

Micellar Scintillators: a Rational Approach to the Design of Stable Assay Solvents for Liquid Scintillation Counting

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Liquid scintillation counting involves the efficient transfer, in solution, of emission energy from a decaying isotope to a transducing molecule and from thence, as photons, to a quantitative photomultiplier system. With the increasing use of liquid scintillation techniques there has been a parallel demand for solvents able to accommodate appreciable amounts of widely differing samples. Contemporary samples often contain low isotopic activity above background. Ideal solvents should therefore have the following properties:

1. Large volumetric capacity for aqueous samples.
2. Low background count-rates at the emission energies of the commonly used beta-emitting isotopes.
3. Temperature-dependent noise should be minimal.
4. Stable background count-rate with increasing sample volume.
5. Minimal change in counting efficiency as sample volume increases.
6. Resistance to quenching effects of a wide range of samples.
7. Dose-response curves suitable for determining counting efficiency by both internal and external standardisation techniques.
8. The light output should be a function of the energy of the sample isotope.
9. No chemical reaction between sample and scintillator or solvent molecules.

Despite the abundance of information about the scintillation properties of organic solvents and solutes in aqueous and non-aqueous environments, there is no coherent theory which will permit both the rational design of 'ideal' scintillation solvents and explain their function. Accordingly, some years ago we initiated a programme broadly designed to examine theories upon which we could base design and predict function of systems possessing features 1 to 9 above. One practical benefit envisaged was that one type of solution would cover most laboratory requirements. In the present communication we briefly review the evolution of this programme; specific details of the several aspects of the work will be reported elsewhere.

Before discussing the development of micellar scintillators, some clarification

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of terms is necessary. 'Scintillator' refers to solute molecules able to transduce radioactive decay energy to photons. This term often loosely refers to a complete system of solvents and solutes to which radioactive samples are added for assay. Such cocktails are referred to as 'scintillants' or 'liquid scintillators'. 'Fluor' is a loose term usefully describing molecules which emit photons in response to radioactive and non-radioactive stimuli such as ultraviolet or visible light, chemical or thermal stimuli. A micelle is an aggregate of molecules bound together in an ordered structure by non-covalent forces. Micellogenic molecules referred to in this review generally consist of two regions containing three parts: an hydrophilic region comprised of charged and hydrophilic moieties, and hydrophobic areas built from hydrocarbons.

EVOLUTION OF SCINTILLANTS

Several transducing systems have evolved for converting decay energy to photons with usable efficiency. The simplest preparations consist of aromatic solvents (toluene, xylene; about 9 molar) containing primary scintillators (about 25 mM) and sometimes a secondary wavelength emitter (about 0.5 mM). Energy from the beta-particle elevates some solvent molecules to their lowest excited pi-electron singlet state. These molecules are among the 350 solvent molecules per molecule of primary solute, and can translocate their energy to excite the latter into a similar state. Excited primary solute molecules emit fluorescence photons for detection by the photomultipliers of the electronic counter.

Reduced to its simplest terms the fundamental problem, then, is to incorporate water containing various solutes into toluene which contains the fluors. There are several milestones in the history of attempts to solve this problem. First, there has been extensive use of short-chain hydrophilic solvents such as 1,4-dioxane,^{1,2} ethanol⁴⁻⁷ or mixtures⁸ admixed with toluene. In some, the freezing point is depressed by polyethers.^{1,3,9} For many purposes these solvents are still satisfactory but they suffer the disadvantages of restricted sample capacity, chemiluminescence and sometimes low efficiency. The former difficulty is inherent in the type of solvent used whilst the latter effects can be partially offset by careful purification and storage of solvents. Also in this group, long-chain hydrophilic solutes such as Triton are effective but are chemiluminescent and unsuitable for assay by external standard techniques.¹⁰

In a second approach, particles of sample are gelled or suspended in the solvent by aluminium stearate,¹¹⁻¹³ Thixcin^{11,14-16} and silica.^{17,18} Apart from difficulties in sample preparation, self-quench is appreciable when particle diameters approximate to the range of the beta-particle. Thirdly, samples have been absorbed by plain or derivatised cellulose or glass-fibre papers, desiccated and counted. Such membrane techniques often have low efficiency for tritium and the assay is dependent upon orientation of the strip in the counting vial.

