

Heterogeneous Counting Systems

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In liquid scintillation counting (LSC), the accuracy of the instrumentation and subsequent measurement is reproducible and optimal only if the sample is correctly prepared. The general requirements are intimate contact between the liquid scintillator and the sample throughout, and absence of colour and substances inhibiting the scintillation process. A major difficulty in the use of LSC is the final form of the sample which often differs greatly from the form in which it was originally collected. I will here describe the preparation of the most frequently occurring samples produced by recent techniques and discuss some typical sources of error and their prevention. At present, more than threequarters of the samples measured by LSC contain tritium or Carbon-14. These nuclides are widely used in biomedical research for measurement of their distribution in tissues and body fluids to determine dynamic effects at the molecular level of the cell, or in routine analysis or diagnostic work. These samples are predominantly aqueous and as such are insoluble in aromatic scintillation systems. This represents the main problem, especially in routine LSC measurements. Therefore these aqueous biological fluids containing Carbon-14 and Hydrogen-3 nuclides will be the main theme discussed here.

A sample in its final form consists of three main components; the material being counted, the solvent and the scintillator. The main sample types can be listed as follows: (1) aqueous solutions or colloids such as body fluids and water soluble substances like buffer solutions and electrolytes; (2) lipophilic compounds, directly soluble in aromatic scintillation solvents, such as lipids, steroids and drug metabolites; (3) solid compounds like protein precipitates, animal tissues and their homogenates, which can be modified into a soluble form and equally distributed into scintillation solvents; (4) insoluble or only partial soluble samples, such as connective tissue, bone, cartilage, certain proteins and polysaccharides.

In an ideal case, the sample is completely soluble in the scintillation solvent. The choice of scintillation solvent is, however, ultimately based on the primary criterion, namely the energy transfer of the tracer in the sample to the scintillator. Unfortunately, the second group includes only a few percent of samples measured by LSC and the incompatibility of the aqueous or solid samples with solvent having adequate energy transfer properties is the chief limitation in sample preparation. Various approaches have, therefore, been used in order to obtain a homogeneous system of the three main components in order to secure the

necessary 4 π counting geometry.

The first group of samples, toluene based systems containing organic solvents such as dioxan, alcohols, alkoxyalcohols and naphthalene have been used to count pure water up to 1/5 of the final sample volume. Figures 1 and 2 illustrate the performance of such systems. Furst and Kallmann,¹ Okita et al.,² Kinard,³ Bray,⁴ Butler,⁵ Bruno and Christian⁶ used more polar solvents to count water, together with toluene or naphthalene. The disadvantages of these systems are

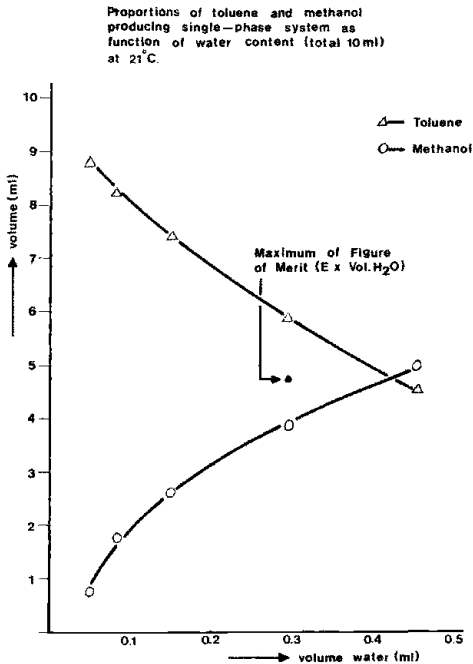


Fig. 1. Behaviour of a multi-component solvent system based on methanol and toluene. ³H-toluene as an internal standard.

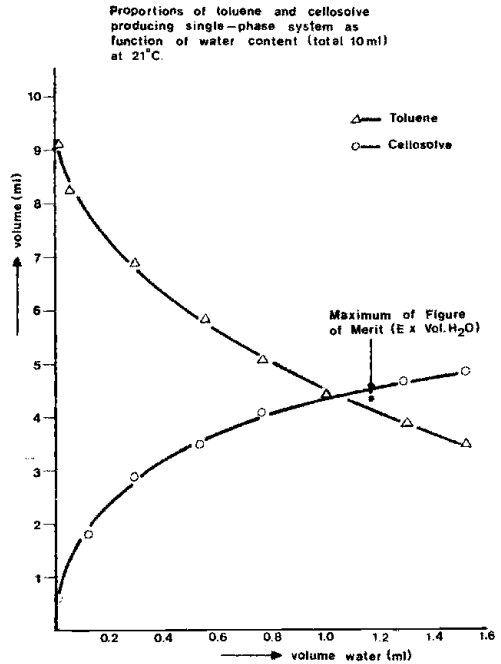
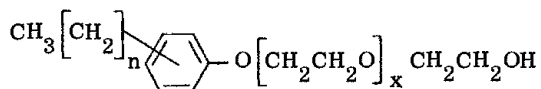


Fig. 2 Behaviour of a multi-component solvent system based on ethylcellosolve and toluene. ³H-toluene as an internal standard.

low counting efficiencies at water concentrations above 5 percent and inability to count samples containing dissolved solids. If the suitability of these systems had been tested for the counting of fluids, for instance for competitive protein binding techniques, they would not have been accepted as standard solutions for such a long period of time. Precipitation, like turbidity and even phase separation, have been accepted in the final sample form. Therefore, LSC has not been as precise a method as it should and can be, if the first step in the measurement, the sample preparation, had been treated correctly instead of as described in statements such as 'the radioactivity was measured after addition of a standard scintillator'. Figure 3 illustrates the typical deviation in the count rate observed when multi-component systems based on organic solvents of various types are used for the counting of proteinaceous systems. For comparison a modern heterogeneous (colloidal) counting system is given.

From the foregoing and from the general inability of organic solvents to dissolve aqueous biological systems it was obvious that other types of approach were needed for ultimate solutions. Thus, a fourth component, other than an additional organic solvent, scintillation solvent and scintillator is needed in order to meet the objective for reproducible measurement of samples represented by groups 1, 3 and 4. Problems associated with the third and fourth group of samples have generated various efforts to obtain reagents to degrade intact samples like tissue into a form which is soluble in the scintillation solvents. These solubilising reagents are various types of quaternary ammonium hydroxides dissolved in alcohol or in a scintillation solvent. Thus when dissolved in toluene they can be used to produce completely homogeneous counting systems for both homogenates and dry or wet biological samples. The sample, up to a few hundred milligrams, would normally undergo a solubilisation procedure probably involving a heating period of one or more hours. The numerous attempts to find a 'fourth compound' for group 1 samples — the most common group — have frequently failed due to excess of quenching, limited sample size and complicated preparation.

Developments of heterogeneous counting systems, those in which the bulk of the sample is not in the same phase as the bulk of the scintillator, were not widely accepted when presented by Erdtmann⁷ and Meade⁸ in 1962. The following reasons for this non-acceptance have been cited: substantial changes in counting results caused by temperature and pH dependences of the systems, self-absorption phenomena, discontinuities in reproducibility and recovery, and chemiluminescence. However, following a later systematic study investigated by Patterson,⁹ these heterogeneous systems have now been recognised as realistic possibilities in obtaining final samples with high load and efficiencies. They are also easy to prepare and many recent publications indicate that numerous efforts have been directed to the further development of previous ideas based on the utilisation of such heterogeneity. The first heterogeneous systems were based on sorbitol esters and castor oil derivatives as the fourth component to effect a process of solubilisation of the aqueous sample into toluene through formation of emulsions. Reasonable results were reported by Shapira¹⁰ and Gordon,¹¹ using large surface area silicon oxide (Cab-O-Sil and Aerosil) with toluene or dioxane/naphthalene to obtain a suspension of samples like barium carbonate, body fluids and tissue homogenates. Apparent self-absorption, especially for tritium, caused by the original sample or the silica, and instability due to the use of these kinds of thixotropic systems, were not adequately overcome even after further investigations. A basis for further development was the discovery of the usability of ethylene oxide polycondensates of alkylphenols,^{7, 8, 9} which were capable of dissolving aqueous samples in the presence of toluene or xylenes by forming colloidal systems which were seemingly clear in certain proportions, and to a given extent, thermodynamically stable. The general formula of these alkylphenoxy-polyethoxyethanols is



Patterson and Greene used a polyethoxyethanol of the above general formula, where $n = 7$ and $x = 9$ (Triton-X-100). They showed initially the useful limits of application of this emulsifier with toluene (in ratios 3:1 to 1:1) for various samples, frequently derived from biological experiments. Subsequent publications have confirmed these earlier studies or have reported refinements of the systems such as varying the length of the polyethoxy or the alkyl chain of the tenside molecule,

the solvent or the scintillator.

For the described type of tenside wherein the hydrophobic portion contains polyoxyethylene, a quantitation of emulsifying properties is given by the so-called HLB value. This value is computed by dividing the oxyethylene weight percentage (EO) of the total molecular weight of the tenside by five. Thus the $HLB = EO \times 1/5$. Tensides having an HLB value lower than 7 are suitable for water-in-oil (w/o) emulsions, where the water is finely dispersed in a hydrophobic solvent. Molecules with higher HLB value form emulsions of the type oil-in-water (o/w). For Triton-X-100 the calculated value is 13.6, but the practical value varies.

Special attention should be paid to the terminology relating to heterogeneous counting systems as many varying expressions appear in the LSC literature. The usual terms emulsion or suspension are widely used, but these terms are not quite correct because they refer to dispersions of one liquid in another whereby the dispersed liquid is in the form of droplets with a diameter of about 0.1 to 100 microns. Such systems are also thermodynamically unstable. The same applies even for so-called microemulsions^{1,2,3} which are transparent, isotropic systems and should not be referred to as emulsions in terms of the expression above.

