RECENT ADVANCES IN SAMPLE PREPARATION

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The art of good sample preparation is the ability to detect beta-particle emissions efficiently and reproducibly with the minimum of preprocessing.

Instrument design and development has advanced to a greater degree than sample preparation technology over the last decade, and any limitations of liquid scintillation counting appear to be associated more often with the preparation of the counting sample rather than a deficiency in instrumentation. There has however been little apparent progression in the development of new solvents or primary solutes in recent years. Toluene, p-xylene and dioxane-naphthalene are still the most population electron trapping solvents. A useful advance was made by Krumbiegel and Schmidt (1973) who claimed that 97% perdeuterated toluene allows tritium to be assayed almost 90% higher than with normal toluene. A further development in solvent modification was the introduction of organic lead and thallium compounds into scintillant mixtures, first suggested by Ashcroft (1969) as density-increasers to determine γ emitting isotopes in liquid scintillation spectrometers. Such solutions can be readily made by shaking saturated lead acetate with a mixture such as Ready Solv VI (Beckman Instruments Ltd) (Helman and Spiehler, 1974) and using the upper layer. Although these scintillants are used by immersing into it a small minivial containing the sample, a more recent development is the direct use of organic thallium or lead loaded scintillant solutions to improve the separation of ^{125}I and ^{3}H materials mixed directly with them (Lundqvist *et.al.*, 1976). This combination of isotopes is useful in nucleic acid biochemistry using ^{125}I-iododeoxyuri-

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ISBN 0-12-549902-7

dine and ^{3}H-thymidine as simultaneous DNA precursors, or in radioimmunoassay investigations when both hapten and protein required to be labelled.

It is useful to distinguish a sample ready for counting as either homogenous (one phase) or heterogenous (multiphasic). This distinction is not just pedantic, but is also important since all quench correction methods can be applied to the former but not to the latter systems.

I. HOMOGENOUS SYSTEMS

The ideal counting system is when the sample is in direct solution with the scintillant and the hydrocarbon solvent. In biochemical work, this is rarely possible. However, one ingenious application of this principle was employed by Roffmann and Troll (1975) in which they assayed proteolytic activity of such enzymes as trypsin and papain by measuring the rate of release of ^{3}H-aniline from benzyl-DL-arginine (^{3}H) anilide. The aniline was allowed to enter the top scintillant rich toluene layer of a strictly two phase mixture. No physical separation of the two phases during measurement was found to be required, since the top layer was being assayed as a homogenous system. Clearly it would be essential to use a non-polar standard such as toluene or hexadecane, for this method. Another novel procedure is to monitor the liberation of ^{3}H-H_2O from a reaction in which the critical proton in the substrate is tritiated. All materials other than water can be retained on a short Celite column and the eluate assayed as a direct measure of the extent of reaction. Methods of assaying thymine-7-hydroxylase (Liu *et.al.*, 1974) and microsomal aryl hydroxylase (Hayakawa and Udenfriend, 1973) have been described based on this principle. To assay an aqueous solution, an homogenous counting system must contain, in addition to the primary solvent, primary solute and occasionally secondary solvents and solutes, a blender or diluter. The function of the latter is to create a true one-phase solution between the hydrocarbon solvent and the aqueous solution. The presence of salts and other solutes will invariably precipitate out of these solutions however, introducing heterogeneity into the counting system as well as uncertainty as to the extent of quench correction necessary. Older counting systems, designed for earlier machines are still used. A typical example is that described by Bray (1960), which contains a dioxane solution of naphthalene, ethyleneglycol, methyl alcohol, PPO and POPOP. The ethylene-

glycol was introduced to prevent freezing of the sample in the counter compartment originally maintained around 7°C The POPOP was also introduced to shift the fluorescence emission from 365nm to 419nm, to match more favourably the early photomultiplier tubes. Neither of these components are now considered necessary, due to the higher temperatures of sample compartments and to the improved matching parameters of modern photomultiplier systems.