A central factor is that the efficiency with which a fluor emits photons depends upon its environment. This efficiency is a complex function, containing statements about the solute concentration, efficiency of transfer of energy from solvent to solute, fraction of the excited solute molecules emitting photons, efficiency of transmission of photons by the solvent, and match between the energy of the emitted fluorescence photons and spectral sensitivity of the photomultiplier system. For some fluors environmental effects are very important. For example, emission from fluorescein is pH-dependent; the quantum yield from aniline derivatives increases markedly as the molecules move to non-polar environments. Therefore prime requisites for scintillators are that the environment of the molecule should possess excellent energy transfer characteristics yet should also be correctly

properties. Figure 1 illustrates the model in terms of structural principles underlying this programme. Choline phosphoglyceride possesses two hydrocarbon 'tails' of varying length depending upon the source of the molecule. The ethanol-amine and phosphate moieties contribute both an hydrophilic character and zwitterionic charge to that region of the molecule. The hydrocarbon tails are held together by hydrophobic interactions which may total several kcal binding energy per mole. Such forces adequately maintain the hydrocarbon chains in a relatively constant and hydrophobic environment, which seems ideal for the scintillator molecule.

At the periphery of the structure the hydrophilic and zwitterionic regions are free to interact with, and hence form relatively stable (but probably dynamic) structures with charged molecules present in the aqueous phase of the sample. In Fig. 1 a protein molecule is shown performing this function but the protein could be replaced in the model by organic or inorganic structures. Into this ordered structure it is necessary to insert the extra molecules required to render the system suitable for use as a liquid scintillator.

SIMPLE MICELLAR SCINTILLATORS

We have indicated that the properties of the micelle depend upon the structure of the hydrocarbon region and the hydrophilic charged regions of the molecules forming the micellar skeleton. At the outset of this work we had no basis for predicting which structures would yield micellogenic molecules suitable for our application. A simple system was therefore examined in order to ascertain whether the principle was valid and to gain some insight into the structural aspects of the problem.

Particular attention has been given to the properties of micelles formed in aqueous systems. For several reasons, however, we wished to examine 'inverted' micelles which form in non-aqueous solvents. The lecithin-benzene²¹ system was the obvious starting point.

A first complication was that unless the lecithin was rigorously purified there was a progressive rise in the background count-rate (tritium window). Even with purified material, oxidative yellowing was associated with an increased count-rate and seemed to account for much of the quenching observed. The progressive rise in background counts could be eliminated by treatment with borohydride under photolysing conditions. Despite the increasing background count-rate of the untreated system (Fig. 2, left-hand panel) there was little change in the titration curve relating lecithin concentration to the external standard ratio (Fig. 2, right-hand panel). In dissociating the rise in background count from the origin of the quench, the latter profile confirmed the high degree of purity required for efficient function of the system. Also, this type of study, together with aqueous titration data, indicated that the zwitterionic configuration may not be an optimal charge configuration for the hydrophilic region of the molecule. An interesting feature was that addition of water and scintillator components to the lecithin-toluene solution (Fig. 3) decreased the background count rate and marginally improved the quenching characteristics (Fig. 3, right-hand panel).

Micellar structure in the lecithin-benzene system depends upon the lecithin concentration²² so it was important to determine the effects of micelle size upon the counting characteristics of the system. In the ternary system (water-lecithin-toluene), Fig. 4 implies that the counting of tritiated water best occurs when aggregates of large micelles are present. A feature of this system is that the micelles required ultrasonic energy for reasonably rapid production (left-hand panel), and that if produced in this way the ternary micelle required appreciable

