = \frac{\text{MIV}}{1 + \text{coeff. of variation}}$$

Fig. 6.

Fig. 6. The Instrument Corrected Merit Value (MIV) uses the instrument reference efficiency under the conditions employed to attempt to relate results from difference sources. It is necessary to use suitable channel settings for both sample and reference measurements to avoid any cut-off, especially by the upper discriminator. The Instrument Stability Corrected Merit Value (MISQ) employs the percentage coefficient of variation.

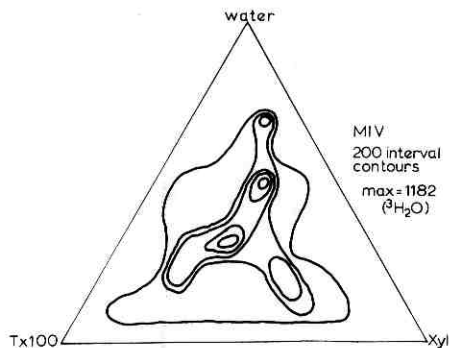


Fig. 7.

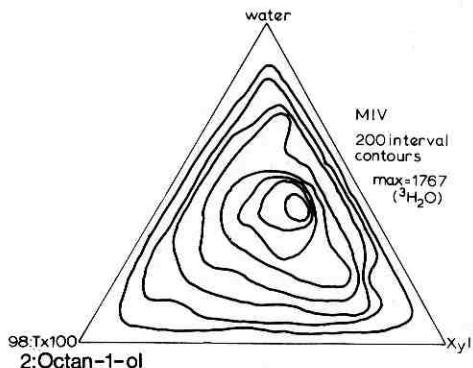


Fig. 8.

Fig. 7. A plot of the MIV values under the same conditions as in Fig. 3.

Fig. 8. A plot of the MIV values under the same conditions as in Fig. 4.

The addition of 2% octan-1-ol, however, spreads out the efficiency contours more evenly (Fig. 8), which suggests that there is a less discontinuous structure within the molecular architecture of the system from one combination to another. The maximum value is also significantly higher (1767), which suggests that a more suitable structure for efficient scintillation counting is present also. At the 5% level (Fig. 9) there is a similar continuity of the merit contours, except that the maximum is now lower. Thus, when 0 and 5% octan-1-ol is present the Triton X-100 layer clearly confers an advantage in terms of both the efficiency and stability by which water is assayed in this system. A more detailed bracketing of these concentrations, and extension to other alcohols would almost certainly lead to real advantages.

Finally, I would like to refer to some work which I am intermittently undertaking with Dr. C. W. Gilbert of our Institute. We have been examining the pulse height distributions throughout the toluene:Triton X-100:water system, using tritiated water and tritiated toluene as standards. When each composition at the 10% level is examined from the point of view of its efficiency in relation to the values of sample channels ratio and external standard ratio, all combinations with the exception of the 10% detergent level lie on the same sample channels ratio:efficiency curve. The contours drawn along sample channels ratio and external standards ratio should be parallel for areas in which they can be both used, but diverge in areas of high heterogeneity. Figure 10 shows preliminary data with regard to these measurements, and it can be seen that at relatively low detergent water levels such divergencies are very great. The efficiency plots closely parallel the sample channels ratio and not the external standard ratio. The degree of divergency, like the adsorption shift of Wigfield quoted earlier, could give a useful parameter by which to measure isotope concentration anywhere within the phase diagram, and overcome the ambiguity created by heterogeneity.

In conclusion, therefore, I think that the art of sample preparation for liquid scintillation counting is to be aware of the proportion of disintegrations that are being measured and to force this proportion to be as high and as constant as possible using the least effort to do so.