Preprocessing for homogenous counting is usually achieved by degradation of large molecules to low molecular weight soluble products or by creating hydrocarbon soluble complexes. The formation of lipophilic salts has been an important method of analysis of inorganic ions. A good example is described by Darrell and coworkers (1973), in which they co-precipitate plutonium ions on $BaSO_4$ and then extract the plutonium into di-(2-ethylhexyl)phosphoric acid with which the plutonium forms a toluene soluble complex salt. The lower limit of detection by this procedure is reported to be about 1pCi. The determination of americium, curium and californium in biological samples has recently been described by Miglio (1978) in which the ashed biological material was dissolved in 8M $LiNO_3$, 10^{-2}M HNO_3 and this solution was extracted directly with a scintillation cocktail containing 20% N,N,N-trioctyl-N-methylammonium chloride in toluene containing p-terphenyl and 1,4bis-2-(5-phenyloxazolyl) benzene. Recoveries of greater than 90% were obtained. Noble gas isotopes can be measured directly in liquid scintillator counting solutions and the influence of temperature and solvent on this solubility has been studied by Cejnar *et.al.* (1977).

A. *Combustion Methods*

The degradation of organic compounds to CO_2 and H_2O is probably the most efficient and reproducible method of assay of ^{14}C and ^{3}H respectively. Combustion in oxygen has been described in over 100 publications, most of which were described as 'improved'. There are three basic systems used:- the bomb, oxygen flask and tube furnace methods. The latter two methods form the basis of well known commercial instruments. The bomb method is particularly useful in radiocarbon dating and Switsur *et.al.*, 1974 have described in detail the technique (and potential hazards) for analysing up to 12g of charcoal. An automated flask combustion system built from standard 500 to 1000cm^3 Erlenmeyer flasks has also been described by Rauschenbach and Simon (1974) and capable of

adaptation in the laboratory where commercial instruments are not available. An interesting tube method has also been described by Baba *et.al.*, 1975, in which 2 oxygen supplies are used and for which there is no requirement of a catalyst. About 3 mins only is required for the combustion of 0.3g sample.

A useful comparison of locally constructed and commercially available flask combustion methods with tube furnace techniques was made by Wegner and Winkelmann (1974) according to whom the oxygen flask appeared to be the most reliable at that time. A fully automated combustion system not using liquid scintillation counting, has recently (Mlinko *et.al.*, 1977) been described for the assay of ^{14}carbon. Although at present less sensitive, this could be a useful method for routine analytical purposes where a high degree of automation is required.

B. *Solublisation Methods*

Following the use of the lipophyllic quaternary ammonium hydroxides such as Hyamine 10X, Primene etc. for CO_2 trapping it was recognised by Vaughn *et.al.*, (1957) that amino acids and proteins could also be brought into solution as a particularly degraded polypeptide complex apparently capable of mixing homogenously with toluene solutions. Although solubilisation is regularly employed in biological and biochemical work, there is a singular lack of information on the fundamental reactions involved in the degradative process. Many commercial quaternary ammonium solubilisers are available however, Dent and Johnson (1974) have critically compared these in relation to a methanolic solution of NaOH, the latter having the advantage of both efficiency and cost.

One of the main problems encountered in the use of such solubilisers appears to be the production of chemiluminescence, especially when dry lyophilisates are used as samples. Laine-Böszörmenyi and Fallot(1974) showed that the peroxidation of lipids was primarily responsible and suggested that these be extracted with 1:2 v/v methanol:$CHCl_3$ before assaying. The chemiluminesence is in fact a complex product of at least three decay components with half lives at ambient temperatures of approx. 1 min, 10 min and 40 min respectively. Cooling reduces the level of chemiluminescence to about 20% of that at ambient temperatures, but lasts much longer. Some solubilisers are also not compatible with certain primary solutes such as butyl PBD owing to excessive colour quenching pro-

duced. (Painter and Gezing, 1973). Some membrane filters containing nitrate esters impart a colour when solubilised but the use of acetate-based esters overcome this problem (Johnsonbaugh, *et.al.*, 1973). There is still considerable scope for the development of new solubilisers especially for such materials as polyacrylamide gel slices, a process which still relies on swelling of gel pores followed by solubilisation of the contained proteins.

II. HETEROGENOUS SYSTEMS

These are by far the most commonly employed amongst sample preparation procedures and have the advantage that preprocessing is usually very simple and larger numbers of samples can be used. Indeed many biochemical experiments could not be undertaken because of the large numbers of complex aqueous solutions that would need to be measured. Heterogenous systems may be divided into two main groups, solid-liquid and liquid-liquid.

A. Solid-Liquid Systems

The scintillating system may be either in the solid or the liquid phase. The former is now rarely used except as a flow-through monitoring material such as detergent coated blue grade anthracene or even POPOP. Although the latter is more efficient, it is normally in a poor crystalline habit for easy flow of liquids.

