

DETERMINATION OF SAMPLE HOMOGENEITY
BY LIQUID SCINTILLATION

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INTRODUCTION

Analytical methods which use liquid scintillation counting involve the measurement of radioactivity by incorporating the radiolabelled analyte into essentially true homogeneous solution with the fluors in the scintillation cocktail. A difficulty encountered when preparing radioactive samples for liquid scintillation counting is the radio-chemical sample compatibility with the scintillation medium. Emulsifer-type scintillation cocktails are designed to incorporate the aqueous sample into intimate contact with the fluors in the organic phase. Different types of radioactive samples and new preparation techniques require an ensured knowledge of sample dissolution in order to obtain meaningful results from liquid scintillation counting experiments. Quench correction cannot be applied to heterogeneous samples which result due to phase separation or a significant change in radionuclide solution geometry. Incorrect quench correction results in nonreproducible determinations of sample activity, DPM.

Few advances have been accomplished in liquid scintillation counting which provide a universal criterion to evaluate true solution counting. The Dual Ratio technique¹ has been the single most important method used to identify nonhomogeneous radioactive analytes in a scintillation cocktail. A shortcoming of this method lies in that it is only applicable in single label analysis. At present, there is a method which checks cocktail phase separation². This method is applicable to multiple radiolabelled analytes in samples which result in two distinct liquid phases, both of which must be scintillating. In dual phase samples, if only one of the two phases scintillates, i.e. adsorption on inert support, this method does not work. This method is based solely upon the accumulated combined Compton spectra

of ^{137}Cs produced in each phase. Because of this principle, this method falsely identifies samples of radiolabelled analytes adsorbed onto solid supports (filter disc, inside vial wall).

The advent of microprocessor controlled instrumentation capable of spectral analysis³ led us to explore the possibility of determining radionuclide homogeneity for multiple radiolabels in a single cocktail.

Heterogeneous samples typically result when a significant change in the counting environment causes loss of physical contact between the radiolabelled analyte and the fluors of the scintillation cocktail. Radiolabelled analytes which have become partially or totally removed from the organic phase produce different radionuclide counting efficiencies depending upon the extent of dissolution in each phase. Hydrophilic cocktails in which the aqueous holding capacity has been exceeded results in phase separation. Nearly all emulsifier-type cocktails have regions of instability in which the aqueous material is not in solution. The extent of these regions will be dependent on temperature, volume of cocktail, volume of analyte, solutes in the analyte, and time. A nonpolar organosoluble radiochemical will always remain in true solution within an emulsifier cocktail although the cocktail appears to be phase separated. Geometry of the radiochemical species is essentially 4π in relation to the organic scintillating phase for true solution samples. In heterogeneous samples, the external standard measurement indicates physical changes occurring within the organic scintillation phase. The external source radiation can not effectively reflect the geometric distribution of the radiocompound(s). Since the radiochemical is in close contact with the scintillation medium, changes in the physical counting environment in relation to the radionuclide distribution would be indicated by a modified sample pulse height energy distribution.

This report describes a new technique to monitor radiochemical homogeneity within the scintillation cocktail by observing the nuclear interactions with the physical environment during measurement. This technique employs both the external standard to describe the cocktail efficiency and the radionuclide(s) contribution, to evaluate

the impact of changing geometry on nuclear emissions in singly- as well as multiply-labelled samples.

EXPERIMENTAL

The pulse height energy distribution spectrum of the external standard source and radionuclides of interest were accumulated using a Tri-Carb® 460 and Tri-Carb® series 4660 liquid scintillation spectrometer with Spectralyzer™ (Packard Instrument Co.). Although initial development of this technique was accomplished using a Packard scintillation counter with a ^{226}Ra external standard source, similar studies involving sources of ^{137}Cs and $^{133\text{m}}\text{Ba}$ were conducted. Both instruments were temperature controlled to 8°C during the experiment. After each measurement the contents of the Spectralyzer were examined and both the external standard first moment of distribution and the observed endpoint of the more energetic radiolabelled analyte were measured.