The alkylphenol derivatives (APPE) with the general formula described above contain a hydrophobic and a hydrophilic portion. Figure 4 shows the arrangement of the hydrophilic ethylene oxide part. On the left we have an oblique-angled and

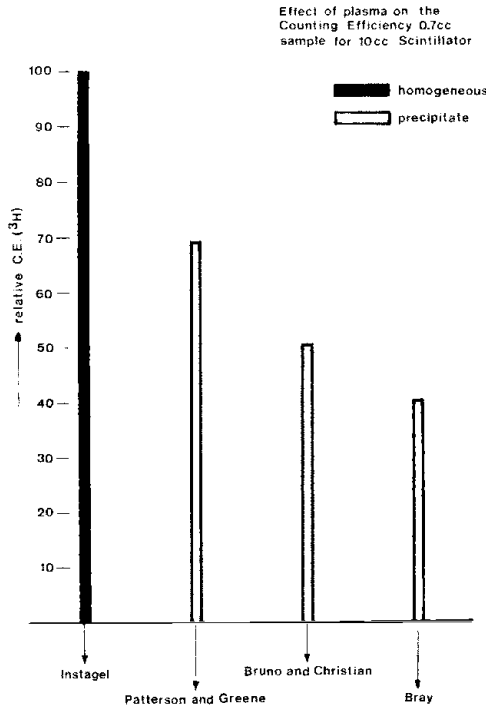


Fig. 3. Effect of phase separation.

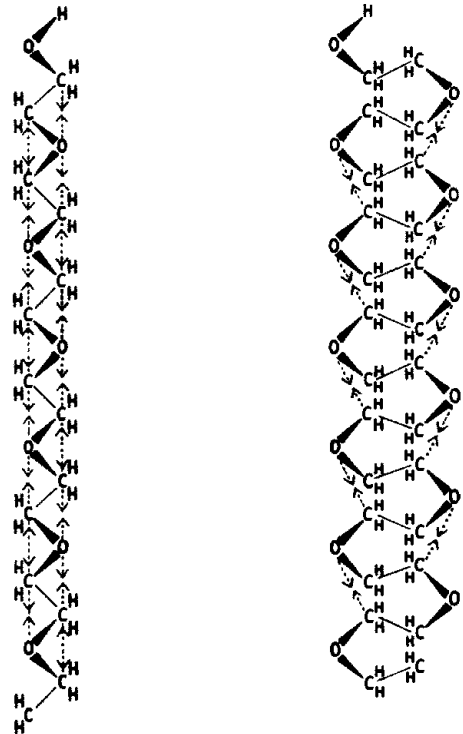


Fig. 4. Arrangement of the hydrophilic polyoxyethylene chain.

equilateral zig-zag line whereby the oxygen atoms are attracted by the adjacent CH_2 -groups. A meander-form (on right) has been proposed as a result of the

contraction of the chain in aqueous solutions. This chain can be hydrated by water by means of hydrogen bonding, thus making the products water soluble and therefore amphiphilic. The ratio of hydrophilic to hydrophobic portion (HLB value), the Van der Waals forces and the hydrogen bonds fundamentally affect the behaviour of these molecules, although they are chemically rather inert; but their major advantage is that they are able to form particles of colloidal size, so-called micelles, which can incorporate insoluble substances into a scintillation solvent. In this connection, Fig. 5 shows different shapes of micelles where the black end represents the hydrophilic part of the tenside molecule. The term

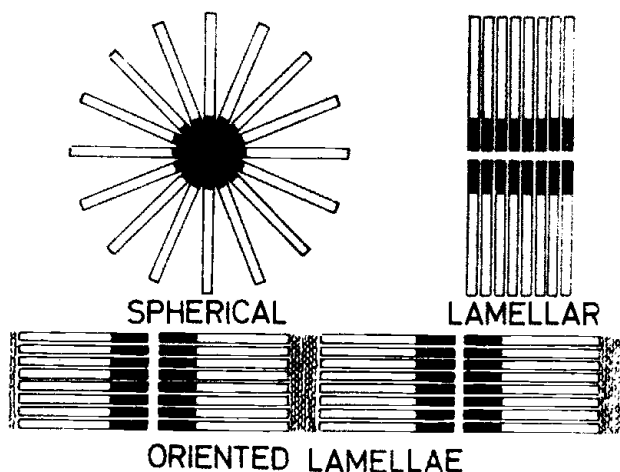


Fig. 5. Shapes of a micelle.

solubiliser is used with LSC for the mentioned quaternary hydroxides with digestive properties. Thus, it would not be correct to adopt the term solubiliser with regard to surface chemistry in order to avoid possible misinterpretations. Figure 6 shows count rates after direct incorporation of equivalent amounts of tritium activity of dry palmitic acid and sucrose. It should be noted that dry hydrophilic samples are not solubilised, while in aqueous solution they can be equally distributed in a few seconds and counted with high figures of merit.

In colloid science, the solubilising molecules were named tensides about 30 years ago. These terms have not been used in LSC for heterogeneous systems, but rather the expressions gel and emulsion are widely accepted. Fox¹⁴ first introduced the most correct term of 'colloid scintillation counting' in 1971. At lower concentrations of aqueous solutions these colloidal systems of APPE form sols and at higher concentrations so-called gels, which are more rigid in form. In contrast to the term liquid scintillator solution, which refers to a true solution, we will name the sols and gels as colloidal scintillator systems.

Conductivity measurements have shown that almost all of the colloidal systems utilised in LSC are of the type of water-in-oil, which means that, in the case of a spherical micelle, the droplet is water surrounded by the organic scintillator solution. It is of importance to understand the term 'heterogeneous counting system' correctly. The inclusion of emulsions and suspensions in this category has led to inaccuracy and non-reproducibility. A colloidal system consists of two discrete phases (bulks) and is, therefore, referred to as 'heterogeneous'. In an ideal colloidal scintillator, however, the contact of the two phases is

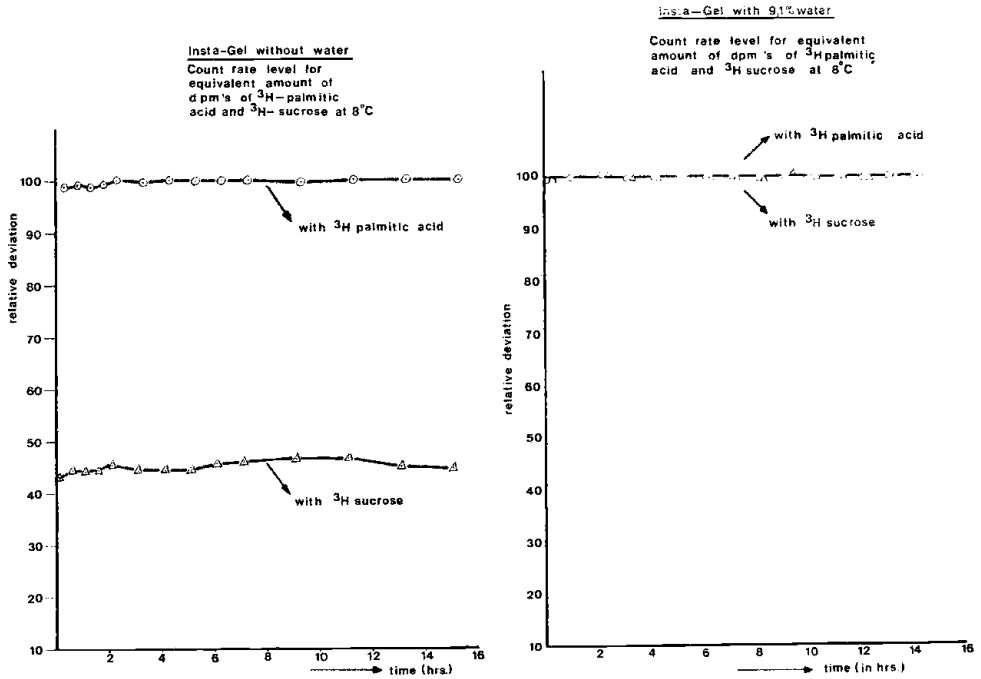


Fig. 6. Demonstration of the necessity of dissolving solid hydrophilic samples in water prior to counting in a colloid scintillator.

equivalent to true solution.

If we consider a tracer in the aqueous phase, little absorption of the beta particle as a function of the mass of the water should occur at low water concentrations. Neither should quenching occur for the scintillator as a function of the aqueous sample load as it is significant in the case of a true solution, i.e. by dioxan. If we compare Fig. 7, where counting efficiency of an aqueous tracer is plotted against its volume percentage of the total sample volume for both a colloidal scintillator and a liquid scintillator solution (i.e. dioxan based Bray solution), we can observe far less quenching for the colloidal scintillator system (i.e. Monophase-40). The rapidly decreasing counting efficiency for a true solution is caused by direct contact of the quencher (water) and the scintillator. In case of the colloidal scintillator the slowly decreasing counting efficiency is to be referred to as a function of the load of the aqueous phase: there are more layers or spherical micelles in the neighbourhood and even if the size of the micelle does not have any measurable effect on the counting efficiency by absorption of beta particles, the radiation must pass through a number of such aqueous layers between the layers of light-producing scintillator solution and result in a certain degree of quenching.

