

Optimisation in Colloid Scintillation Counting

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The colloid counting system has been shown to be a potentially useful means of assaying those solutions derived from biochemical experiments which contain many solutes other than the ones being measured and in which the labelled material is present in very low specific activity. Many such solutions have been examined by this general procedure (for review see Ref. 1), but the composition of the colloid system used has been the result of an empirical application of a composition, often used for a completely different solution than for the one intended.

In an earlier communication,² it was shown that the type of system used depended to a very considerable extent on the composition of the solution and that the final choice of mixture was highly dependent on the composition of this solution.

I wish to make it quite clear at the outset that my intentions are quite different from those of the many manufacturers of these colloid scintillant mixtures. I am attempting to measure very low specific activity solutions, given the fact that several millilitres of the aqueous solution is available and I want to be able to take full advantage of the concept of 'merit value'² to be able to measure the few counts present, where other methods of assaying, by aliquot sampling, precipitation, etc., fail. This aim differs from that of the industrial exploitation of the colloid system in that they require a water accepting mixture, which will also accept a varying type and quantity of other solutions and count with a reasonably high efficiency. However, in all these systems, there is inevitably considerable loss of the counting potential of the system, as the region of maximum value is nearly always confined only to a small area in the total contour diagram. It is clearly useful to be able to compare one ternary system with another from this point of view and to be able to judge the general properties of a scintillant system with regard to its general counting and stability characteristics at a glance.

In order to prepare a contour diagram, a triangular board, cut out and marked as in Fig. 1, is used. In the first instance, 0.5 ml of a concentrated scintillant solution in the primary solvent under study is added to each vial. Starting from the detergent:aqueous sample edge, 0.5 ml of pure primary solvent is added to the first row of vials (representing the 10% primary solvent level). 1.5 ml is added to the next row and each row with an incremental increase of 1 ml, until the last

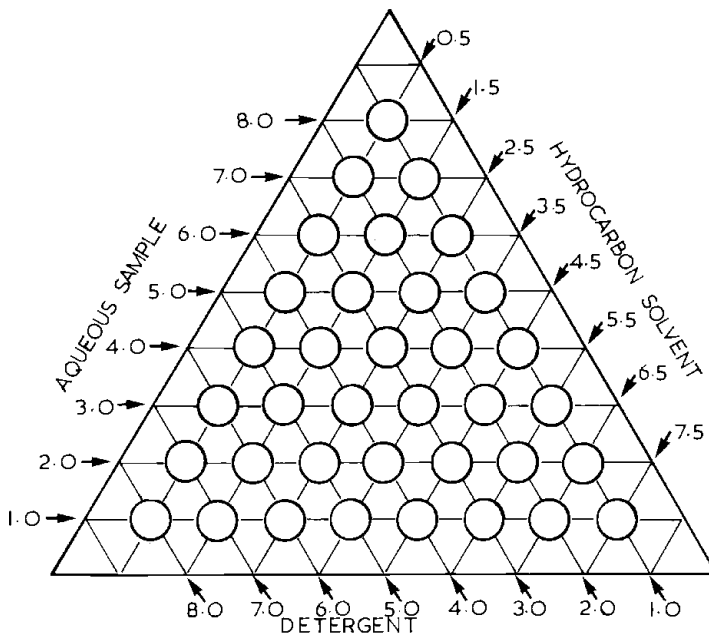


Fig. 1. The construction of a contour diagram. It is usually convenient to regard the lower left-hand corner as 100% detergent, the lower right-hand corner as 100% primary hydrocarbon solvent and the apex as 100% aqueous sample. The additions indicated are those of the pure components following addition of the concentrated scintillant solution (0.5 ml) to each vial.

bottle at the hydrocarbon solvent corner contains 8 ml of primary solvent (i.e. 7.5 ml pure solvent + 0.5 ml concentrated scintillant solution). Then, starting from the primary solvent:aqueous sample edge, 1 ml of detergent is added to the first two, 2.0 ml to the second and so on until the bottle at the detergent corner is reached when 8.0 ml is dispensed. A similar sequence of fillings with aqueous sample is conducted from the detergent:primary solvent border to the aqueous sample corner. The bottles are thoroughly shaken and the backgrounds taken to see if there is any contamination or unnecessary chemiluminescence interference.