In separate experiments a tritium and a ^{14}C -radiolabelled amino acid in 0.01 M HCl (New England Nuclear) was added to 20.0 mL glass scintillation vials containing different emulsifier type cocktails. Various emulsifier type scintillation systems were used in this study. Instagel® and Monophase 40® which are mixed xylene-based cocktails (Packard Instrument) along with PicoFluor 30® pseudocumene-based cocktails (Packard Instrument) were used. Optifluor™ (Packard Instrument), also an emulsifier cocktail, was used in this study. Toluene scintillator (PPO, Me_2POPOP) was another cocktail used in selected experiments. Prior to adding 50 μL of the radiolabelled analytes, each cocktail was loaded with distilled deionized water to produce 1.5% to 48.9% aqueous concentrations in a series of ten vials. Total solution volume ranged from approximately 11.5 mL to 20.0 mL corresponding to the increasing aqueous percentage with a fixed cocktail volume of 10.0 mL. Approximately 150,000 cpm of tritium or carbon-14 were contained in each aliquot. Following the addition of the radioactivity the vials were incubated for 15 minutes at 37°C to ensure maximum homogeneity, then chilled to 8°C. The samples were allowed to dark adapt, temperature equilibrate, and stabilize (emulsion) for 5 hours before starting the assay. The following

experiments which made use of these emulsifier cocktails involve similar preparation techniques.

In another experiment, a ^{14}C -labelled amino acid $1.5 - 2.5 \times 10^4$ cpm in water-containing cocktail (1.5, 12.2, 16.0 and 48.9% v/v water) was counted using Instagel as the scintillation solution.

Similar activity levels of a ^{14}C -amino acid were evaporated in situ on glass membrane filter discs or evaporated on the inside wall of a glass scintillation vial. These samples were designed to simulate adsorption. Toluene scintillator (PPO, Me_2POPOP) was used as the liquid scintillation cocktail in these samples to ensure heterogeneity.

Dual-label samples containing a tritium and a ^{14}C -labelled amino acid (tritium activity from 1074-20637 dpm and carbon-14 activity from 728-13217 dpm) were mixed in different ratios, then added to an emulsifier-type cocktail containing 30% v/v water.

Triply-labelled samples were prepared using equivalent amounts of activity (approximately 30,000 cpm) of tritium, ^{14}C and ^{36}Cl . Each of the radiolabelled analytes was organosoluble. Increasing amounts of nitromethane were used to induce cocktail quenching in 15.0 mL of toluene scintillator. Although both the dual and triply-labelled samples were homogeneous in nature, they were used to demonstrate the applicability of this technique in multiple label experiments.

In separate experiments samples of iodine-125 (sodium iodide in sodium thiosulfate solution) and phosphorous-32 (orthophosphoric acid in 0.01 N HCl) were prepared using Instagel and PicoFluor 15. Each quench series for a given cocktail contained a different level of iodine-125 or phosphorous-32 activities. These levels of activity ranged from approximately 100 cpm to 150,000 cpm. Both cocktails were loaded with distilled deionized water (1.5% to 48.9% v/v). Each vial contained 10.0 mL of cocktail.

The radioactivity in each sample described was measured by the Tri-Carb scintillation spectrometer for a statistical level of precision in the total accumulated counts not to exceed 0.5 at 2 sigma percent, or 10 minutes.

RESULTS AND DISCUSSIONS

The initial experiments set out to characterize the accumulated pulse height energy spectrum for ^{226}Ra , the external standard. The accumulated pulse height energy distribution spectrum was obtained by interrogating the stored contents of the Spectralyzer after termination of each sample measurement. A shift in the ^{226}Ra external standard apparent endpoint in phase separated samples was observed.

As increasing amounts of aqueous quenching agent were added to the emulsifier-type cocktails, the spectral endpoint shifted to lower levels. This result was expected since an energy spectrum will naturally compress to lower energies as the degree of cocktail quenching increases. By increasing the amount of water to produce a break region in the cocktail, the anticipated external standard endpoint would have been at still lower energies. However, in these particular samples which phase separated, the external standard endpoint shifted to higher energies. In solgel emulsifier-type cocktails which produce stable emulsions (gels) at high water concentrations (25 - 50%), the external standard endpoint followed a continually-decreasing pattern as first observed with small amounts of water (1 - 15%). Other common external standards such as ^{137}Cs and $^{133\text{m}}\text{Ba}$ were evaluated for similar spectral behavior. Each external standard produced a monotonic quench efficiency correlation using SIE as the quench indicating parameter. In multiphase samples the observed spectral endpoint increased with a concomitant increase in the first moment of distribution (SIE)³ using each external standard. A more pronounced endpoint shift was observed with the moderate-range external standards (^{137}Cs and $^{133\text{m}}\text{Ba}$) than was observed with ^{226}Ra . It was apparent that phase separation was related to a simultaneous increase in both the measured SIE and the external standard endpoint. Since the external standard could not provide sufficient information relating to radionuclide dissolution, we observed the behavior of the sample spectrum by pulse height analysis. With aqueous soluble radiolabelled analytes in emulsifier type cocktails, increasing the degree of aqueous quenching resulted in a continual decrease of the sample endpoint, as expected. In many of the samples which had phase