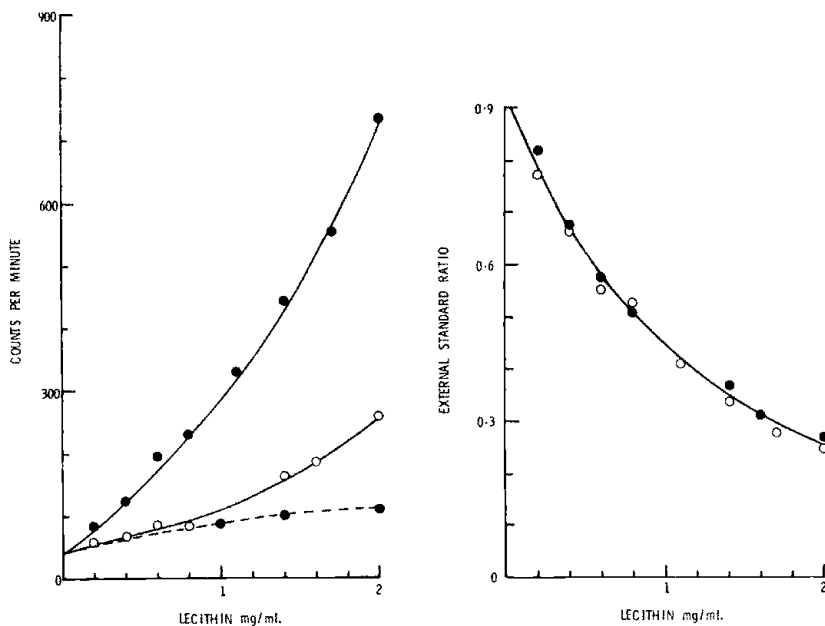


Fig. 2. The instability of lecithin associated with background count-rate in hydrocarbon micelles. Vials contained 10 ml toluene, 85 mg butyl-PBD and stated amounts of lecithin. A 'wide' tritium window was used to obtain the background count-rate in a Nuclear Enterprises 8312 Scintillation Counter (—●—●—). Vials were left for 24 h under aerobic conditions at room temperature in the dark and recounted (—○—○—). To vials, 25 mg potassium borohydride was added and the lecithin degradation products reduced for 24 h prior to adding butyl-PBD and counting (---●---●---). The latter vials are not shown on the external standard profile, but gave a similar response curve.

time to stabilise. The slight second peak at 10 h indicates that there may be an intermediate heterogeneous micelle population able to equilibrate more quickly than other classes of micelle. Stabilisation was marked by a gradual increase in count-rate and transition in the external standard ratio profiles from a rather flat response to a useful linear profile (right-hand panel).

In the binary (lecithin—toluene) system, the amount of water imbibed depends upon both the nature and concentration of the micellogenic molecule and upon the organic solvent used. As water is progressively added an end-point is reached marked by formation of macroscopic aggregates. Solution structure at this point is not precisely identified but for convenience may be viewed as two phases. The upper phase, rich in organic solvent, is often of good optical clarity. The lower phase, however, contains opaque hydrated aggregates of lecithin. We would therefore expect both phases to show different counting characteristics.

A common finding in later experiments has been that the end-point in many binary systems is preceded by formation of a clear gel. Some of these gels are unstable and subject to separation of as many as four phases so that in practise the end-point is less clearly identified in such cases. Consequently the term 'gelation—precipitation point' is used to describe a region in which phase separation occurs.

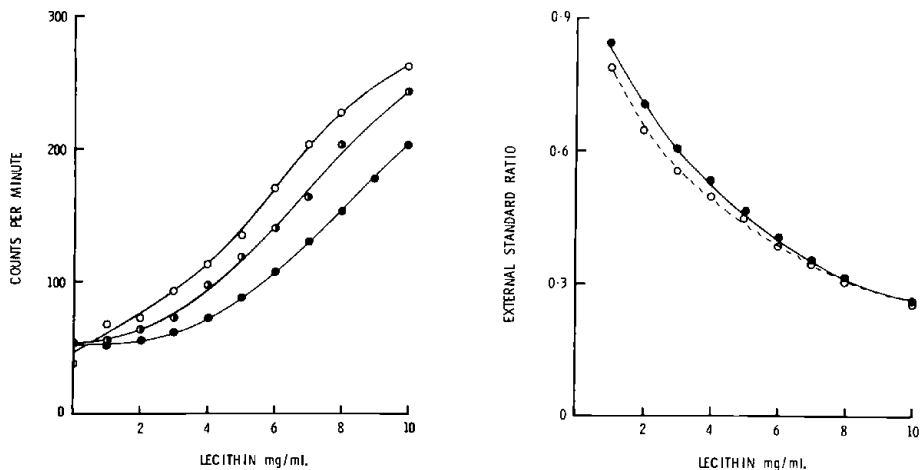


Fig. 3. Effects of scintillator and water upon the background count-rate of the lecithin-toluene micellar system. The composition of vials is given in the legend to Fig. 2, except that 200 μ l water was added ($\text{---}\circ\text{---}\circ\text{---}\circ\text{---}$) to vials containing butyl-PBD and lecithin ($\text{---}\bullet\text{---}\bullet\text{---}\bullet\text{---}$). The latter were prepared by adding 85 mg butyl-PBD to each vial after the first assay ($\text{---}\bullet\text{---}\circ\text{---}\bullet\text{---}$). Counting conditions are as for Fig. 2.