Following spiking 10 μ l of tritiated water to all the samples and thorough mixing, they are again counted after assessing for opacity by an empirical system (from 0 to 5 indicating clear to completely opaque). The degree of viscosity is also assessed on a 0 to 5 scale varying from completely fluid, like toluene, to a solid gel which remains rigid on inversion of the vial. Colloid stability (not counting stability) is assessed by a similar empirical set of numbers from completely stable for the duration of the experiment (1) to a rapid separation into two phases (4). If these values are independently obtained and are then plotted on triangular co-ordinate papers, it can be seen that contours may be constructed around the figures. Where apparently great changes in character occur in an unusual region, sub-division around that region may reveal a local change in this parameter.

The resulting diagrams for the system Triton X-100:xylene:water (PPO and POPOP as scintillants) are shown in Figs. 2, 3 and 4 for appearance, viscosity and colloid stability respectively. The resulting contours are by no means

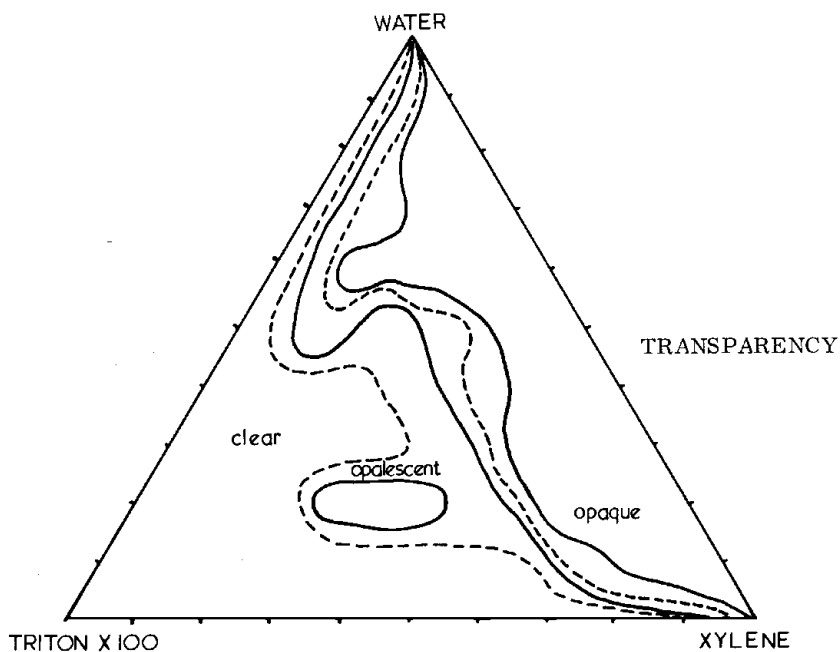


Fig.2. The contour diagram of the appearance of the system Triton X-100: xylene:water at 21°C. The contours are constructed to enclose areas of similar appearance (see text).

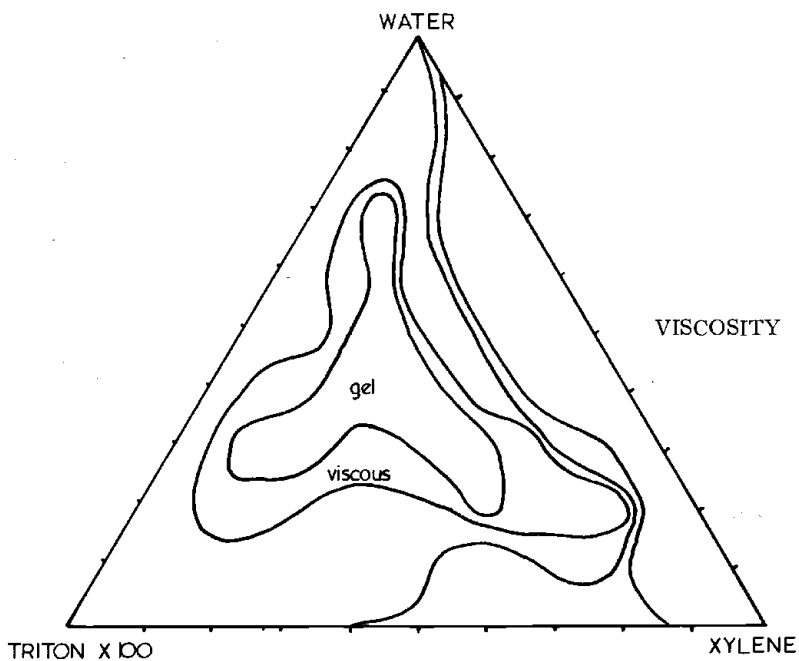


Fig.3. The contour diagram describing the viscosity of the samples (see text)

