

LIQUID SCINTILLATION COUNTING
RECENT APPLICATIONS AND DEVELOPMENT
VOLUME II. SAMPLE PREPARATION AND APPLICATIONS

THE IMPORTANCE OF PHASE CONTACT IN SOLGEL
SCINTILLATOR-AQUEOUS SAMPLE SYSTEMS

Royal H. Benson

Monsanto Chemical Intermediates Co.
Texas City, Texas

INTRODUCTION

It is now well accepted that a two phase system exists at all significant sample concentrations in the solgel scintillator-aqueous sample system. Further, phase contact between the aqueous sample phase and the organic scintillator phase has been shown to be virtually identical to true solution contact in order to give the excellent counting efficiencies and nearly linear water quenching curves which are obtainable with the better solgel scintillators (Benson, 1976). Most investigators have assumed either a constant degree of phase contact or phase contact equivalent to true solution contact throughout the usable concentration range afforded by the commercial solgel scintillators. In most cases this is an error.

For our purposes, I have defined perfect phase contact as the intimate and uniform dispersion of the aqueous sample in the solgel scintillator in dimensions of nearly molecular size which results in no detectable phase-specific absorption of the energy of the tritium beta particle. Achievement of this ideal contact should be indicated by identical counting efficiencies for all soluble tritium-labeled compounds at any given aqueous sample concentration. For example, with perfect phase contact, the counting efficiency for tritium-labeled organic molecules and tritiated water samples should be the same at any concentration, 1 through 55% by volume, of water in the presently available solgel scintillators. Use of a

scintillator with bad phase contact characteristics can give mysterious errors in results regardless of the type of quench correction technique being used. This is true because the counting efficiency of the sample will be a function of the distribution coefficient of the tritium-labeled compound. This results in an error not correctable by external standardization or by internal standardization unless the internal standard has a distribution coefficient identical with the labeled molecule in the sample. Further, since phase contact in some solgel scintillators varies with the aqueous sample concentration, the accuracy of the quench correction will vary with sample size. In short, phase contact in solgel scintillators is at present an undefined variable which can result in large and unsuspected errors in experimental results in the radioassay of tritium-labeled compounds.

EXPERIMENTAL

Early attempts to define phase contact by use of tritiated water and tritium-labeled organics were unsuccessful due to a variety of problems ranging from variations between vials to sample matrix, absolute efficiency measurement problems and lack of recognition that phase contact variations can occur over surprisingly small concentration ranges. In the method presented here, all of these common problems, with the exception of statistics and 48 hours spectrometer stability, are eliminated.

Stock solutions of tritium-labeled water and tritium-labeled toluene of about 1 $\mu\text{Ci/mL}$ are used. The solgel scintillator samples, 10 mL (Volumetric pipette), are pipetted into each of twenty four vials (Wheaton Glass Company). Into vials 1 through 12 are added 100 μL of tritium-labeled water (100 μL Hamilton syringe with Chaney Adapter) and 100 μL of inert toluene. Into vials 13 through 24 are added 100 μL of tritium-labeled toluene and 100 μL of inert distilled water. The vials are capped, shaken and the entire group of 24 are placed in the liquid scintillation spectrometer (Packard Tri-Carb, model 3310). After cooling and dark adapting, they are counted (100 sec. counting period) at least ten times each and preferably overnight which should give about twenty measurements on each. The average count rate of each sample is computed and recorded. The vials are removed from the spectrometer and distilled water is added (Class A burette, 25 mL with .05 mL subdivisions having a Teflon stopcock and fine point tip) to vials 2 through 12 to give water concentrations of 5 through 55% by volume in 5% increments. The

procedure is repeated for vials 14 through 24, giving two sets of samples identical in every respect except for the identity of the tritium-labeled compound. The amount of distilled water added should take into account the 0.10 mL already present in each vial and should be rounded off to the nearest .05 mL subdivision. This will make it easier to reproduce exactly the amount of water in vials having the same nominal concentrations. The vials, which should be at room temperature by this time, are shaken well and returned to the scintillation spectrometer for a second series of counts. A minimum 6 hour cool-down and equilibration period is recommended (Benson, 1976) before counting overnight. For the second series of counts, about twenty determinations per vial are recommended and the new average count rate of each sample is computed and recorded.