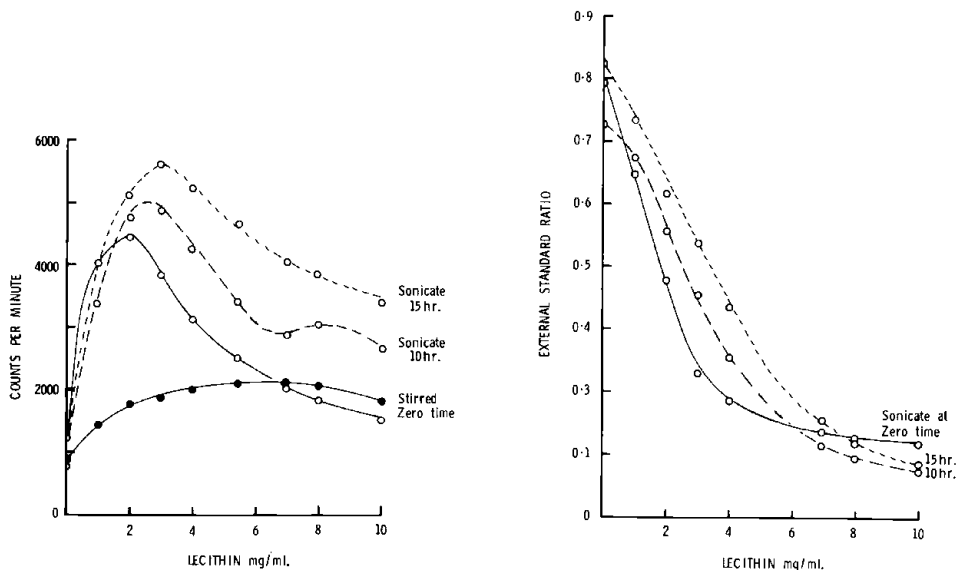


Fig. 4. Efficiency of counting tritiated water in ternary lecithin micelles produced by ultrasonic energy or mechanical stirring. The composition of vials is given in the legend to Fig. 2, except that each vial contained 3.6×10^4 d.p.m. tritium as tritiated water (100 μ l). After adding the isotope, vials were stirred mechanically for 30 min and counted ($\text{---}\bullet\text{---}\bullet\text{---}\bullet\text{---}$). Duplicate vials were sonicated at 28 kc/s, probe amplitude 5 μ for 1 min, and counted immediately ($\text{---}\circ\text{---}\circ\text{---}\circ\text{---}$), at 10 h ($\text{---}\circ\text{---}\circ\text{---}\circ\text{---}$) and finally at 15 h ($\text{---}\circ\text{---}\circ\text{---}\circ\text{---}$). A wide tritium window was used in the 8312 Counter.

Figure 5 confirms that both phases have different counting characteristics and that optimal counting in each phase occurs at different lecithin concentrations of the initial-binary solution as might be expected from partition effects. A prime feature of these experiments is that at the gelation-precipitation point the sum of

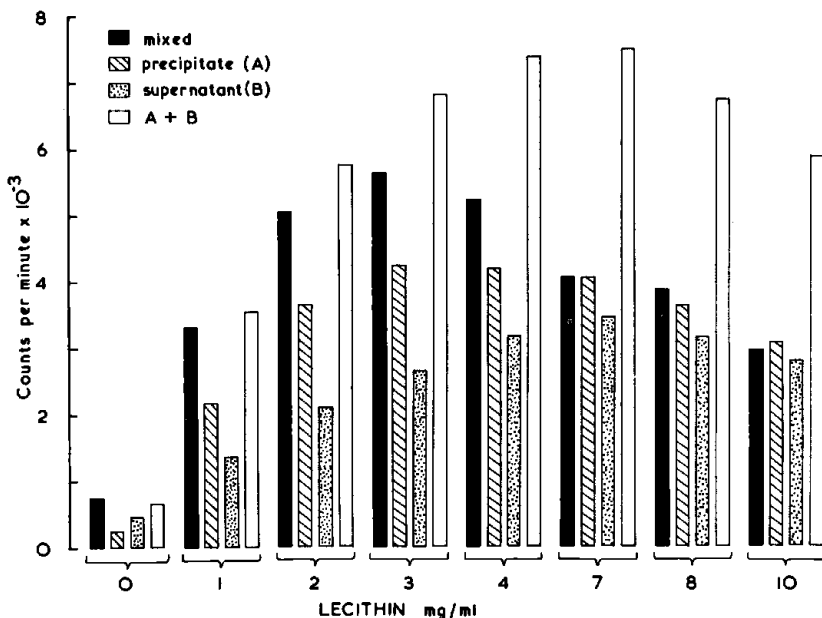


Fig. 5. Partition effects and aberrant counting at the gelation-precipitation point. Vials similar to those described in the legend to Fig. 4 were titrated with unlabelled water until the end-point was reached (see text). Each vial was thoroughly mixed and assayed (= 'mixed'). Phases were then separated under gravity for 20 h. The supernatant and precipitate of each vial was assayed separately as shown, and the separate count-rates summed (A+B).

the counts in the two individually assayed phases yields improved counting efficiency when compared with assays of the physically admixed phases. Figure 5 shows that there can be as much as 75% difference when the system is evaluated in this way.