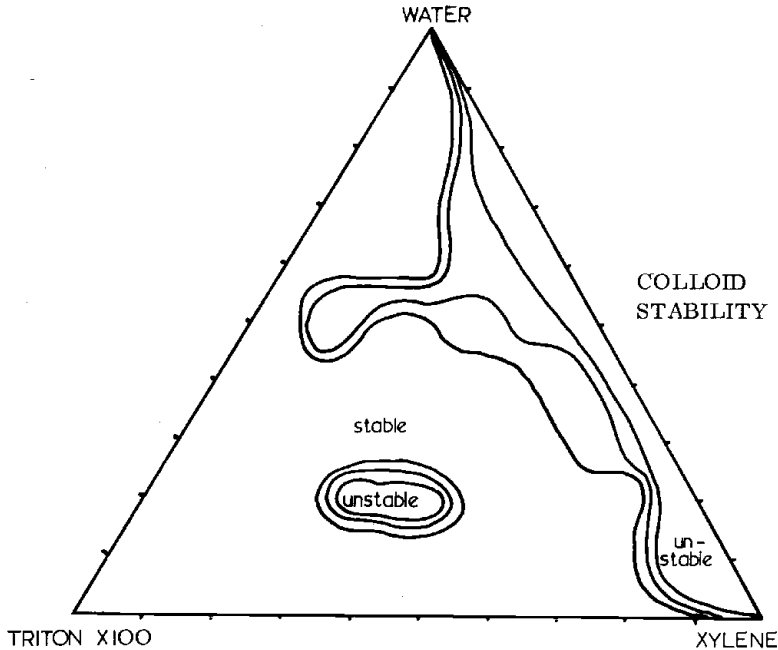


Fig. 4. The contour diagram illustrating the regions of colloid stability (not counting stability) ranging from an unstable region, separating very soon after mixing, to a stable composition which remains the same after several days.

superimposable, and bear no obvious relationship to one another, but are clearly continuous within one parameter consideration. It is therefore not possible to construct such diagrams on vague descriptions employing two or more of these parameters, such as a cloudy gel or transparent fluid.

However, none of these parameters are really important from the point of view of liquid scintillation counting. Only two criteria are primarily important: (a) the merit value (i.e. the product of percentage efficiency and the percentage of sample) and (b) the counting stability. For the purpose of assaying this latter quantity, at least five recycled samples spread evenly over 48 h were taken. The standard deviation of the repeated counts were computed and in order that each sample could be compared with each other on an equal basis, the coefficient of variation was determined, i.e. the quotient (expressed as a percentage) of the standard deviation to the mean counts. Plotting both these parameters gives the contour diagram combination shown in Fig. 5. It can be seen that it is possible to select either a point which has a high merit value with some sacrifice in counting stability (A) or the reverse, high counting stability with some loss of merit value (B). The choice will depend on the operator's particular requirements and tolerances. When the appropriate point is chosen, the level of scintillant most suitable may be determined by varying the proportion of the concentrated scintillant solution to that of the primary solvent in that particular sample. The secondary solute appears to play a much more important role in this system than in an equivalent homogeneous system but the reason for this difference is not yet clear.