The phase contact ratio for each concentration is calculated in two steps. First, the relative counting efficiency for each sample in both groups is calculated using the following equation:

$$\% \text{ Relative Counting Efficiency} = \frac{\text{final count rate}}{\text{initial count rate}} \times 100$$

A plot of these data, if desired, give the familiar water sample quenching curve for the solgel scintillator. The phase contact ratio for each concentration is then calculated by dividing the relative counting efficiency for tritiated water at each concentration by the relative counting efficiency for the tritium-labeled toluene sample at the same concentration:

Phase Contact Ratio =

$$\frac{\% \text{ Relative Counting eff. for Tritiated Water}}{\% \text{ Relative Counting eff. for Tritiated Toluene}}$$

The values of phase contact ratio are then plotted against the water sample concentration to give a curve where problem areas are readily apparent. Obviously a phase contact ratio of 1.00 at all concentrations represents ideal performance, showing perfect phase contact and equal counting efficiency for soluble tritium-labeled compounds at all concentrations regardless of partition coefficient.

RESULTS

The following figures (1,2) are computer-fitted plots of phase contact ratio for several of the better commercially

available solgel scintillators. The figures are largely self-explanatory. It should be noted that any deviation from a phase contact ratio of 1.00 represents a potential problem area. The high value at 55% for Aquasol II (New England Nuclear Corp.) and the low value for PCS (Amersham Corp.) scintillator result from separation problems, also observed visually, at high aqueous sample concentrations. Scintiverse (Fisher Scientific Co.), using Triton X-100 as a gelling agent, shows an exceptionally short usable concentration range. The dip exhibited by Instagel (Packard Inst. Co.) appears to be a characteristic problem associated with the inflection region of the water quenching curve which is believed to be the phase inversion region. Poor phase contact is only one of the problems observed in the 15-20% concentration region for most solgel scintillators. This is a range to be avoided whenever possible. Some of the scintillators exhibit considerable variation in phase contact ratio at different concentrations, making their application very difficult for radioassay where the highest accuracy and best possible precision are required.