In addition to demonstrating the validity of the concept, experiments such as these showed several encouraging features:

1. Aqueous samples could be assayed efficiently in a micellar scintillant.
2. The background count-rate could be eliminated by purification of the lecithin.
3. Acceptable dose-response profiles were obtained by external standardisation techniques.
4. Profiles obtained by external standard techniques were in good accord with those obtained by internal techniques using either tritiated water or toluene.
5. Information was obtained about the molecules required for forming the necessary micellar structures in the solvent. Stability of lecithin was a particular limiting factor in the further development of this binary system. A second difficulty was that the lecithin structure possessed characteristics which required appreciable expenditure of energy in order to establish the ternary (water-lecithin-toluene) micelles.

6. Binding energies in the micelle were sufficient to sustain stable ternary structures. Destruction of the micellar structure occurred when the aqueous capacity of the system was exceeded. This was associated with phase separation and aberrant counting.

It seemed likely that two solvent structures occurred during conversion of the system from binary (lecithin—toluene) to ternary (water—lecithin—toluene) micellar modes by addition of an aqueous sample. Figure 6 illustrates the transition from a phased solution of toluene and water (panel A) to micellar mode. When lecithin is added to the upper (toluene) phase, the bifunctional lecithin molecules tend to arrange randomly (panel B) prior to formation of micelles (panel C). Admixture of water from the lower phase doubtless produces some hydrated lecithin molecules (panel B1), but is more likely to yield an ordered ternary micelle (panel C1). At present we know very little about the static or dynamic features of these micelles. Answers to such questions are important for the rational design of micellogenic molecules for use in future systems.

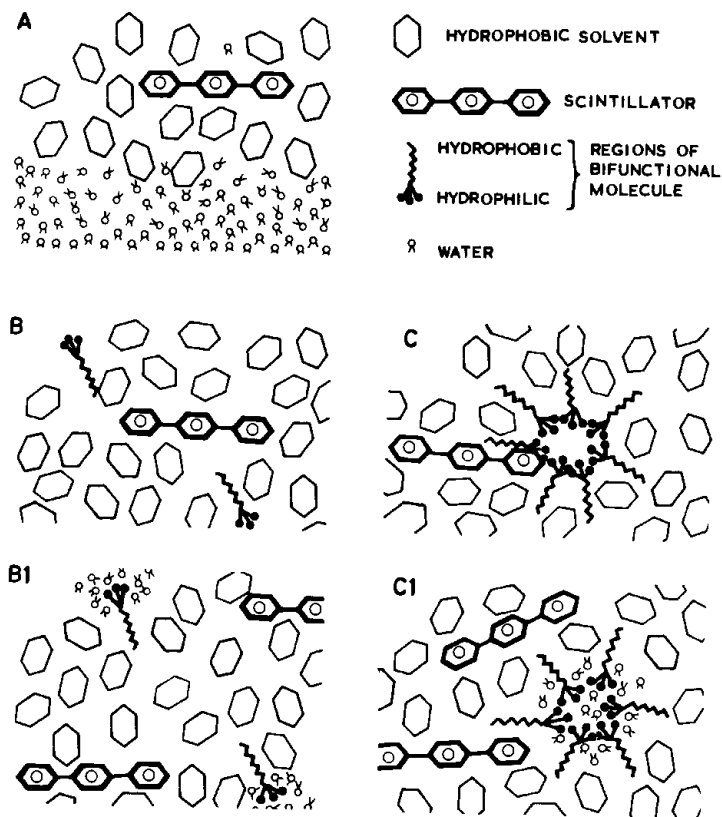


Fig. 6. Models illustrating the tendency of bifunctional micellogenic molecules to form binary and ternary micelles. The effects of micellogenic reagents upon a phased solution of toluene and water (A) are described in the text.

It was apparent from the lecithin—toluene system that in order to realise the potential of this approach it would be necessary to examine synthetic micellogenic reagents as replacements for the naturally occurring choline phosphoglyceride used in the first-generation experiments.

SECOND-GENERATION MICELLAR SCINTILLATORS

The initial problem was to dissect the lecithin molecule to determine the structural features required for optimal function in the ternary micellar system. Several questions arose: How many hydrocarbon chains are required per molecule and how long should the chains be? Are aromatic structures advantageous? Which hydrophilic groups are most appropriate? What type of charge, if any, is required at the hydrophilic terminus? These five questions gave rise to a wealth of possible experiments and organic syntheses, the number approaching Avogadro's Number in magnitude!