In an attempt to describe such a system with a unique value, a 'merit stability

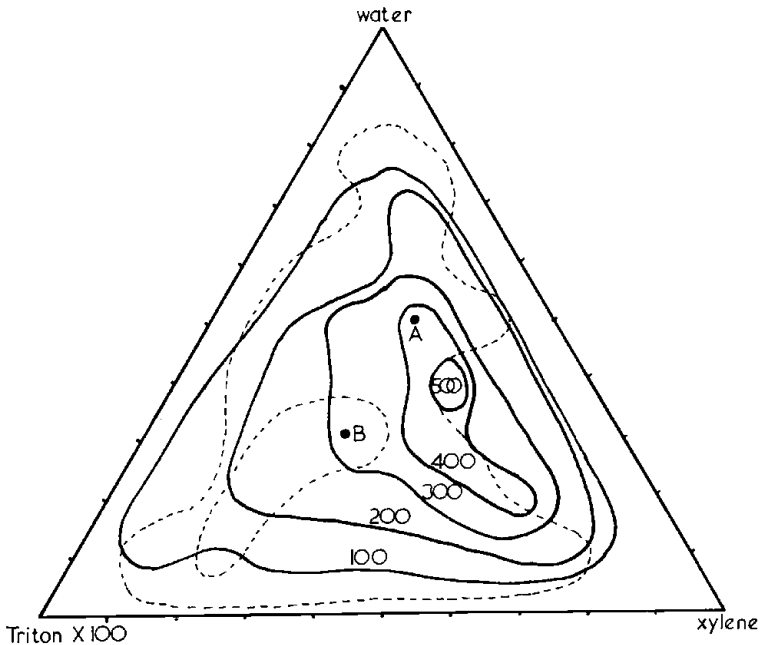


Fig. 5. A composite contour diagram for the system Triton X-100:xylylene:water, showing merit values in contour steps of 100(—) and coefficient of variation (----). Inner contour less than 0.5%, outer contour encloses the area less than 1.0%. Position 'A' has a merit value of 447 and a coefficient of variation of 0.98%, whereas position 'B' has a merit value of 329 with a coefficient of variation of 0.33%. The highest merit value of 552 has a coefficient of variation of 7.5% and is clearly unsuitable for counting.

quotient' (MSQ) is suggested which is simply the merit value/coefficient of variation corrected for machine efficiency, i.e.

$$\text{MSQ} = \frac{\text{Merit value}}{\text{Coeff. of variation}} \times \frac{100}{\text{Machine eff. (Ref.)}}$$

The machine efficiency could be the reference standard for that particular isotope supplied with the machine or a 4 g/litre solution of PPO in toluene, with or without 0.02 g POPOP depending on photomultiplier type.

In view of the considerable sensitivity of this figure to small changes in coefficient of variation, it is tentatively suggested that the logarithm of this figure could be used. The figure is still not satisfactory, however, since it is only meaningful if there is always some instability. When the log values are plotted on the triangular co-ordinate system, there is a clear distinction between two systems, one showing instability over a wide area (Fig. 6) and the other (Fig. 7) showing a relatively larger area of stability, and would be presumably more favourable to the requirements of the manufacturer. However, the pattern can only give a broad impression about the system, and to obtain the more exact conditions the superimposition of the two parameters as indicated in Fig. 5 must remain the most useful method of selection.

However, Table 1 shows some maximum \log_{10} MSQ values for some systems tried. It must be emphasised, however, that these conditions and values are

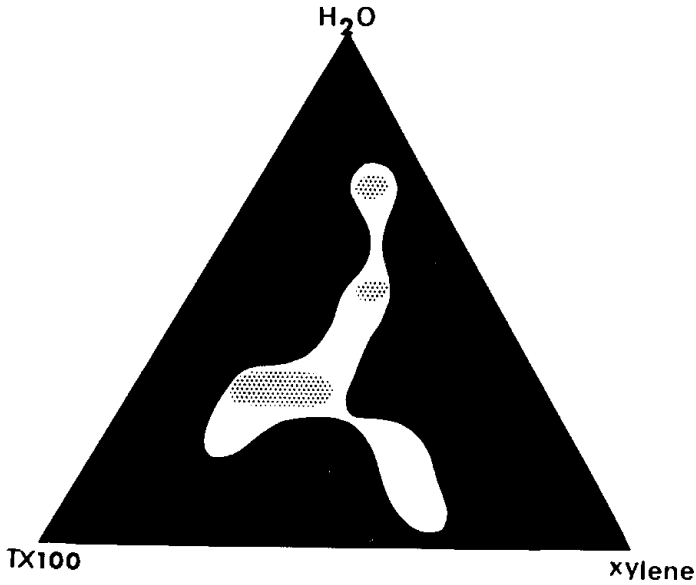


Fig. 6. Plot of \log_{10} MSQ for the system Triton X-100:xylylene:water, with PPO/POPOP as scintillant. The three small areas enclosed within the clear area are regions where the value is greater than 3.0, whereas the black outer area is less than 2.5 and represents either low stability or poor merit values.