It is evident that the thickness or diameter of the aqueous micellar phase is of the order of less than 20 nm for an ideal colloidal scintillator. In the case of turbidity or opalescence the size approaches the visible regions or near 400 nm. This can be demonstrated by comparing the counting efficiencies of hydrophilic and lipophilic tracer solutions. In a good colloidal system both give identical results within the deviations of the accuracy of counting of the tracer available. Therefore, it is important that the size of the micelle is the smallest possible

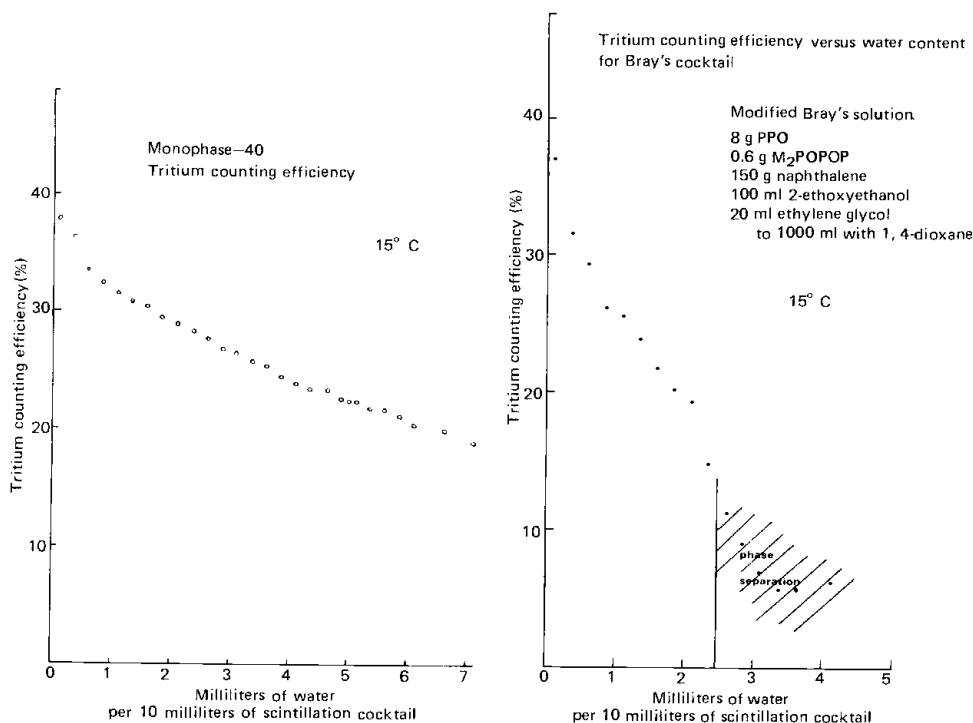


Fig. 7. Counting efficiency versus sample load of a modern colloidal counting system (left) and a dioxan-based scintillation fluid.

and subject to variations to the lowest possible extent. Fluctuations in the shape of the micelle can be caused by the ratios of the aqueous sample, scintillation solvent and the tenside. Furthermore, the electrolyte content of the sample and temperature changes have similar effects, resulting in thermodynamic instability of the systems.

Obviously, preference in LSC should be given to systems which are less affected by these instability factors. Therefore, knowledge of any remaining instability factors of the colloidal system are of importance in obtaining greater benefit from this most promising technique.

The most frequently occurring samples for LSC contain various combinations of inorganic salts and organic anions as buffer solutions, together with proteins. The property of organic scintillation fluids has been used to separate low energy beta emitters, soluble in organic solvents, from insoluble biological substances. In contrast, many assays of immunology and enzymes require the counting of an isotope bound to protein and any precipitation, even if invisible but detectable by the instrument, alters the bound/free substrate ratio and the validity of the radio-tracer method. It is not in the scope of this paper to compare different organic scintillator systems but rather to give comparative data on the properties of available heterogeneous counting systems, capable of reproducible counting.

Experiments made by Van der Laarse¹⁵ using phase diagrams of three components showed the necessary concentrations of tensides for various purposes. Fox¹⁴ extended this work with studies on the appearance or stability of various systems of tensides and samples. It was not possible to predict the appearance, and even slight changes of the sample consistency or temperature variations

unpredictably influenced the counting efficiencies; hence a universal system is obviously most preferable in routine experimental work.

As there is no exact instrumental method to compensate the faults caused by self-absorption (sedimentation, turbidity, phase separation) of scintillation fluids containing, for example, dioxan or Triton, and as the composition of biological material is not constant, these fluids are of limited use. The following nomograms (Figs. 8–11) illustrate comparative studies on the usability of formulations frequently cited in the literature such as toluene–Triton-X–100 (2:1) with 6 g PPO

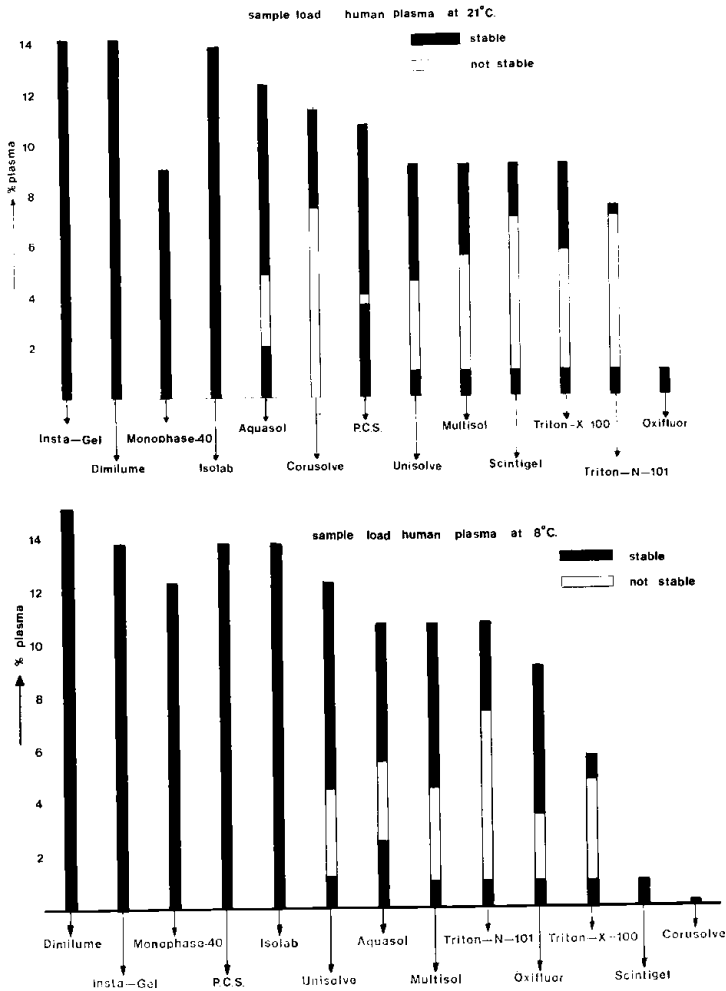


Fig. 8. Stability of commercial and experimental formulations for human plasma samples for counting temperatures of 8°C and 21°C.

and 0.5 g POPOP per litre and p-xylene–Triton-N–101 (2.5:1) with 7 g PPO and 1.5 g bis-MSB per litre and of the available commercial scintillation fluids. The usability of these systems is referred to as the reproducible stability of the count rate of the actual sample in the colloidal scintillator within a one percent

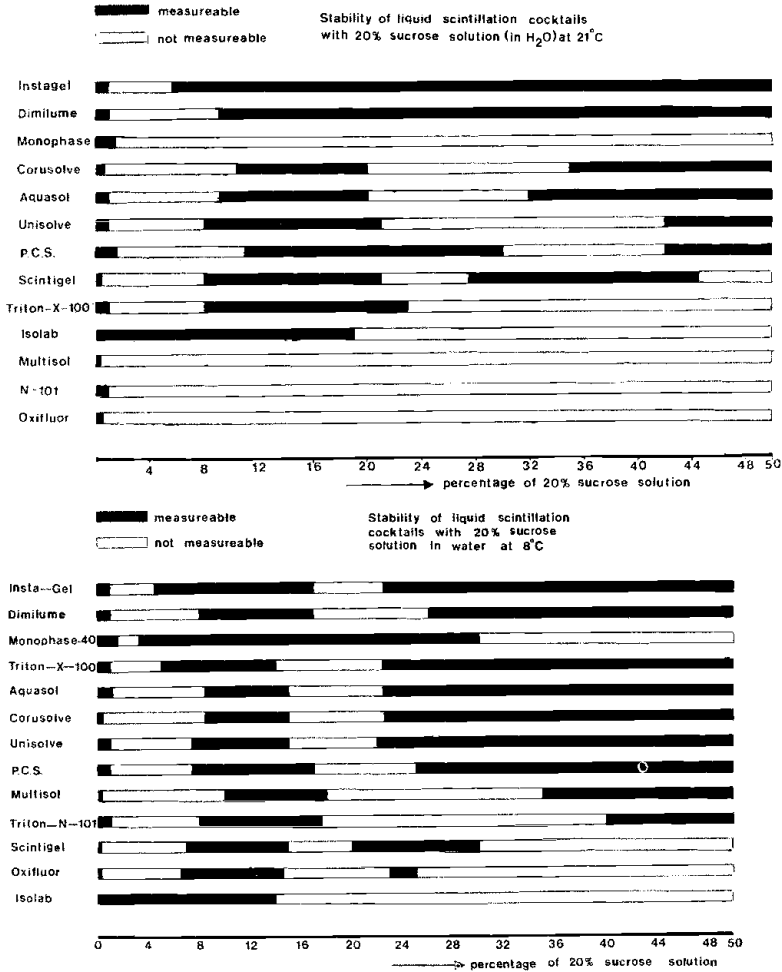


Fig. 9. Stability of various formulations with 20% aqueous sucrose solution at 8°C and 21°C.

error during a period of 170 hours following temperature equilibration for 2 hours in the sample compartment of a Packard Tri-Carb model 3380. When separation of the systems into two layers occurs (also referred to as two-phase range) the results are invalid and further counting statistics are not meaningful. The filled part of each nomogram indicates a reproducible counting system. First, Fig. 8 illustrates the ability to count human plasma after a mixing procedure with various colloidal counting systems and Fig. 9 similarly shows a sample of 20 percent sucrose in water. Figure 10 shows the influence of electrolytes, in this case 0.1 M sodium sulphate in water. Incubation of electrolytes lowers the repelling energy within the micelles, resulting finally in the two phases visibly separating into two layers. The influence of multivalent ions is greater than that of monovalent and therefore, it can be demonstrated that the colloidal systems can solubilise up to twenty times more concentrated sodium chloride than sulphate (Figs. 11 and 12).