At the outset the experimental approach was therefore confined to studying simpler molecules and restricted to questions concerning the length of the hydrocarbon chain, the type of charge and requirements for the hydrophilic region of the molecule. As the ground was cleared we were able to examine other questions such as: What are the effects of aromaticity in the hydrocarbon moiety?

Findings from these experiments may be briefly summarised: In many situations a terminal cationic charge was particularly useful. Other solvent and sample conditions demanded charge of opposite polarity. A well-defined hydrophilic terminus is obligatory; the type of charge depends upon the samples to be added and the length of the hydrocarbon chain is to some extent dictated by the nature of the sample and the solvent of the organic phase.

Some of these features are illustrated by the data in Table 1. Here, alkylphosphate esters have been examined as micellar reagents in conjunction with different organic solvents. Similar data are obtained for certain cationic derivatives but the alkylphosphate esters are shown for they demonstrate interesting features when examined in fluorinated solvents.

In hydrocarbon solvents the major determinant of water capacity of the binary system is the structure of the organic solvent rather than the structure of the incoming water. In fluorocarbon solvents, however, there was greatly increased capacity for water, and the structure of the incoming water now contributes noticeably to the capacity of the system. Although data is not shown, structure of the alkylphosphate is also important for determining the aqueous solvent properties.

When the binary solutions are examined as scintillation reagents, hydrocarbon solvents produce high efficiency when assessed with either tritiated toluene or tritiated water. Although efficiencies are similar, there is perhaps some reduction in efficiency using tritiated water as the standard. The previous models (Fig. 6, panel B versus panel C1) indicate that there may be some less efficient energy transfer from tritium in the hydrated core of large micelles to scintillant molecules in the hydrophobic domain around such micelles.

Methyl substitution of the aromatic solvent decreases water capacity and increases counting efficiency. This is true for both the hydrocarbon and fluorocarbon series, though anomalous behaviour is observed with the latter as will be seen below. The basis for such effects may lie in non-covalent interactions between fluor, alkylphosphate esters and solvent, though this remains to be explored in detail. The ternary solutions have similar optical clarity in the visible range so an as yet unidentified modification of micellar structure, in turn dependent upon solvent structure, may be responsible for improved energy transfer in the system.

Fluorocarbon solvents show markedly improved water capacity when compared with hydrocarbon analogues (Table 1) but show appreciable quench for tritium. Similar reduction of efficiency for both standard tritiated toluene and tritiated water implies an appreciable impedance to energy transmission through the

Table 1. Relationship between hydrophilic properties and tritium counting efficiency in alkylphosphate micellar systems.

Solvent	End point by titration with ml		Background (c.p.m.)	Tritium added as		Ratio B/A
	H ₂ O	D ₂ O		Toluene A (c.p.m.)	Water B (c.p.m.)	
∅H	0.25	0.22	50	9,557	47,625	4.98
∅Me	0.155	0.152	45	11,890	55,386	4.66
m∅Me ₂	0.071	0.067	50	12,645	54,553	4.31
∅F	1.2*	0.9*	38	2,246	12,501	5.57
∅CF ₃	0.55	0.43	45	3,535	10,833	3.06
m∅(CF ₃) ₂	T	T	48	4,789	2,615	0.55

Alkoxydecylphosphate esters were examined at a concentration of 75 g/litre in the solvents shown. ∅ refers to the C₆H₅ structure except where prefixed by m, in which case it refers to the C₆H₄ nucleus in the meta-configuration. Me refers to methyl. Assays were performed with a wide tritium window in order to demonstrate the quenching effects of the fluorocarbon series upon the background count-rate. All solutions contained butyl-PBD 8.5g/litre. The end-point in titrations with water and deuterium oxide is the gelation-precipitation point of the system. T refers to an indefinite, turbid end-point occurring soon after starting the titration. * indicates that the titration end-point had not been reached. Efficiencies were determined in 7 ml solution containing water (first column) plus the stated isotope.

hydrophobic domain of these systems. An important exception to this seems to be trifluoromethylxylene which shows some eleven-fold greater counting efficiency for toluene compared with water at the end-point.

Several proposals can be advanced to explain such findings (Fig. 7). Stabilised layers, composed of scintillator- and toluene-rich hydrophobic regions, could be sited upon a scintillator-poor lamina. As shown in the diagram, the micelle may invert and produce increased light output despite the increased amount of water. Exploration is necessary to understand and perhaps take advantage of this curious phenomenon.