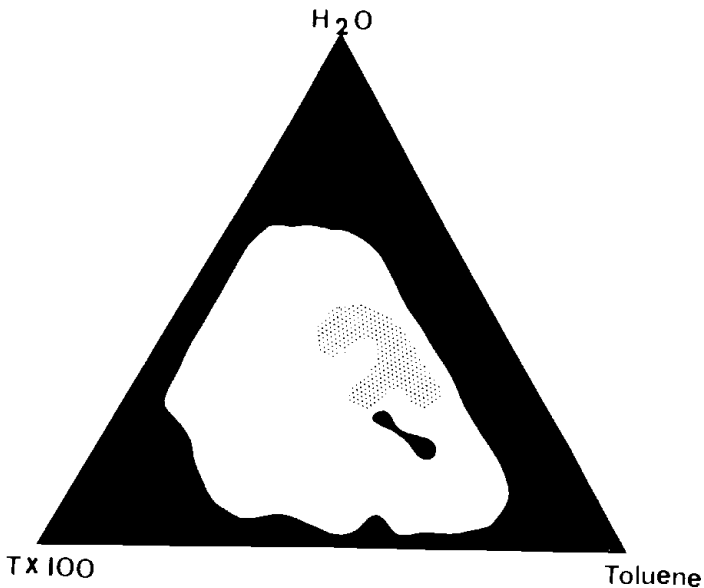


Fig. 7. Plot of \log_{10} MSQ numbers for the system Triton X-100:toluene:water (PPO and POPOP as scintillants). It can be seen that the unshaded area representing \log_{10} MSQ between 2.5 and 3.0 indicate that a greater region of the area is considered to be potentially useful for counting.

Table 1. The relative merit stability quotients of a number of ternary systems.

| Aqueous solution | Detergent | Primary solvent | Secondary solvent | Primary solution | Secondary solution | MSQ | \log_{10} MSQ |
|------------------|-----------|-----------------|-------------------|------------------|--------------------|------|-----------------|
| Water | Tx 100 | Toluene | — | PPC | POPOP | 2395 | 3.38 |
| Water | Tx 100 | Xylene | — | PPO | POPOP | 2073 | 3.32 |
| Water | Tx 114 | Xylene | — | PPO | POPOP | 1436 | 3.14 |
| Water | TN 101 | Xylene | — | PPO | bisMSB | 1333 | 3.13 |
| Water | Tx 57 | Toluene | — | PPO | POPOP | 960 | 2.98 |
| Water | Tx 45 | Toluene | — | PPO | POPOP | 593 | 2.77 |
| Water | Tx 100 | Xylene | Naphth | PPO | POPOP | 542 | 2.73 |

valid in these cases only for water containing tritiated water standard. Recently, the factor MSQ has been modified to take into account its considerable sensitivity to low coefficients of variation. A more realistic figure, abbreviated to MISQ, is defined as follows:

$$\text{MISQ} = \frac{\text{Merit value}}{1 + \% \text{ coeff. variation}} \times \frac{100}{\text{Ref. efficiency}}$$

When the coefficient of variation is zero, the MISQ becomes an instrument efficiency corrected merit value and will thus be capable of being used as a comparative figure to describe the potential efficiency of a colloid scintillation mixture. The maximum MISQ for toluene-water detergent systems would thus be