However, the incorporation of a sample in the solid phase and the scintillator mixture as a liquid phase is one of the most common applications of the heterogenous counting system.

B. Disc Counting

Disc and suspension counting have been used since the early development of sample preparation technology. The use of 2.4mm diameter discs of filter paper, glass fibre, cellulose esters, and extruded polystyrene have all been used. Glass fibre has been particularly useful since the sample cannot penetrate the actual fibre itself. This contrasts with filter paper where differential absorption into the fibre of beta emitting samples in a mixture could lead to incorrect evaluation of the true ratio present. The advantage of disc

counting lies in the fact that after drying and provided the beta emitter is insoluble in the scintillant, a stoichiometric relationship between the counts on each disc occurs. In modern counters, with improved light trapping systems, the discs need only be just covered with a simple non-polar scintillant. As with all heterogenous counting, the use of external sources for quench correction will give only the efficiency of the scintillant and does not take into account the quenching due to the physical entrapping of the beta emitter, on the support, or from the solutes co-precipitated with the sample. Sample channels ratio techniques however would give a more true account of the degree of quenching present. In some cases as in the case of monomeric and polymeric carbohydrates, a greater efficiency of counting is achieved by first adding water to the disc and assaying in blended scintillant mixtures (Sandford and Watson, 1973). A similar addition of ethyl alcohol has been recommended for labelled lipids (Pyrovokakis *et.al.*, 1974).

Cation exchange paper has been used in a number of different ways to separate charged from uncharged molecular species and has proven particularly useful in the assay of enzyme system that effect such conversion. Methionine adenosyl transferase activity has been assayed using both phosphocellulose (P81) (McKenzie and Gholson, 1973) and carboxymethylcellulose (Wilson, 1970). It is not always necessary to regard the disc that has been counted as a sample lost. Murphy and Roux (1974) have described a method of recovering aminoacids and t-RNA for further processing from filter paper discs after radioassaying. The collection of double stranded DNA on glass fibre discs, then removal by means of a quaternary ammonium base such as NCS followed by assay in a toluene based scintillant, was considered by Schrier and Wilson (1973) to be the most efficient method of measuring of this high molecular weight polymer, especially when co-precipitated with bovine serum albumin. However, bovine serum albumin does not appear to assist the collection of single-stranded DNA and for the latter, nitrocellulose filters were preferred. More recently, DNA repair problems requiring a measurement of the proportion of double to single stranded DNA, and also the level of protein-DNA crosslinking have been assayed on DEAE discs in a similar manner (Kohn *et.al.*, 1976).

Whereas the orientation of the disc is now no longer a problem in modern scintillation counters for ^{14}C carbon and tritium, this is not the case with ^{32}P (Blasius and Sparmhake, 1973). The amount of scintillation fluid between the sample on the disc and the wall of the glass vial becomes more impor-

tant with the more energetic beta emitters.

B. Thin Layer Plates

Thin layer plates can be assayed by the use of an ingeniously devised formulation referred to as 'Stripmix' by the authors Redgewell, Turner and Bieleski (1974). It consists of 7g cellulose acetate, 3g diethyleneglycol, 2g camphor, $25cm^3$ n-propanol and $75cm^3$ acetone. A pool of approx. $20cm^3$ is spread evenly with a glass rod over the plate and the surface dried for 5 to 10 mins. When a section is cut with a blade, the bound thin layer material curls up and can be transferred to a vial. It is particularly useful when polar materials are being separated since it avoids the use of water which would cause them to diffuse.

C. Suspension Counting

Suspension counting now appears to be less used than previously due to the development of alternative preprocessing procedures which lead to a scintillant formulation in which relatively efficient quench correction methods can be applied.

A useful observation has been made by Larsen (1973) that $Ba^{14}CO_3$ which results from trapping $^{14}CO_2$ in baryta, need not be assayed as a suspension but it can be dissolved (~6mg) in $1cm^3$ of 0.05M EDTA tetra sodium salt in vial and then can be colloidally suspended in a toluene:Triton X-100 scintillator.