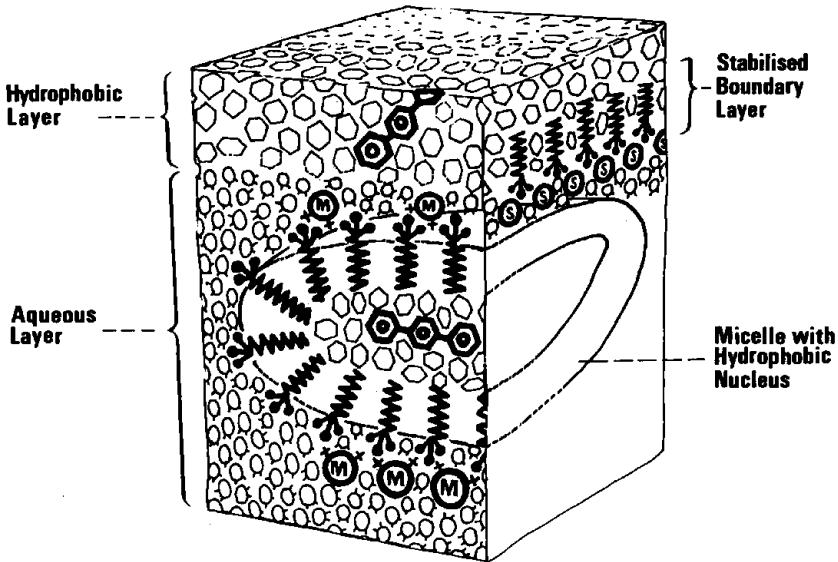


Fig. 7. Some stable structures of the ternary (water-lectithin-toluene) system at the gelation-precipitation point. An inverted micelle is shown with the hydrophilic charged regions stabilised by a complementary cation (M) from the sample. At the boundary between the upper toluene and lower aqueous layers the micellogenic reagent is shown to be stabilised by molecules of high affinity (S) derived also from the sample. In both cases the scintillator is located in the toluene phase. Symbols are identical with those shown in Fig. 6.

CONTEMPORARY AND FUTURE MICELLAR SCINTILLATORS

During evolution of this programme it was evident that the micellar concept not only provided highly efficient scintillants with good capacity for a wide range of samples, but also offered a useful theoretical approach to the design of functional systems. Experiments of the type outlined above delineated the following important design principles.

The structure of the micelle is an important determinant of both counting efficiency and the fidelity of external standardisation. Binary and ternary micellar systems are relatively free from unwanted thermal noise components of the background count-rate. Macroscopic phase transitions are undesirable because of their association with anomalous counting and unreliable response to standardisation by both internal and external methods. To determine isotope dose with absolute veracity in such a situation would require an internal standard of isotope

molecules identical with those in the sample. Kinetic characteristics of molecules forming the micelle matrix should be such that stable ternary micelles rapidly form on addition of sample. The scintillant must be stable for use and storage at room temperature.

A series of contemporary micellar scintillators has evolved²³ from these studies. The solutions show extensive tolerance to a wide range of samples produced in current biochemical and clinical practice. For example, protein solutions such as human plasma, or chaotropic reagents such as urea are absorbed and counted with ease. An important feature is that good efficiency is sustained as sample volume is increased. Figure 8 (left-hand panel) shows the titration profiles for plasma in a micellar scintillant compared with a commercial product currently available. Identical values of quench are obtained when tritiated water and tritiated toluene are used as internal standards. Hence the external standard can be used to advantage to monitor efficiency (Fig. 8, right-hand panel). Other samples seem to follow this example.