Figure 13 shows the advantage of high counting efficiency and resistance against

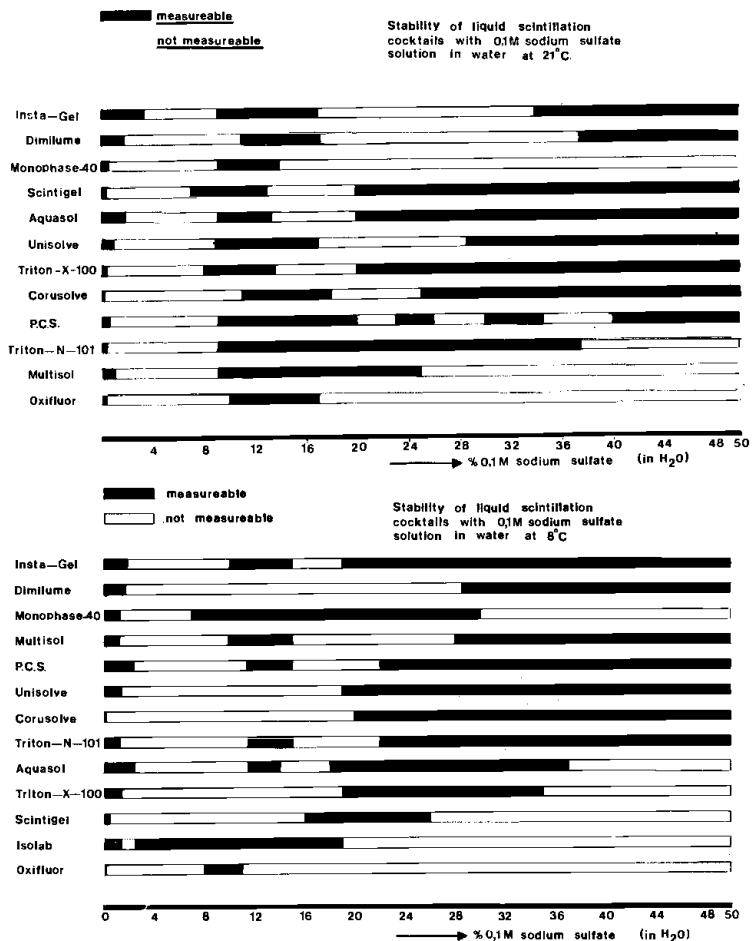


Fig. 10. Stability of various formulations with 0.1 M sodium sulphate.

quenching with equal loads of various inorganic and biological samples. This demonstrates the advantage of the presence of the sample in a phase other than the scintillator. Even here the phase contact is equal to that in a true solution for radioactivity measurement because no difference of recovery of tritiated water and tritiated toluene is detectable.

The earlier mentioned temperature dependence can be seen in Figs. 14, 15, 16, 17 and 18 where one can observe a discontinuity divided into one or more regions as the sample load or temperature changes. The sample measured contains only water and it is to be expected that even more peculiarities in the pattern of usable sample loads will occur for practical biological samples. At lower water percentages there is an apparent liquid form which results in the organic solvent and aqueous sample separating out into two distinct layers with a more rigid, gel-like, appearance. This phenomenon is influenced by changes in the shapes of the micelle. The blank area noted as 'two-phase' or 'frozen' gives different counting efficiencies for hydrophilic tritium (HTO) and for an equivalent

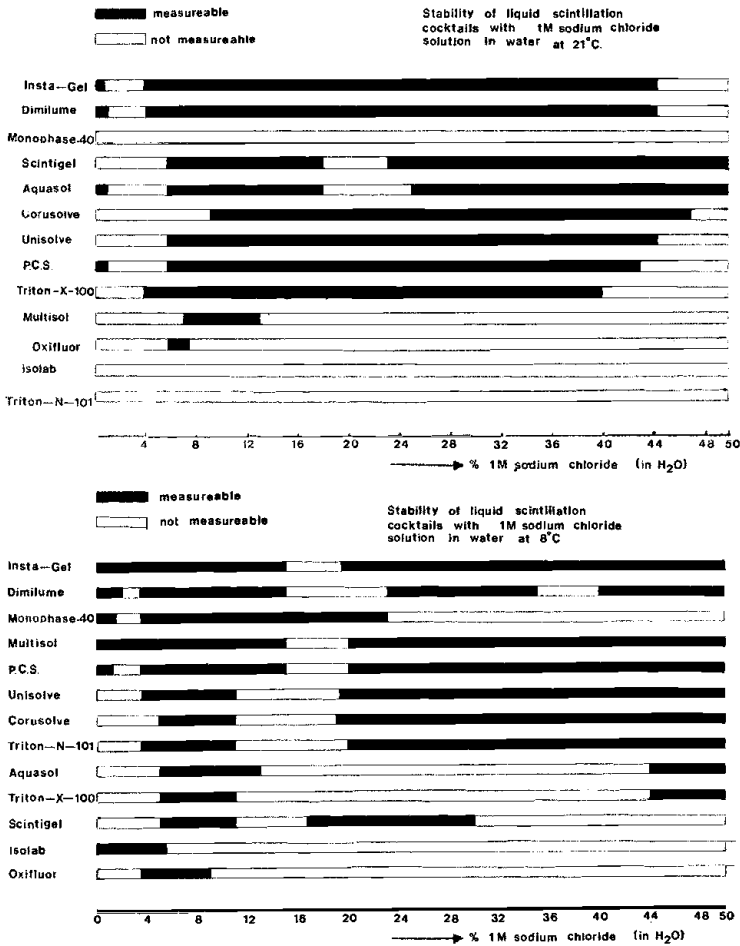


Fig. 11. Stability with 1 M sodium chloride solution.

amount of hydrophobic tritium (³H-hexadecane) with count rates which also vary during the measurement. The micellar structure is found to be of the water-in-oil type at lower water concentration, changing at higher loads to the so-called reversed micellar structure of the oil-in-water type. This is demonstrated by conductivity measurement of water, with dissolved electrolytes: water-in-oil results in a marked conductivity increase compared with the reverse type.

Optical characteristics of these systems indicate a controversial structure of lamellar-type micelles. This would give a better classification to the usability of the colloidal system for reproducible counting at higher water load, where the appearance is more rigid and consists of the hydrophobic phase of scintillator dispersed in the aqueous sample.

Temperature dependence was observed following an extended study by Williams.¹⁶ She recommended warming of the colloids to 40°C followed by several hours temperature stabilising in order to obtain the desired accuracy for low level tritium counting at high sample loads of water. By using a simple

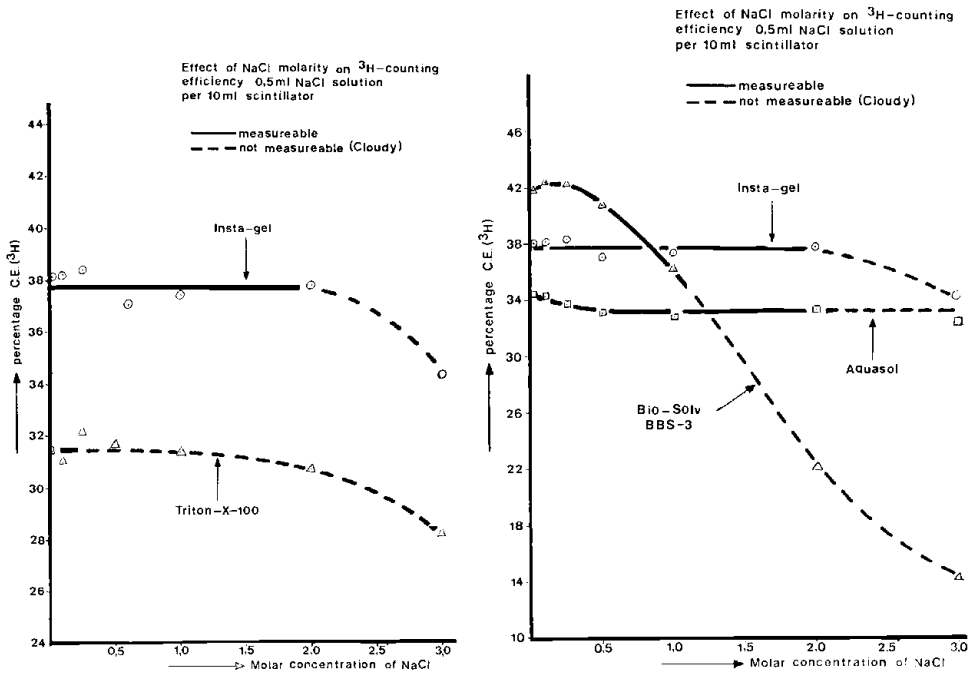


Fig. 12. Effect of sodium chloride concentration on the counting efficiency of tritium (as HTO).

mixing procedure at room temperature followed by a temperature equilibration of one hour maximum in the sample compartment of a temperature stabilised LSC spectrometer, we have observed adequate accuracies and stability over the range in which the system is generally used. It is desirable that the intimate phase contact is reached rapidly and this can be reached by the recommended sample preparation, i. e. for Insta-Gel: by the addition of the aqueous sample into the scintillator, followed by vigorous mixing.