D. Polyacrylamide Gels

The assaying of labelled proteins and macromolecules entrapped in polyacrylamide gels have depended on the ability to simultaneously swell the polymer matrix with SDS or peroxide followed by the release macromolecules for subsequent solubilisation in an ammoniacal solubiliser. A simple method was described in 1977 by Albanese and Goodman, where the slice is dried in a Pyrex culture tube and treated with a $0.25cm^3$ volume of freshly prepared and ice-cooled mixture of 1 vol conc. ammonium hydroxide and 99 vols of ice-cold 30% hydrogen peroxide and incubated at 37^oC for 2-3 hrs and then measured in a commercial solubiliser (1 vol, BBS3) and 5 vols of 0.4% PPO-toluene base scintillator. A similar procedure

where gels are soaked in 10% acetic acid and then sliced, dehydrated in alcohol and saturated with a butyl PBD-toluene based scintillant and treated with a solubiliser has been recommended by Gezelius (1977).

E. Liquid-Liquid Systems

Although, at first sight, the use of two phase liquid systems may not appear attractive, if the partition coefficient of the sample is known and sufficiently different from its contaminating solutes, considerable purification and concentration can be achieved into a non-polar scintillant-rich phase. This principle has been extensively exploited in the field of inorganic chemistry and is typified by the analysis of the transplutonium actinides by extracting the lipophyllic complex formed with 1-nonyl-decylamine sulphate into the scintillant phase (McDowell, 1972).

F. Colloidal Scintillation Counting

By introducing a concentrated detergent into the toluene:water two phase system in an appropriate proportion the two phases can be dispersed into a micellar or colloidal structure. The nature of the colloid, i.e. whether organic micelles in an aqueous environment or the reverse, or the lamellar and usually liquid crystalline intermediate form, will determine both counting efficiency and stability of the structure. Many of the published and one suspects, commercial, mixtures have been achieved by an empirical choice of components. The result is a multitude of recommended formulations for salt, sugar and protein laden solutions. The structure of the hydrocarbon solvent:detergent:water system was systematically examined by triangular plotting procedures by Winsor (1960) and from a scintillation counting point of view by van der Laarse (1967) and in greater detail by Fox (1968). The most useful amphiphilic non-ionic detergent is the iso-octylphenoxypolyethoxyethanol, containing approximately 10 ethoxy units, known as Triton X-100 (Rohm and Haas). A critical evaluation of many closely related detergents including Triton X-114 and Triton N101 have failed to demonstrate any improvement in efficiency. The criteria for the evaluation of the comparative efficiency of these systems, taking into account the counting efficiency, counting stability as well as instrument standardisation have been stressed (Fox, 1974).

The colloid scintillation counting system is of greatest

value where aqueous solution of salts, protein or other solutes need to be assayed and where addition of even a small volume of blender would lead to precipitation in an homogenous counting system. The technique is especially valuable where an assay of many fractions needs to be made, e.g. from column chromatography or caesium chloride gradient centrifugation. Some examples of optimal composition are listed in Table I. The scintillant composition is improved by the use of a primary solute which is least susceptible to quenching such as butyl-PBD. Dobrota and Hinton (1973) recommended a mixture of 31.5g butyl PBD in 3L Toluene, 0.5L methanol and 1.5L Triton X-100. Up to 0.5cm^3 of 2M sucrose or of 60% w/w caesium chloride could be added to 10cm^3 of scintillant provided that 1.5cm^3 of water is added at the same time.

Further empirical modifications have been described such as the use of HCS solubiliser to increase stability of a toluene:Triton X-100 (2:1) system for trichloracetic solution, a notorious quenching agent (Chow, 1974) or of ethylene glycol and ethanol for similar reasons (Fricke, 1975). However, more detailed triangular plotting will be necessary to fully exploit these claims. Lahmann and Ninzpete (1974) also claimed that a mixture of 66% w/w lauryldimethylamineoxide and 33% polyethylene glycol-mono(p-'nonyl'phenyl)ether and a toluene based scintillant (1g:1.5cm^3) gives a scintillant composition capable of accepting 25% of its volume of aqueous solution with a "high counting efficiency".