separated the sample endpoint decreased abnormally for the corresponding amount of quenching agent. Samples, with compositions near the break-region concentrations, in hydrophilic cocktails produced a higher observed radionuclide endpoint than in phase separated samples. The decrease in the observed radionuclide endpoint and the concomitant increase in the measured SIE immediately revealed the possibilities of this technique.

Various methods used to determine the radionuclide endpoint were evaluated. Two basic approaches were initially considered. The first approach involved the determination of radionuclide endpoint by significant counts per channel above nominal background. The other method involved curve fitting techniques. In liquid scintillation spectroscopy almost all beta emission spectra produce a similar pulse shape in the upper 1/3 of the energy distribution region. This spectral feature was used in estimating the radionuclide endpoint. The relationship which resulted from this work would correlate the observed radionuclide endpoint to the measured SIE. Each of the endpoint determinations worked reasonably well. Using curve fitting techniques, the endpoint determination was reproducible and applicable to all spectral shapes encountered, as the energy distributions compressed with increasing quench. Some radionuclides produce complex pulse height spectra, i.e. ^{125}I , but curve fitting analysis can be accomplished. Since the radionuclide spectrum was now involved in the analysis, our original goal was fulfilled. Heterogeneity in multiple labelled samples would be determined by the measured endpoint of the more energetic emitting radionuclide in the sample regardless of the dissolution of the lower energy radionuclide(s).

Homogeneous solutions of a given radionuclide produce a nearly linear relationship between the higher energy measured endpoint and SIE (Figure 1). This correlation applied to all tested cocktails, hydrophobic and hydrophilic, over a wide range in cocktail quenching. This relationship was defined by a simple equation for each radionuclide. Deviation from this relationship by greater than $\pm 4\%$ indicated a heterogeneous sample. Alteration of radionuclide counting geometry not related to phase separation, i.e. adsorption, also produced data points which deviated from the homogeneous relationship.

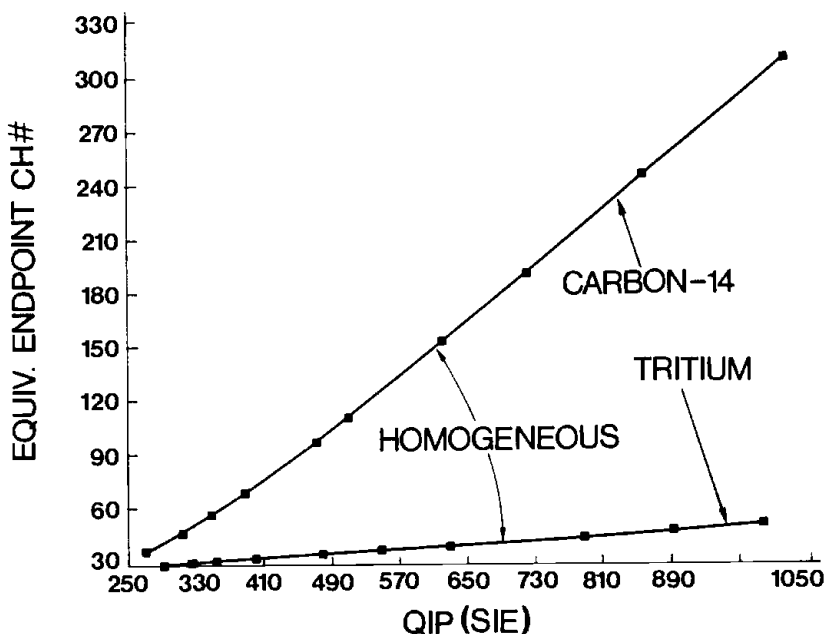


Figure 1. ^{14}C endpoint vs QIP (SIE)

In a similar set of experiments using both ^{137}Cs or $^{133\text{m}}\text{Ba}$ as the external standard, a nearly linear relationship between the measured radionuclide endpoint and the external standard first moment was observed. Although this study was conducted using a Packard instrument which supports the use of ^{226}Ra as an external standard source, similar results may be obtained using other manufacturers' instruments. There is a unique correlation for each radionuclide to the maximum observed energy for a particular external standard. However, the liquid scintillation counter must have the capability to accurately determine the radionuclide spectral endpoint in order to utilize this technique.