The use of external standardisation for determination of counting efficiency for colloidal scintillators has generated many questions. The usable counting ranges versus temperature and sample concentration, as described here in various examples, are compatible with the automatic external standardisation (A. E. S.) method for quench correction. By using the recommended sample preparation method and the usable sample load ranges the behaviour of a modern colloidal scintillator is similar to any ideal homogeneous sample. This was not the case when Triton-X-100 was used,¹⁷ probably due to the temperature sensitivity and to the already mentioned influences of slight alterations^{14,15} in sample composition. Studies of Iwakura¹⁸ are of interest in this connection. He used the ratio external standard to internal standard (Se/Si) as an indicator of the degree of phase contact for colloidal scintillators. The best phase contact yielded a factor Se/Si equal to 1. This fact was found to be dependent on the counting time, temperature and on the kind of tenside.

The temperature dependence and the stability of the count rate are influenced by the tenside system in a modern colloidal scintillator. Figure 17 shows absolute dependence of tritium counting efficiency as a function of temperature and similarly the AES ratio plotted against temperature. It can be observed that these curves

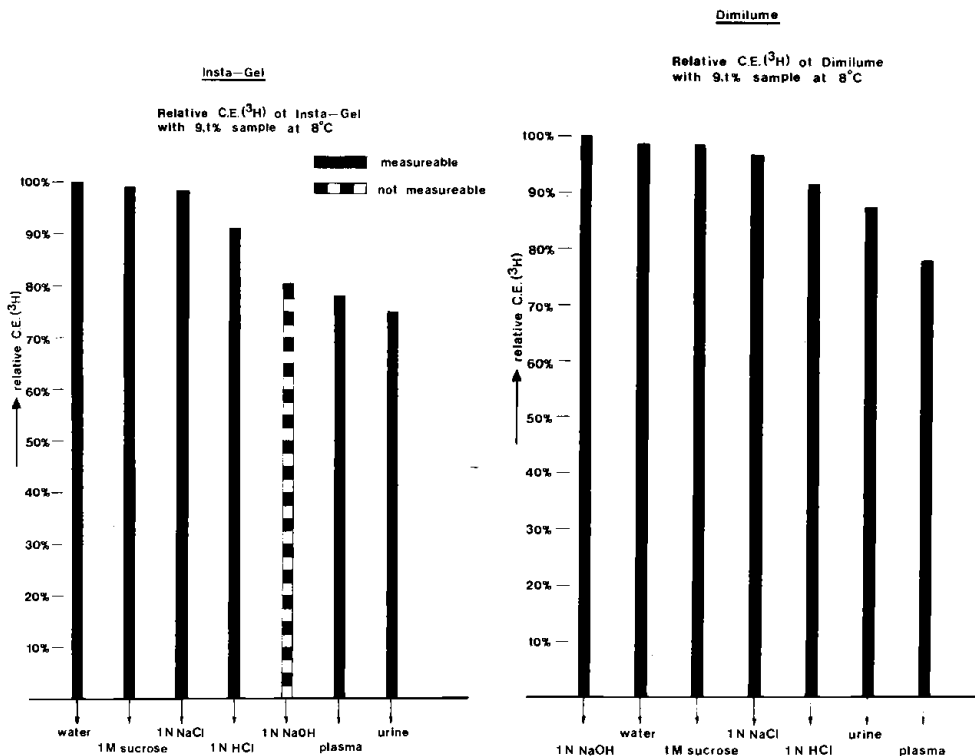


Fig. 13. Comparison of relative counting efficiencies of equivalent loads of various samples in Insta-Gel and Dimilume. 1 ml sample added to 10 ml scintillator.

match each other very well in shape and the external standard is usable, independent of the temperature, provided that the correct load and sample concentration are used as illustrated earlier. It can be seen that the external standard is applicable for Insta-Gel. However, there are some colloidal systems for which the physical changes are so temperature dependent that the correlation of changes in AES ratio with temperature does not match with the changes in efficiency, thus making the determination of counting efficiency inaccurate.

A practical standardisation curve for biological fluids shown in Fig. 20 illustrates the usable range of a typical colloidal scintillator for routine samples. The external standardisation method has been criticised by Rauschenbach¹⁹ in terms of the type of standard used and the type of vials. Discrepancies due to these variables were reported when standards, other than Radium-226, were used. Also the use of polyethylene vials was not recommended. Figure 21 clearly shows the penetration of scintillator and/or activity through the wall of a plastic vial. For periods longer than a few hours there is little or no difference between colloidal scintillators and solutions of toluene and scintillator in terms of this penetration.

Stability of colloidal samples can be affected if the composition of the scintillator is incorrect and by variations in counting efficiency during the time of measurement or recounting after storage. No enhancement in count rate was detected by Handler²⁰ when counting mixtures containing up to 33 percent aqueous

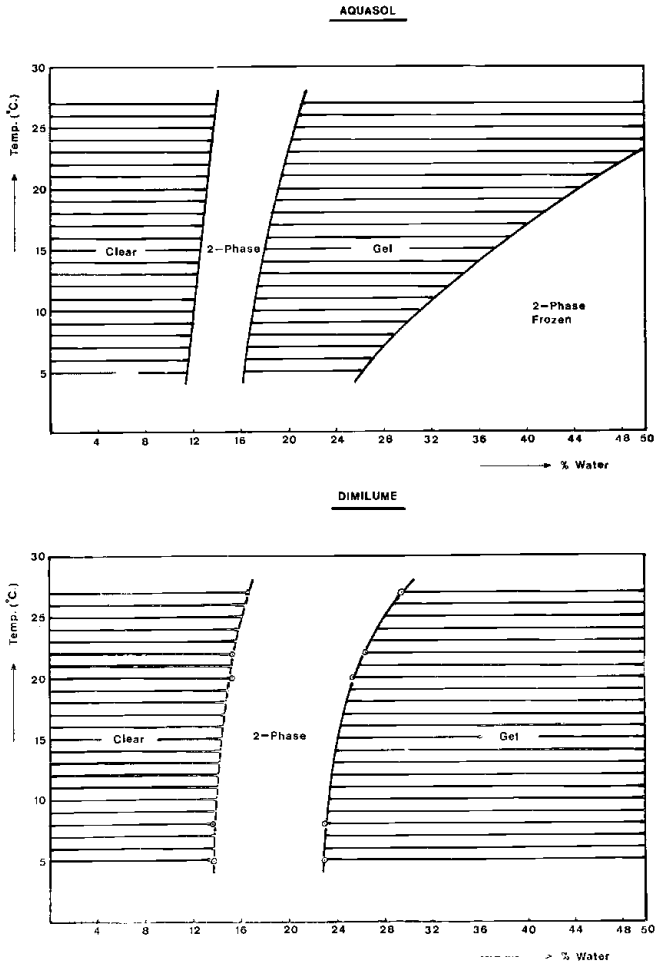


Fig. 14. Dependence of physical characteristics on temperature and sample load (water) for Aquasol and Dimilume.

sample in Insta-Gel, measured after 30 days storage. Figure 22 shows measurement of one regular and two other sample loads of water in Insta-Gel in terms of count rate versus time. Using temperature stabilised instruments it is of importance to allow the sample to reach the counting temperature of the compartment.

Chemiluminescence has been reported in the literature as one of the major problems when alkaline samples are to be counted with colloidal systems containing alkylphenol-polyethoxy-ethanols. Although the origin of the luminescent reaction has not been investigated, purification methods involving possible removal of these interfering compounds have been studied. None of these has been satisfactory. A method describing storage treatment of sodium carbonate for possible elimination of luminescent phenols, has been patented.²¹ In contrast, I have not found this method to be of significant value. The phenomenon interfering luminescence, investigated by our laboratory has been found to be a direct function of the peroxide content of the tenside molecule. The ethylene oxide chain of the tenside molecule is known to form relatively stable hydroperoxides, which are attributable to chemiluminescence under alkaline conditions. The alkylphenol

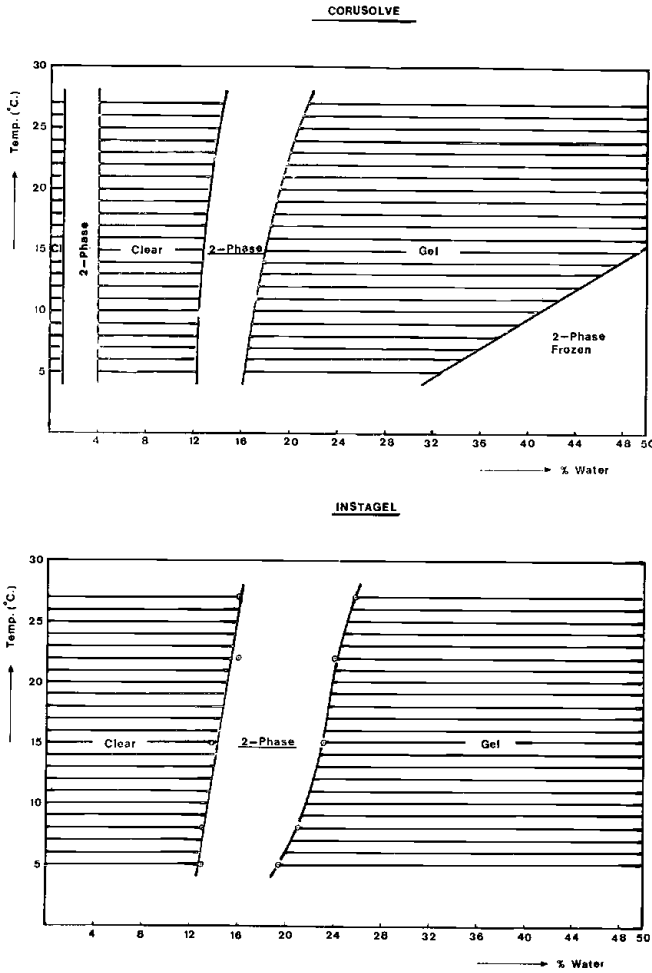


Fig. 15. Dependence of physical characteristics on temperature and sample load (water) for Corusolve and Insta-Gel.

derivatives as such have reasonable transfer energy and compete for spectral emissions, thus interfering with the scintillation measurement in the form of counts from spurious luminescence. Alkaline samples yield thousands of spurious counts with colloidal scintillators and decay of these counts can require several days. If alkaline samples are to be counted with colloidal scintillators the pH should be adjusted to neutral or slightly acid in order to obtain a normal background level within reasonable time.