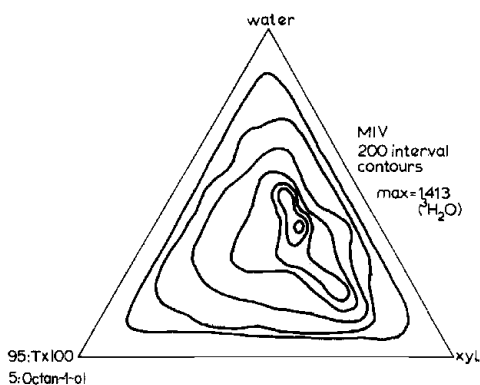


Fig. 9.

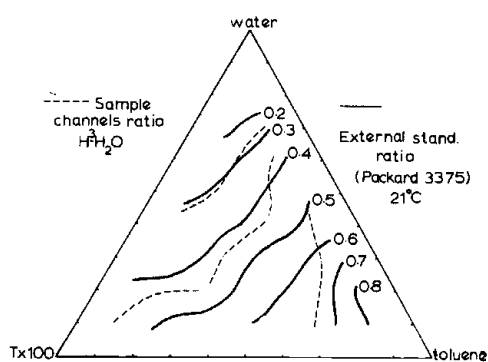


Fig. 10.

Fig. 9. A plot of the MIV values under the same conditions as in Fig. 5.

Fig. 10. A plot of contours for external standard ratio and sample channels ratio for the toluene:Triton X-100:water system using tritiated water as standard. The mixture contained both PPO and POPOP.

ACKNOWLEDGEMENTS

I would like to thank Dr. C. W. Gilbert for helpful discussions and Mr. Robert Caffrey for excellent technical help. The work was supported by grants from the Medical Research Council and the Cancer Research Campaign.

REFERENCES

1. Elizabeth Bush Mueller, in *Liquid Scintillation Counting*, Vol. 3 (eds. M. A. Crook and P. Johnson), Heyden, London, 1974, pp. 47-64.
2. R. Steele, W. Bernstein and C. Bjerckness, *J. Appl. physiol.* **10**, 319 (1957).
3. G. B. Jones and N. F. Henschke, *Intern. J. Appl. Radn. Isotopes* **14**, 618 (1963).
4. J. Vikari, *Anal. Biochem.* **63**, 566 (1975).
5. L. Sankaran and B. M. Pogell, *Anal. Biochem.* **54**, 146 (1973).
6. D. R. White, *Proc. 1967 Beckmann Summer School*, Beckmann Instruments Ltd., 1967, 72 pp.
7. G. A. Bray, *Anal. Biochem.* **1**, 279 (1960).
8. S. Baba, U. Baba and J. Konishi, *Anal. Biochem.* **66**, 243 (1975).
9. A. A. Moghissi, E. W. Bretthauer, E. L. Whittaker and D. N. McNellis, *Intern. J. Appl. Radn. Isotopes* **26**, 339 (1975).
10. M. Laine-Boszormenyi and P. Fallot, *Intern. J. Appl. Radn. Isotopes* **25**, 241 (1974).
11. A. Dunn, *Intern. J. Appl. Radn. Isotopes* **22**, 212 (1971).
12. K. Painter and M. J. Gezing, *Intern. J. Appl. Radn. Isotopes* **24**, 361 (1973).
13. L. R. Wetter and J. Dyck, *Intern. J. Appl. Radn. Isotopes* **24**, 430 (1973).
14. R. E. Johnsonbaugh, J. O. Kleiman and J. Sode, *Anal. Biochem.* **54**, 490 (1973).
15. P. A. Sandford and P. R. Watson, *Anal. Biochem.* **56**, 443 (1973).
16. R. M. McKenzie and R. K. Gholson, *Anal. Biochem.* **53**, 384 (1973).
17. F. G. Winder and G. R. Campbell, *Anal. Biochem.* **57**, 477 (1974).
18. P. O. Larsen, *Intern. J. Appl. Radn. Isotopes* **24**, 612 (1973).
19. D. C. Wigfield, *Anal. Biochem.* **59**, 11 (1974).
20. D. C. Wigfield and V. Srinivasan, *Intern. J. Appl. Radn. Isotopes* **25**, 473 (1974).
21. W. G. Crouthamel and K. Van Dyke, *Anal. Biochem.* **66**, 234 (1975).
22. P. Rauschenbach and H. Simon, *Z. Anal. Chem.* **256**, 119 (1971).
23. F. Gogan and P. Gogan, *Anal. Biochem.* **60**, 363 (1974).
24. D. L. Horrocks, *Intern. J. Appl. Radn. Isotopes* **26**, 243 (1975).
25. J. W. McBain, R. C. Merrill and J. R. Vinograd, *J. Amer. Chem. Soc.* **62**, 2880 (1940).
26. C. Neuberg, *Z. Elektrochem.* **42**, 289 (1936).
27. J. C. Turner, *Intern. J. Appl. Radn. Isotopes* **20**, 499 (1969).
28. B. W. Fox, *Intern. J. Appl. Radn. Isotopes* **25**, 209 (1974).