G. *Quenching*

One of the most important aspects of liquid scintillation counting is to appreciate the nature and extent of quench present. Interference with the number of photons arriving on the photocathode can be from a variety of different sources. Some of these are within the source itself, such as the physical nature of the source, unlabelled solute associated with the sample, the solid support used with the sample or the structure of a colloidal micelle containing or associated with a sample. The primary solvent may also contain an associated energy sink such as dissolved oxygen, associated blenders or solvents added with the sample. In some cases, physicochemical modification of the primary solvent could occur. Similarly, there may be physico chemical damage to the primary solute, a higher concentration of which could also quench. The emitted light could be trapped by absorption by coloured materials present (colour quenching), or by associated unintentionally added waveshifters. The photocathode

TABLE I. Shows the recommended composition of Triton X-100: Toluene for optimal counting of different aqueous samples of biological interest

Sample	*Scint.comp. Triton X-100: Tol (v/v)*	*PPO g/L*[a]	*Counting Mixture: Scint (ml)*	*Sample (ml)*	*Merit Value (MIV)*[b]
Water	*1:1*	*8*	*6*	*4*	*1231*
8M urea	*1:1*	*8*	*6*	*4*	*1142*
5% sucrose	*2:3*	*6*	*5*	*5*	*989*
2M NaCl	*7:3*	*8*	*7*	*3*	*989*
Am.formate(.03M)	*2:3*	*5*	*5*	*5*	*778*
" " (1.0M)	*3:4*	*8*	*7*	*3*	*736*
TCA (5%)	*13:7*	*10*	*8.5*	*1.5*	*662*
PCA (5%)	*3:1*	*3*	*6*	*4*	*1148*
Formic Acid (0.1N)	*6:11*	*10*	*8.5*	*1.5*	*706*
HCl (1.0N)	*2:5*	*8*	*7*	*3*	*1030*
HCl (3.0N)	*5:11*	*5*	*8*	*2*	*748*
Fischer's Medium (+ 20% Horse serum)	*7:9*	*6*	*8*	*2*	*456*
Tryptone:yeast glucose (TYG)	*1:1*	*10*	*8*	*2*	*536*
Nutrient broth	*1:1*	*4.5*	*6*	*4*	*448*
Eagle's MEM	*1:1*	*3.3*	*8*	*2*	*313*
Cow's Milk	*3:5*	*3.3*	*8*	*2*	*664*
Human urine	*1:1*	*4.5*	*6*	*4*	*965*
Human plasma	*2:7*	*6*	*9*	*1*	*388*

[a] *The concentrations of PPO are not optimised for maximum efficiency in all cases.*

[b] *The Merit Value (MIV) is standardised for the instrument efficiency and is equivalent to:*

$$\frac{\%\ \text{counting efficiency} \times 100}{\%\ \text{sample value in mixture} \times \%\ \text{efficiency of machine references standard}}$$

The values of MIV are given for tritiated water only.

sensitivity could be affected by poor spectral matching and finally geometry changes within the vial could lead to light loss. It was shown by Takine and Ishikawa (1974) that the degree of colour quenching experienced corresponded to the

degree of overlap of the quencher absorption spectrum and the scintillator emission spectrum. Impurity quenching on the other hand could not be related to any structural features of 30 or more quenchers examined, but some similarities between isomers were noted. Some interesting data was obtained by ten Haaf (1974) however, who showed that the 350-450nm maximal absorption by carotenoids did not correspond with the most powerful quenching effect and that other geometry parameters were also involved.

Liquid scintillation counting is clearly being employed in increasingly diverse fields associated with archaeology, climatology, hydrology, the movement of lubricating fluids in engineering, leaks in gas containing equipment, marine biology and in psycho-pharmacology. The study of bio- and chemiluminescence has been advanced by the development of the automated spectrometer, and I feel that it is in these latter fields we may see a future increase in the development of the spectrometer applications.

ACKNOWLEDGMENTS

Thanks are due to the University of California for travel support and to Ms Gillian A. Simpson for the tedious typing of the final manuscript.

REFERENCES

Albanese, E. and Goodman, D. (1977) *Anal.Biochem.*, *80*, 60.

Ashcroft, J. (1969) *Int.J.appl.Rad.Isot.*, *20*, 555.

Baba, S., Baba, Y., and Konishi, T. (1975) *Anal.Biochem.*, *66*, 243.

Blasius, E. and Sparmhake, N. (1973) *Int.J.appl.Rad.Isot.*, *24*, 301.

Bray, G.A. (1960) *Anal.Biochem.*, *1*, 279.

Cejnar, R., Wilhelmová, L. and Vrzala, P. (1977) *Int.J.appl. Rad.Isot.* *28*, 281.