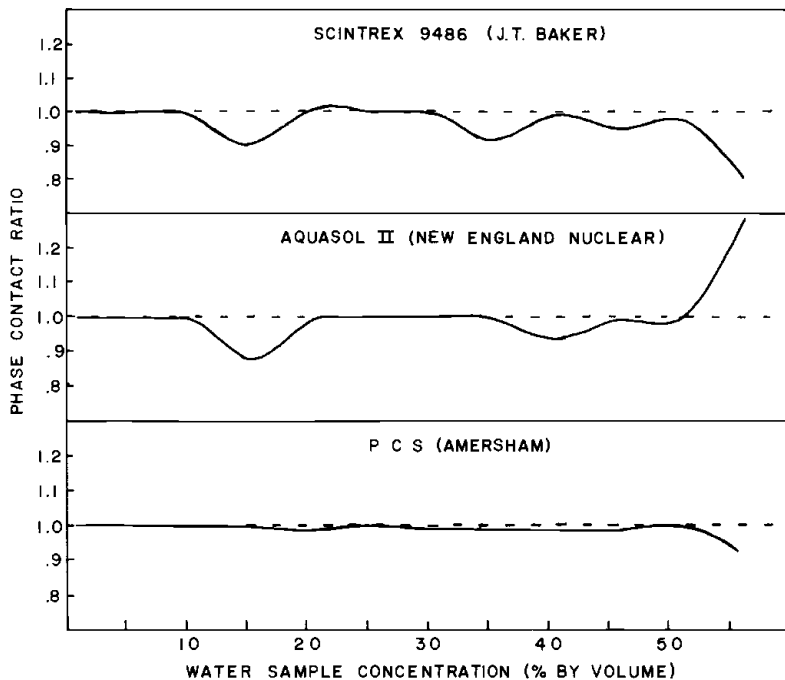


FIGURE 1. Variation in Phase Contact Ratio For Various Solgel Scintillators

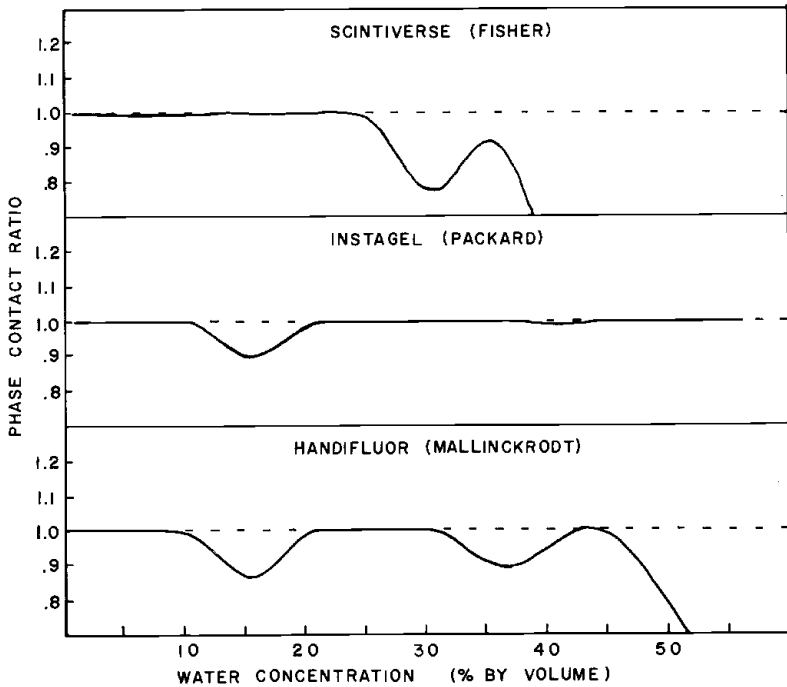


FIGURE 2. Variation in Phase Contact Ratio for Various Solgel Scintillators

It should also be pointed out that the phase contact ratio is independent of absolute counting efficiency. Wide variations in counting efficiency were observed during the collection of these data for the different commercial products. One of the best phase contact ratio curves was given by a solgel scintillator having relatively poor absolute efficiency. This is almost certainly the result of a manufacturer's decision to sacrifice efficiency to achieve linearity by the use of the higher gelling agent concentration observed in the product.

DISCUSSION

The precision of the method described here depends primarily on counting statistics and spectrometer stability. The phase contact ratios obtained should be accurate to about 0.5% at 95% confidence limits. The technique described here cancels out most of the common minor sources of error in liquid scintillation counting, for example:

1. Vial differences are eliminated as a source of error since the relative counting efficiencies are determined on samples in the same vial.
2. Differences in the amount of tritium in each vial do not cause an error since the relative counting efficiency results from the change in count rate and is independent of the initial value.
3. Vial positioning errors in the spectrometer are eliminated by averaging a large number of determinations.
4. Counting statistics are based on the average of at least ten determinations where, for the worst case, a total of about one million counts are accumulated. Statistically, the average should be good to $\pm 0.2\%$ (95% confidence).
5. The Hamilton syringes with Chaney adaptor deliver the same amount of sample each time to within 1%. The errors resulting from even these minor variations in sample size are self cancelling.
6. The errors resulting from the addition of distilled water to reach the various concentrations should be negligible if done with care. For example, to reach a concentration of exactly 10% by volume, 1.022 mL of water should be added. In order to avoid the errors resulting from attempting to extrapolate between divisions, a volume of 1.05 mL is used which results in a true concentration of 10.22% by volume. This is designated at the 10% sample. Since exactly the same volume is used on the tritiated water as on the comparable tritiated toluene sample, the final concentrations are the same in each vial and the errors cancel. Adding quantities of water that are measurable exactly on burette calibration marks tremendously improves the ability to add equal amounts to comparable vials.

Assuming perfect spectrometer stability, which is monitored by the values for the 1% concentration samples, the most likely source of error is in counting the samples for the second round before they are completely equilibrated.