DISCUSSION

V. Tarkkanen: I wish to thank Dr. Fox for his contribution showing how important it is to solubilise a sample into a scintillator homogeneously. In your example showing addition of octanol into Triton-xylene the tests are only described for water. Adding the octanol showed an improvement in this case for pure water, but our experience is contradictory whenever true samples (i.e. those of buffers or any aqueous samples) are counted; addition of octanol destroys the stable micelle systems required for a correct sample. In practice the properties of most samples are not comparable with the properties of the water sample. Pure water samples are rare in liquid scintillation counting and are mostly found in hydrology or following sample combustion.

B. W. Fox: The system I described is simply designed in order to understand the basic principles involved at the level of the molecular architecture of optimal counting conditions. Water is used only as an example to study and it is not intended to suggest that

this is the best method of counting, even water. Salts will introduce further changes in this architecture, but this must be the subject of further study.

S. Apelgot: I would like to say that liquid scintillators are able to extract lipids not only from liquids like plasma, but also from any material, even cells and tissue. With a liquid scintillator containing dioxane, it is possible to extract from such materials a lot of aqueous soluble compounds.

In the case of labelled tissue, it is possible to obtain results by using an enzyme, pronase, which are the same as those obtained by the combustion technique. These results were presented two years ago at the Sydney meeting.

I know from many years experience that accurate measurements are obtained in heterogeneous phases (solid-liquid) without elution. What happens in the case of ^3H on paper has nothing to do with elution. In my laboratory we were able to show that this is related to the structure of the paper, and never occurs with membranes or glass fibre papers. A paper on this topic will appear at the end of this year.

B. W. Fox: Substances adsorbed on to glass fibre discs often differ from their absorption on paper and membranes. In the latter cases, different substances, especially tritium-labelled, will count with different efficiencies depending on the extents to which they have penetrated the paper. In some cases, e.g. with nucleic acid precursors on paper discs, differential absorption of nucleosides and nucleotides has led to differences of counting efficiency which in turn gives erroneous data as to the extent of, say, an enzyme conversion of one to another. When absolute measurements are required, the only efficient way to count is to elute or combust. Glass fibre discs, of course, do not suffer from problems due to absorption within the fibre itself, and in this case the thickness of the deposited film, bearing in mind also that the counting is near 2π , will determine counting efficiency. In this case, higher counts which are reliably corrected for quenching can be obtained by elution, but for most practical purposes it is more convenient to assay the discs in non-polar scintillants following standardised disc preparation procedures.

N. G. L. Harding: At the last symposium we adduced structures for these micellar systems and were very pleased to have these confirmed by your diagrams of the micelles using polarised light. Since then we have produced further evidence strengthening the view that these are dynamic, rather than static, structures. This accounts for stabilising and destabilising effects observed with a variety of agents added to micellar systems. Have you explored the usefulness of such reagents for studying the solution structure?

B. W. Fox: In the present work we used octanol in an attempt to do this, but there is clearly considerable scope to extend to further alcohols to explore this system.