Chow, P.N.P. (1974) *Anal.Biochem.*, *60*, 322.

Darrall, K.G., Hammond, G.C.M., and Tyler, J.F.C. (1973) *Analyst*, *98*, 358.

Dent, J.G. and Johnson, P. (1974) Liquid Scintillation Counting, Vol.3. (M.A. Crook and P. Johnson, eds), p122.

Debrota, M. and Hinton, R.H. (1973) *Anal.Biochem.* *56*, 270.

Fox, B.W. (1968) *Int.J.appl.Rad.Isot.*, *19*, 717.

Fox, B.W. (1974) *Int.J.appl.Rad.Isot., 25*, 209.

Fricke, U., (1975) *Anal.Biochem. 63*, 555.

Gezelius, G. (1977) *Anal.Biochem. 80*, 2.

Haaf, F.E.L. ten (1974) Liquid Scintillation Counting, Vol.3. (M.A. Crook and P. Johnson, eds), p41.

Hayakawa, T. and Udenfriend, S. (1973) *Anal.Biochem., 51*, 501.

Helman, E.Z. and Spiehler, V. (1974) *Clin.Chem., 20*, 516.

Johnsonbaugh, R.E., Kleiman, J.O., and Sode, J. (1973) *Anal.Biochem., 54*, 490.

Kohn, K.W., Erickson, L.C., Ewig, R.A.G. and Friedman, C.A. (1976) Biochemistry, *15*, 4629.

Krumbiegel, P. and Schmidt, H. (1973) *U.S.P.* 3711421.

Laarse, J.D. van der., (1967) *Int.J.appl.Rad.Isot, 18*, 485.

Lahmann, W. and Hinzpete (1974) *Int.J.appl.Rad.Isot., 25*, 515.

Laine-Böszörmenyi, M. and Fallot, P. (1974) *Int.J.appl.Rad. Isot., 25*, 241.

Larsen, P.O. (1973) *Int.J.appl.Rad.Isot., 24*, 612.

Liu, T.Z., Wong, C.H., and Shohet, S.B. (1974) *Anal.Biochem., 62*, 408.

Lundqvist, H., Johanson, K.J. and Jonsson, G. (1976) *Int.J. appl.Rad.Isot, 27*, 233.

McDowell, W.J. (1972) Organic Scintillators and Liquid Scintillation Counting (D.L. Horrocks and C.T. Peng, eds) Academic Press.

McKenzie, R.M., and Gholson, R.K. (1973) *Anal.Biochem., 53*, 384.

Miglio, J.J. (1978) *Int.J.appl.Rad.Isot., 29*, 581.

Mlinkó, S., Dobis, E., Payer, K., Ottinger, J., Bánfi, D., Palágyi, T. and Turi, A. (1977) *Anal.Biochem, 83*, 7.

Murphy, M.R.V., and Roux, H. (1974) *Anal.Biochem., 58*, 89.

Painter, K., and Gezing, M.J. (1973) *Int.J.appl.Rad.Isot., 24*, 361.

Pyrovokakis, J.A., Harry, D.S., Martin, M.J. and McIntyre, N. (1974) *Clin.Chim.Acta., 50*, 441.

Rauschenbach, R. and Simon, H. (1974) 'Liquid Scintillation Counting' *3*, 153.

Redgewell, R.J., Turner, N.A. and Bieleski, R.L. (1974) *J.Chromatog. 88*, 25.

Roffmann, S. and Troll, W. (1975) *Anal.Biochem. 61*, 1.

Sandford, P.A. and Watson, P.R. (1973) *Anal.Biochem., 56*, 443.

Schrier, B.K. and Wilson, S.H. (1973) *Anal.Biochem., 56*, 196.

Switsur, V.R., Burleigh, R., Meeks, N., and Cleland, J.M. (1974) *Int.J.appl.Rad.Isot., 25*, 113.

Takine, M., and Ishikawa, H. (1974) *Nucl.Instr.&.Methods. 118*, 51.

Vaughn, M.D., Steinberg, D. and Locan, J. (1957) Science, *126*, 446.
Wegner, L.A. and Winkelmann, H. (1974) Liquid Scintillation Counting, Vol.3. (M.A. Crook and P. Johnson, eds) p150.
Wilson, R.H. (1970) *Biochem.J. 118*, 16.
Winsor, P.A. (1960) *Chem.&.Ind.* 632.