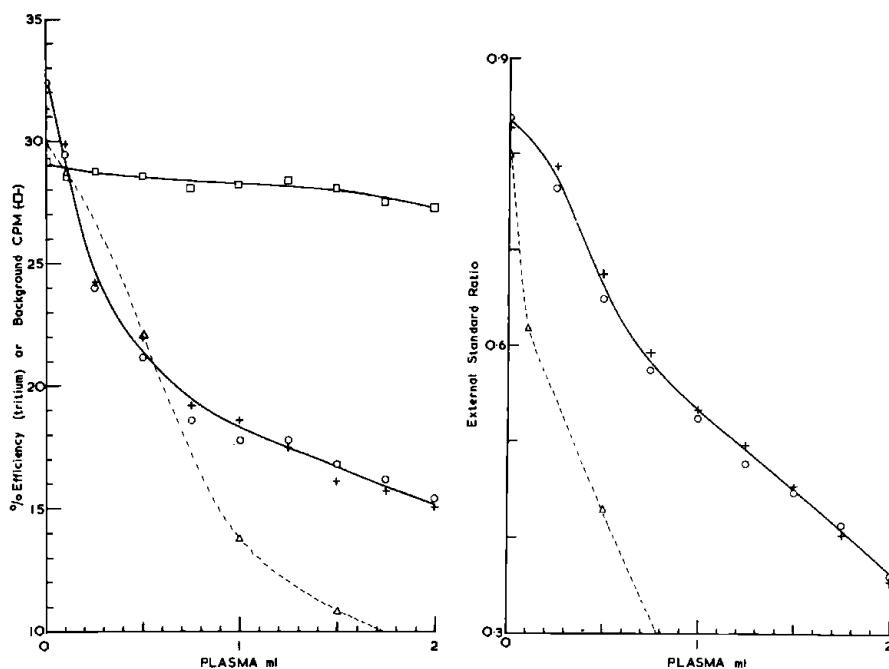


Fig. 8. The assay of human plasma in a micellar scintillant. The indicated volumes of fresh plasma were added to a series of vials containing 10 ml of a micellar scintillant²³ and efficiency determined by addition of standard tritiated water (○) or tritiated toluene (+), and the External Standard Ratio determined for each set of vials. Tritium window, ambient temperature, N.E. 8312 Counter. A currently available gel scintillator is shown for comparison (----), the efficiency in this case being determined by tritiated toluene.

There seem to be several directions which future work may take. Some of the unexplained findings with experimental micellar scintillants need to be analysed in detail to improve our understanding of the structure and function of these systems. These avenues lead towards the design of micellogenic molecules possessing an

intrinsic fluor region. At the same time a generation of Reticular Scintillants is envisaged. In these, the solution structure appears rather like a liquid crystal (Fig. 9) containing scintillators and isotopes in their traditional domains. Large sample capacity and high light output are attractive theoretical features of these structures.

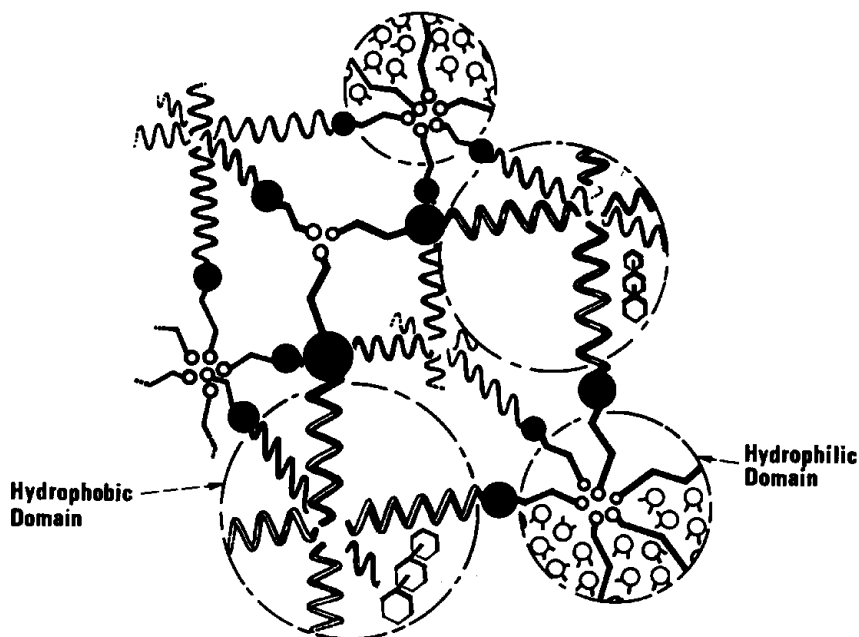


Fig. 9. A model of the solution structure of a Reticular Scintillant. Symbols are similar to those used previously (Fig. 6), except that the molecules of the lattice are shown with three defined regions rather than the two regions shown in the diagrams of the micellogenic reagents.

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DISCUSSION

C.J. Palais: External standard ratio was correlated to lecithin concentration. Did the authors also try to correlate this ratio to counting efficiency for the various concentrations? If so, what were the results?

N.G.L. Harding: As I have indicated, lecithin provided us with a means for exploring the scintillant properties of inverted ternary micelles. Tritium efficiency was between 15–25% depending upon the purity of the lecithin and mechanism by which the micelles were produced. If the lecithin was rigorously purified under anaerobic conditions, counting efficiency was independent of lecithin concentration in the working range. However, I have shown our profiles with the external standard values to emphasise first that quench can arise from instability of the micellogenic reagent, that this quench is concentration-dependant and, finally, that in the micellar system such quench can be competently quantified by the external standardisation technique. I should perhaps add that during our work we have been concerned to examine the response of micellar scintillants to the external standard for a couple of reasons. First, as an isotope user I am very conscious of the value in not having to dispense aliquots of standard calibrated isotopes and then recount one's vials. Secondly, as a simple-minded computer user I find that the less curvilinear are response profiles, the more easy it is to devise programs to analyse the data.