The only colloidal scintillator having the general usability similar to the most universal colloidal systems with the advantage of rapidly reducing luminescence is Dimilume. The data in Fig.23 show that using Dimilume as scintillator for a sample containing strong alkaline solubiliser with digested tissue, only a 45 min background stabilisation period is required compared with a much longer time for the usual type of colloidal scintillator.

The sensitivity of colloidal scintillators is best illustrated by studies of counting of low levels of environmental tritium in rainwater by Schell.²² The minimum sensitivity level was as low as 193 picocuries per litre of water for

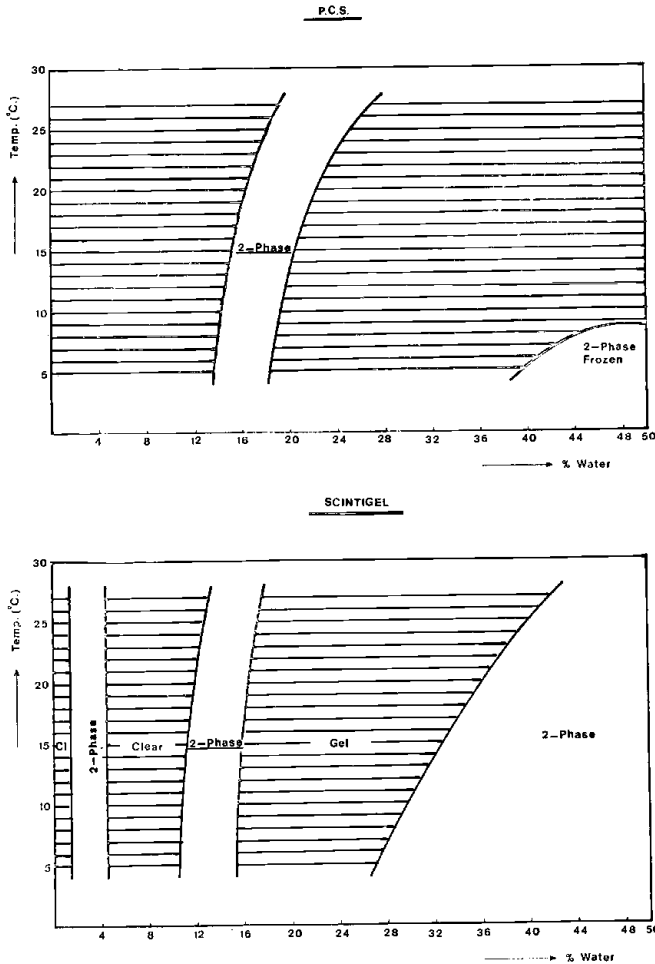


Fig. 16. Dependence of physical characteristics on temperature and sample load (water) for P.C.S. and Scintigel.

Insta-Gel, which represents a sensitivity improvement factor of 3.6 compared with dioxan.^{2 3} There is no way to make similar comparisons for dioxan based scintillation fluids containing biological samples, because the organic solvent as such, as described earlier, is not suitable for scintillation counting of such samples.

The Figure of Merit, as a product of the counting efficiency (in percent) multiplied by the percentage of the sample load, has been used in comparative studies of counting systems of aqueous samples. Figure 24 shows such comparisons for tritium in water. It appears clearly from this study that the system Insta-Gel shows the highest Figure of Merit and the best universal usability as is demonstrated in the studies described earlier in this paper.

In conclusion, we can say that colloidal counting today represents the most universal system. It allows experimental measurements of low level radioactivity in environmental monitoring and routine determination of activity in body fluids for any particular purpose with high efficiency and accuracy. Together with sample preparation by automatic combustion techniques I see these counting systems

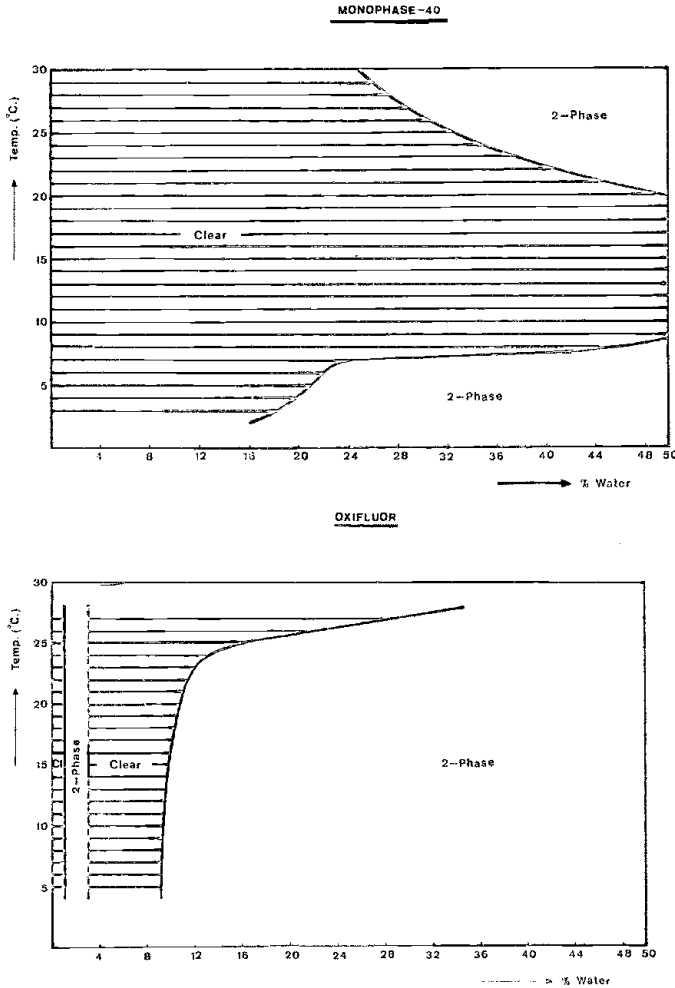


Fig. 17. Dependence of physical characteristics on temperature and sample load (water) for Monophase-40 and Oxifluor.

contributing to LSC as an exact method in analytical science. The largest drawback — difficulties in sample preparation — will soon belong to history.

Materials and trade names

The commercial products referred to were usually obtained through the owner of the trademark; if not, as otherwise specified as follows: Aquasol, Oxifluor: NEN Chemicals GmbH, Germany; Corusolve: ICN-Tracerlab, Belgium; Isolab: Isolab Inc., U.S.A.; Dimilume, Insta-Gel, Monophase: Packard Instrument Inc., U.S.A.; Multisol: Euratom, Centre Nuclear, Mol, Belgium; P.C.S.: Amersham-Searle Inc., U.S.A.; Scintigel: Karl Roth KG, Germany; Unisolve: Koch-Light Laboratories, U.K.; Triton N-101, X-100: Rohm & Haas, U.S.A. The vials used for these measurements were of the lowest background type glass (Packard Instrument Part No. 6000128).

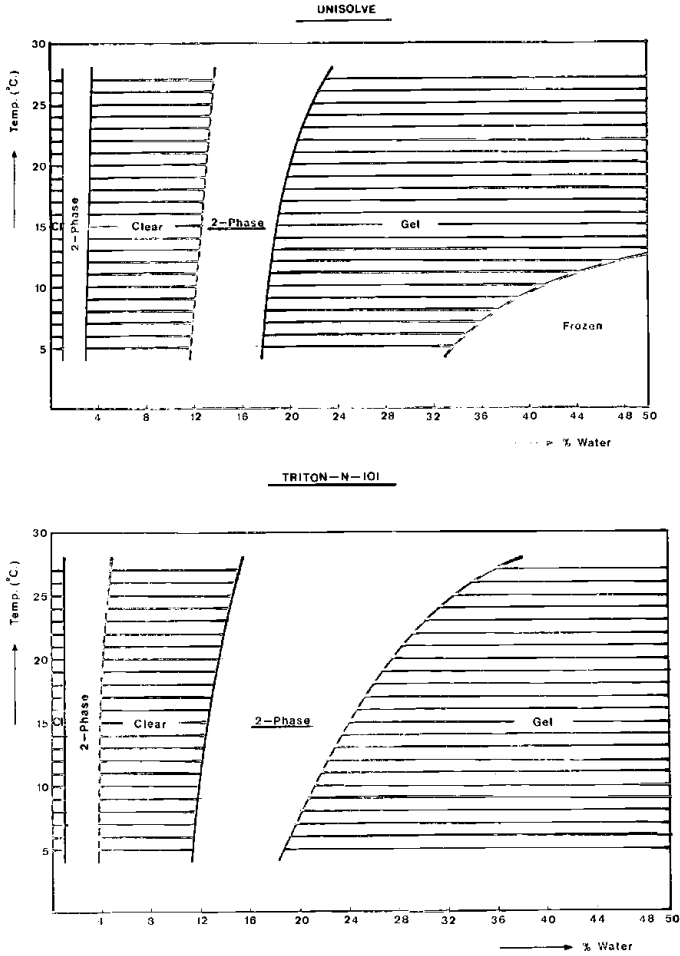


Fig. 18. Dependence of physical characteristics on temperature and sample load (water) for Unisolve and Triton N-101.