It should be pointed out that the selection of tritiated toluene and tritiated water to measure phase contact was an attempt to select labeled molecules having extreme dissimilar partition coefficients. Toluene has virtually zero solubility in water and should be totally confined to the organic scintillator phase of the counting sample system. Tritium-labeled water should have essentially zero solubility in the

aromatic scintillator phase and should be confined to the aqueous phase of the system. Possibly the selection of tritium-labeled xylene, rather than toluene, would be even more ideal since the manufacturers must use xylene as the primary solvent. Although not widely known, the selection of xylene avoids really insurmountable problems in phase contact which result when practically any other aromatic solvent is used to formulate solgel scintillators. The better energy transfer characteristics of xylene are incidental and are not the compelling reason for the selection.

When this method was first applied to solgel scintillators which gave results showing perfect phase contact existed up through 55% by volume of aqueous sample, a conflict appeared. Earlier work (Benson, 1976) had shown that a plot of the relative figure of merit (RFM), vs weight % aqueous sample deviated significantly from the theoretical curve which should result from a solgel scintillator with perfect phase contact. Plots of the water quenching curves used to obtain data showing perfect phase contact ratios continued to show deviations identical to the earlier data from the theoretical curve. Here then, was a baffling puzzle. How was it possible for a solgel scintillator with perfect phase contact to

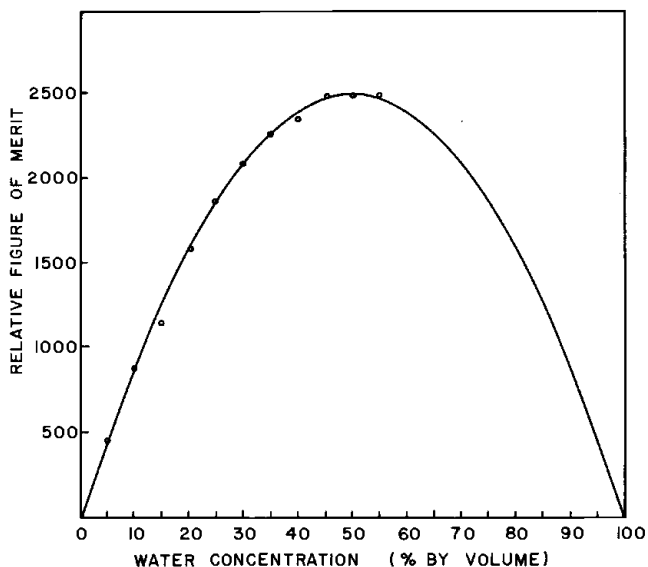


FIGURE 3. Data Plot of the RFM from 5 mL Scintillator Samples on the Theoretical Curve for Perfect Phase Contact

deviate from the theoretical curve? Both sets of data were incontrovertible and readily reproducible. After considerable thought, the water quenching curves were rerun over the 1-55% concentration range using 5 mL of scintillator. The total volume of the 1% sample was 5.050 mL and the 55% sample was 11.111 mL. These new data for the solgel scintillators now fit precisely on the theoretical curve (Figure 3). The earlier deviation which I had reported was actually the result of increasing scintillation light losses from the counting vial as the sample volume was increased from 10.10 mL to 22.25 mL and was not the result of less than perfect phase contact. In the new method just described here for the phase contact ratio, errors due to reduced counting efficiency resulting from light losses from the vial are cancelled out and the method correctly indicated that ideal phase contact existed.

CONCLUSIONS

A unique method for the precise measurement of phase contact in solgel scintillators has been developed. When applied to various commercial scintillators, some have shown concentration regions where large deviations from perfect phase contact occur and where conventional quench correction techniques may not result in accurate corrections. Lack of knowledge of these regions and of the potential problems associated with them can lead to considerable experimental error in routine use. The new method has pointed out an earlier error in solgel scintillator quenching curves, compared to a theoretical ideal curve, which was due to light losses from counting vials at large sample volumes. Both phase contact ratio and the comparison of water quenching curves to theory now show phase contact equivalent to true solution contact over most of the 1 through 55% by volume concentration range for the better solgel scintillators.

REFERENCES

Benson, R. H., *Int. J. App. Rad. and Isotopes*, 27, 667 (1976).