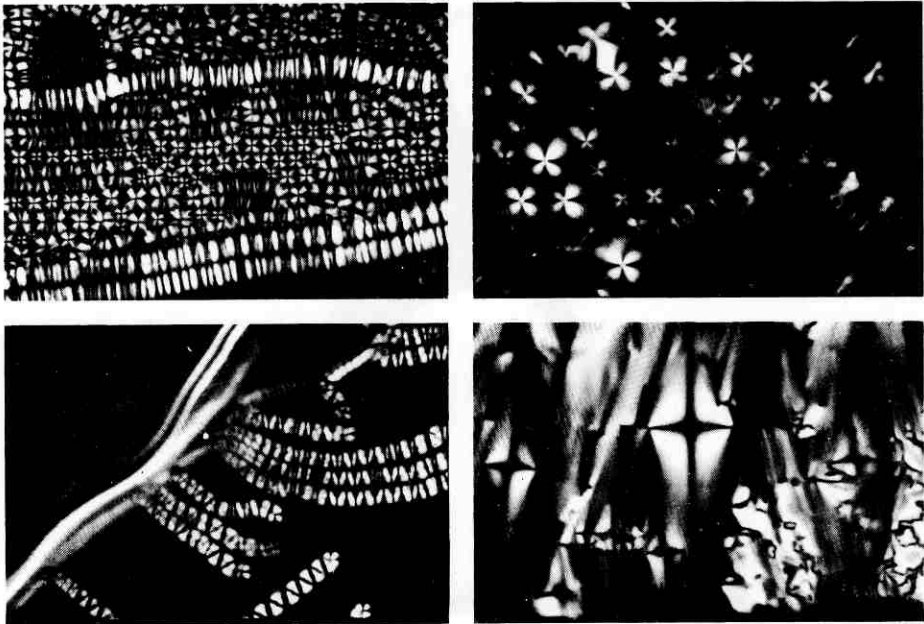


Fig. 8. The plates show four of the many different appearances under crossed polaroid optics of regions of the birefringent zone of the system Triton X-114:xylene:water (approx. 5:1:4). The fluctuating temperature of the microscope stage can change the complex structure dramatically in short periods of time (magnification approx. 600).

related to the detergent as follows, Triton X-100 (1014), Triton N101 (507), Triton X57 (368), Triton X45 (297) and clearly indicates that amongst these detergents, with PPO/POPOP as solutes, Triton X100 shows the best potentialities for the most sensitive detection of $^3\text{H-H}_2\text{O}$.

As an added interest, a number of selected regions from a ternary combination of Triton X-114:xylylene:water were examined through crossed polaroid filters at approximately 600 magnification and a number of these are indicated in Fig. 8. It can be seen that a remarkable ordered arrangement of these colloid systems exists which exhibits a great variety of liquid crystalline structures. The variation of such structures clearly go a long way in accounting for the extreme versatility of the system and the potential value of the system in studying membrane and similar structures can be envisaged.

REFERENCES

- 1 E. Rapkin, 'Gel and Emulsion Counting of Aqueous Solutions', Digitechniques published by Intertechnique.
- 2 B. W. Fox, in Liquid Scintillation Counting, Vol. 2 (Eds. M. Crook, P. Johnson and B. Scales), Heyden & Son, London, 1972, p.189.

DISCUSSION

J.F. Stoutjesdijk: You add 0.5 ml of a concentrated PPO-solution and so the PPO-concentration in the organic phase is not constant. Cannot this give differences in counting efficiency?

B.W. Fox: When you are undertaking this kind of analysis using phase diagrams, you must decide whether to have the same level of PPO in each vial or the same concentration of PPO per ml toluene in each vial. From a practical point of view, therefore, we decided on the first approach to attempt to modify the level of PPO in the chosen vial to determine final optimal working conditions. If the second method is chosen, it would be very difficult to interpret the results due to self-quenching effects in the toluene-rich regions.

J.F. Stoutjesdijk: Did you investigate Insta-Gel with different amounts of H_2O ?

B.W. Fox: Yes.

F.E.L. ten Haaf: You mentioned a particular sample which even showed phase separation but nevertheless had good counting stability. Wouldn't this depend rather strongly on the phase in which the marker is dissolved?

B.W. Fox: Yes, but I included it as an example to show that good stability may sometimes be achieved even under such conditions. It also demonstrates that the visual appearance is no index of the true structure, since it is likely that both so-called phases are probably colloidal.

K. Painter: We have noted variations in efficiency and stability of Triton systems based upon the order of addition of components. Have you noticed any similar variations?

B.W. Fox: No. We always add the aqueous component last and ensure that the whole is thoroughly mixed, either on a rotary mixer or by warming and cooling, before counting. We have not examined possible effects due to order of mixing.

P.E. Stanley: Batches of Triton X-100 vary somewhat. Do your MSQ values change with different batches of the detergent?

B.W. Fox: We buy our batches of Triton X-100 in bulk and consequently we have not been able to compare batches since the same batch has been used throughout. This is an interesting point and it should be done.