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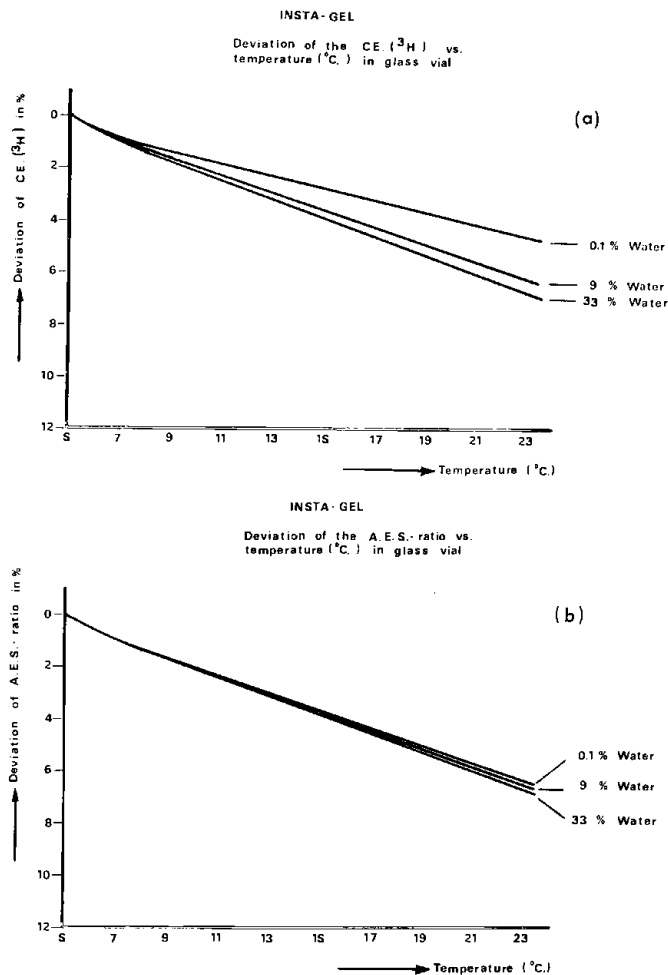


Fig. 19. Absolute dependence of tritium (HTO) counting efficiency on temperature for Insta-Gel with water as a sample. (a) Comparison of the deviation with AES ratio for the determination of disintegration rate (b). Sample volume 10 ml. Radium-226 as external standard.

- 14 B. W. Fox in *Liquid Scintillation Counting*, Vol, 2, M.A. Crook, P. Johnson and B. Scales (Eds.), Heyden & Son, London, 1972.
- 15 J. van der Laarse, *Intern. J. Appl. Radiation Isotopes* 18, 485 (1967).
- 16 P. Williams, *Intern. J. Appl. Radiation Isotopes* 19, 337 (1968).
- 17 R.H. Benson, *Anal. Chem.* 38, 1353 (1966).
- 18 Tetsuo Iwakura, Aiko Maebayashi and Yoshiko Kasida, *Radioisotopes* 18, 506 (1969).
- 19 P. Rauschenback and H. Simon, *Anal. Chem.* 256, 119 (1971).
- 20 L.H. Handler and J.A. Romberger, *Intern. J. Appl. Radiation Isotopes* 24, 129 (1973).
- 21 Royal H. Benson, U.S. Patent 3, 573, 219, 30 March 1971.
- 22 G. Sauzay and W.R. Schell, *Intern. J. Appl. Radiation Isotopes* 23, 25 (1972).

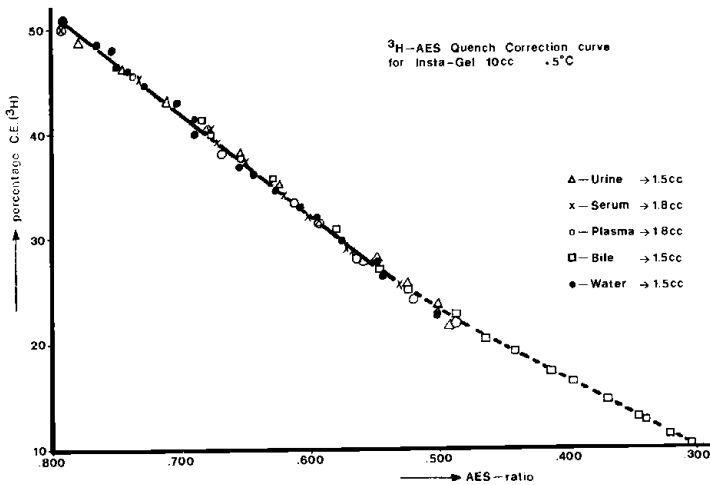


Fig. 20. AES quench correction curve for Insta-Gel with various biological samples labelled with Tritium (Battig).^{2 4}

- 23 P.H. Williams and T. Florkowski in Proceedings of the Symposium on Radioactive Dating and Methods of Low-Level Counting, International Atomic Energy Agency, Vienna, 1967, p.703.
- 24 F. Battig, personal communication.

DISCUSSION

K. Painter: I hope everyone enjoyed the Packard commercial as much as I did. Certainly, Dr. Tarkkanen, somewhere there must exist a sample in the universe which works better in something other than Insta-Gel? Also, all your graphs show Insta-Gel taking aqueous samples from zero percent water on. Yet your literature states one should not use samples less than 1 ml (ref. Packard catalogue).

V. Tarkkanen: As to your first comment, this should be possible. The referred catalogue notice claims 0.1 ml, not 1 ml. This is easily understandable; the minimum amount of water is needed in order to obtain equal distribution of a sample within the detecting liquid.

B.W. Fox: As a person who has no connection with an instrument firm, I feel that this discussion demonstrates that with secret recipes there is no possibility of rational scientific discussion.

K. Painter: Could I ask Dr. Fox if he has worked out the MSQ value of Insta-Gel?

B.W. Fox: I have, but owing to the fact that I was limited to the basic composition of colloid scintillant, I could only work out the maximum MSQ value for that composition, which came to 2.70. I recognise, however, that the requirements of manufacturers are for a wide spectrum application, and they are prepared to sacrifice some efficiency for this parameter, which for these purposes is a correct approach. My aim is to find conditions where I can observe the maximum number of counts with the greatest counting stability. Dr. Tarkkanen, over what period do you regard counts as being stable?

V. Tarkkanen: If we cannot detect visible, physical instability like formation of two layers and freezing, the observed period is a minimum of 170 h.

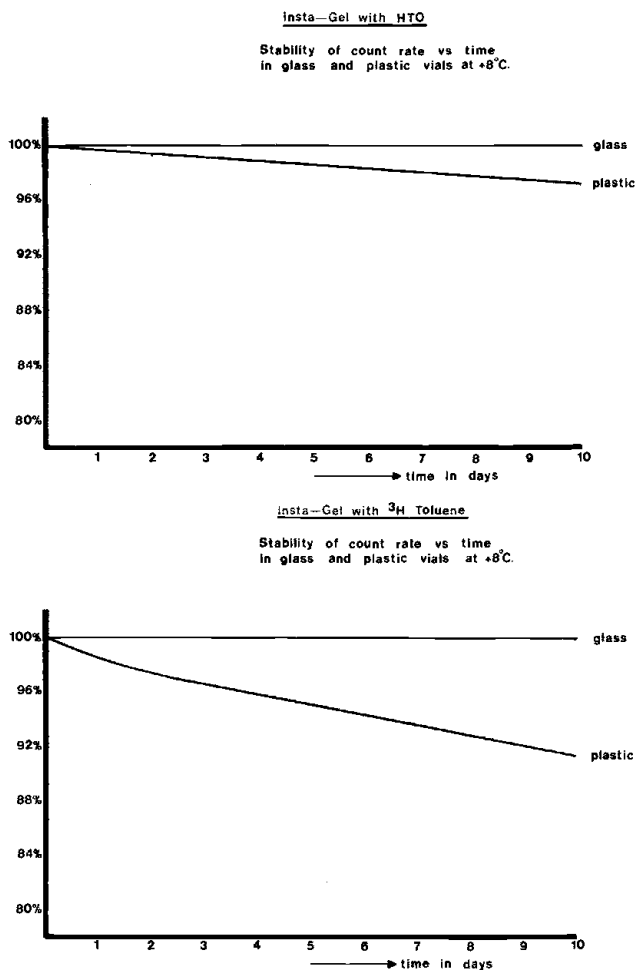


Fig. 21. Influence of the penetration of radioactivity through a plastic vial.
Sample volume 15 ml.

E. Gunther: In dioxane-based scintillator some nuclides are adsorbed on the walls of the vials. Therefore one adds silica to have a controlled adsorption. What about the case of Insta-Gel? Is it also useful to introduce silica in such cases?

V. Tarkkanen: Addition of silica has only a negative influence in the form of self-absorption in Insta-Gel in the case of organic polymer samples. We have not enough experience concerning nuclides.

E. Gunther: Is the shape of the pulse spectrum in Insta-Gel the same as in the scintillator systems? For example, have you experience in determining the β -activity by an extrapolation method?

V. Tarkkanen: The pulse height spectrum is similar to a normal fluorescence emission spectrum. We do not have special experience using extrapolation for the determination of β -activity in Insta-Gel.

R. Dierckx: I have samples of highly concentrated lithium acetate $[(CH_3COOLi)nH_2O]$; would they be soluble in Insta-Gel?

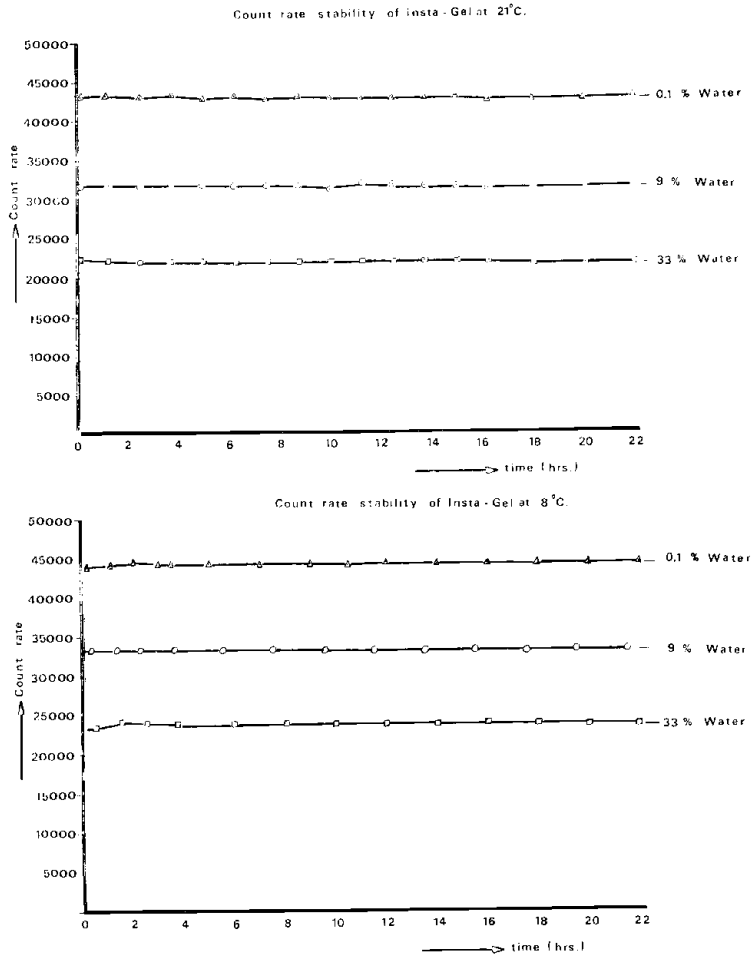


Fig. 22. Count rate stability of Insta-Gel with varying amounts of water at 8°C and 21°C. Final sample volume 15 ml.

V. Tarkkanen: I don't know, but if you like to count neutrons, the necessary formulation is available.

D.A. Ginger: You drew attention to an alleged product of my company, called Unisolve 3. Can you amplify your remarks regarding this product, as it has never been released for sale?

V. Tarkkanen: We tested Unisolve 1, 2 and 3 of which the Unisolve 2 was not usable due to abnormal low counting efficiency. Unisolve 3 gave better results than Unisolve 1. Therefore the presented results are most favourable for Unisolve 3.

D.A. Ginger: I must emphasise that only 1 litre of Unisolve 2 and 3 was ever manufactured at the express wish of a customer, not Packard-Becker, and comparisons obtained in this way are odious.

E. Mueller: Do you get the same efficiency for ^3H -water and ^3H -toluene in all the composition regions you describe as stable?

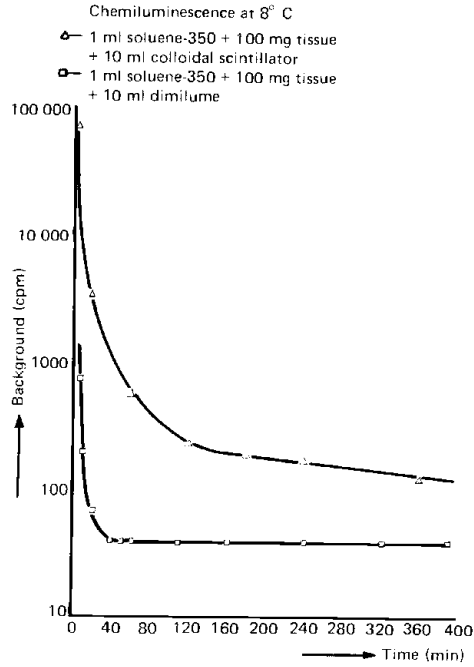


Fig. 23. Chemiluminescence decay in colloidal counting systems. Final sample volume 11 ml.

Triton-X-100

Figure of merit (CE X % water) for tritium (+ 8° C)

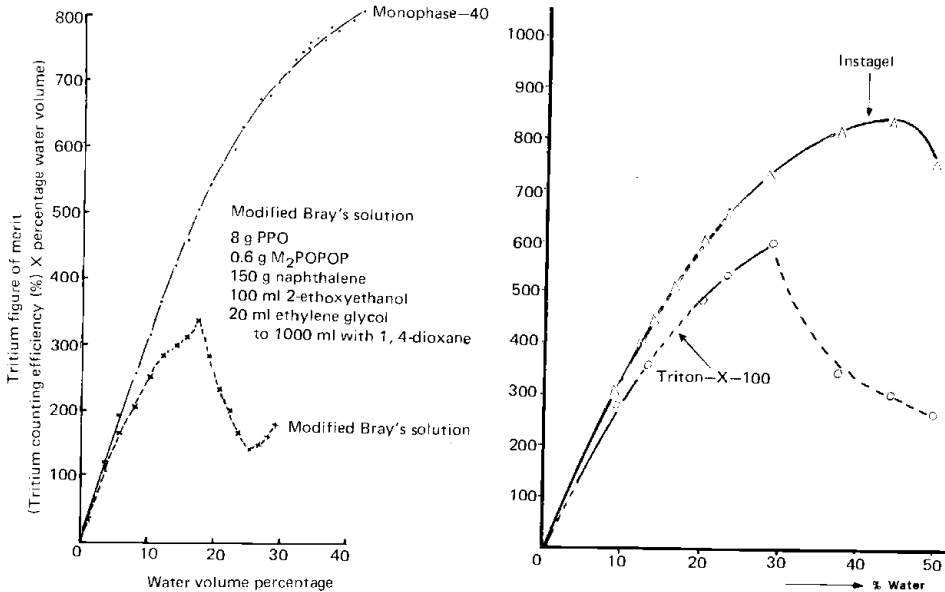


Figure 24.

V. Tarkkanen: We did this experiment only in the Insta-Gel and PCS solutions, and we did get the same efficiency in those parts of the stable regions which we tested.

M.W. Hollingsworth: You showed the difference between the chemical and colour quench curves for toluene systems. In the Insta-Gel system the points lay on the same curve. Is this generally true for these colloidal systems; for example, is PCS similar to Insta-Gel in this behaviour?

V. Tarkkanen: Insta-Gel shows similar correction curves with CHCl_3 and dinitro phenylhydrazine (yellow, the most usually occurring optical quench band) for tritium, but not for Carbon-14 when the instrument settings are optimal for counting efficiency. PCS shows different correction also for tritium when measured in the absence of an aqueous sample.

D.E. Bowyer: Insta-Gel showed little difference between chemical and colour quenching as determined by the external standard channels ratio method as given by Dr. Tarkkanen. This was presumably determined with a high energy external standard (Radium-226). Is this similarity also seen with a low energy external standard?

V. Tarkkanen: This experiment has not been performed by us using other than Radium-226.

B.W. Fox: Does Dr. Bowyer have any evidence, with respect to Insta-Gel, that there is a difference between standardisation by high energy external standard compared with low energy external standard. There is a difference in the Triton system.

D.E. Bowyer: No, I have no such evidence.

K. Painter: Users of commercial cocktails should be worried when counting tritiated plasma or serum samples that the weak β -particle may be self-absorbed by globular proteins and inaccurate d.p.m. values may result. I believe there is a publication in the literature critical of Insta-Gel on this very point. Although the data presented here is very complimentary to PCS, we would recommend to our users that digestion or combustion be used to test for self-absorption when counting tritiated serum or plasma samples.

V. Tarkkanen: As I mentioned, the term solubiliser in connection with colloidal scintillators is correct only regarding terminology within surface activity, not liquid scintillation counting: the sample must be in solution or in a colloidal form or in some cases so finely homogenised, i.e. in water or in alcohol-water, that the activity is eluted into the solution. There is one publication in which an attempt was made to count a concentrated, coarse homogenate in Insta-Gel. This is, of course, not correct and against our recommendations.

B. Scales: I am amazed that you obtain virtually identical calibration curves with chemical (thermal) and coloured quenching agents. In my experience even different chemical quenchers, let alone different colour quenchers, can give rise to different E.S.R./efficiency curves. I would like to ask if any members of the audience have any experience in the use of Insta-Gel and the production of quench correction curves? Also, how would Dr. ten Haaf explain the results?

V. Tarkkanen: Yes, different chemical quenchers can certainly give rise to different calibration curves. One should not make calibration curves with, for example, chloroform or acetone, but use water or other authentic materials as

quencher. Also various instruments give different quench curves, depending on which external standard is utilised. The curves of colour and chemical quenching can be fairly close to each other if the settings of the instrument are adjusted accordingly.

F.E. ten Haaf: I should like to point out that numerous parameters have influences on the potential difference between colour and chemical quench curves and that it would not be correct to connect this only with the sample solvent. For instance, colour and chemical quench curves for Carbon-14 diverge much less if a rather narrow measuring channel is used, than in the case of a wide or integral channel. What channel width did you use?

V. Tarkkanen: We used optimal settings regarding counting efficiency, which were not optimal for separation of colour and chemical quench. For tritium, 55% gain, discriminator 50 to 1000; and for carbon, 6% and 8 to 1000 respectively.