Water-soluble tritium and ^{14}C -labelled analytes were used to

generate normal and phase-separated samples in various emulsifier-type cocktails. The colloidal counting systems were capable of maintaining intimate contact between the organic fluor phase of the cocktail and the water-soluble radiolabelled analyte under recommended conditions. Since each radioactive sample contained approximately 150,000 cpm the total assay count time to accumulate the pulse height spectrum was approximately 1.5 minutes. The radionuclide equivalent endpoint and SIE were measured for each sample. The results, when plotted, were nearly identical to the results obtained using the sealed toluene standards. Data for all emulsifier cocktails were plotted on a single graph to demonstrate the homogeneous nature of the counting environment at water concentrations ranging from 1.5% to 48.9% (Figures 2,3). Nonstable samples (phase separation) in which the aqueous holding capacity had been exceeded resulted in data points which deviated significantly from the correlation established using known homogeneous counting solutions. For each emulsifier cocktail studied, similar heterogeneous samples containing tritium or ^{14}C were identified. Essentially the same water concentrations in various emulsifier cocktails produced different degrees of quenching. The average measured endpoint for tritium and ^{14}C in moderately quenched homogeneous samples is shown in Table 1. Several emulsifier-type cocktails produced similar SIE values under different aqueous quenching conditions with water-soluble radiolabelled analytes. Tritiated and ^{14}C -labelled toluene in toluene scintillator were also included in this comparison.

Carbon-14 labelled amino acids (approximately 150 cpm and 25,000 cpm) in water-containing (1.5%, 12.2% and 48.9%) cocktail were counted as homogeneous solutions (Table 2). A radiolabelled ^{14}C -amino acid evaporated in situ on glass membrane filter discs or on the inside walls of glass vials was identified as heterogeneous mixtures (Figure 3).

Iodine-125 produces a complex pulse height energy distribution in liquid scintillation spectroscopy. However, the same method of quench correction used for beta emitters can also be applied to iodine-125 in liquid scintillation counting⁴. The maximum measured endpoint

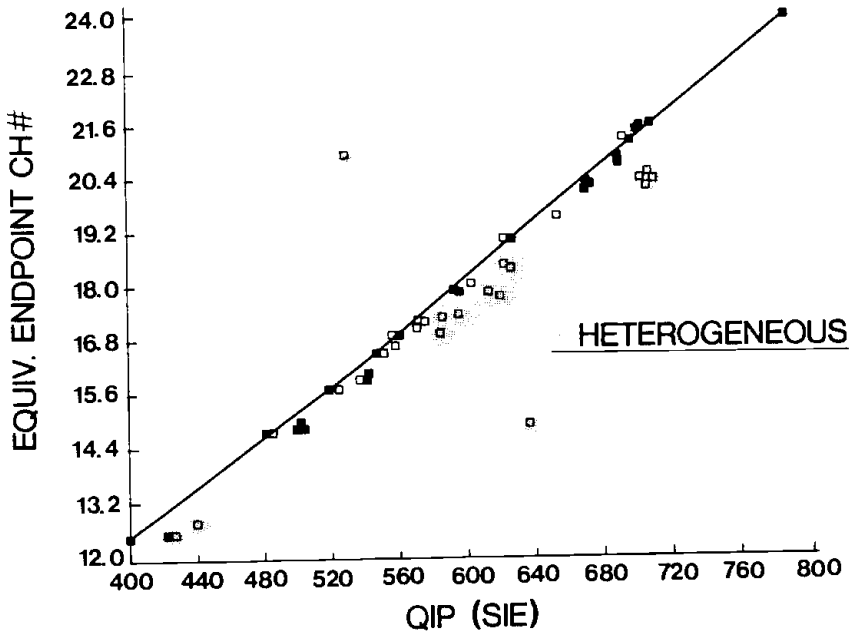


Figure 2. ^3H endpoint vs QIP (SIE) emulsifier cocktails, 1.5 48.9% aqueous

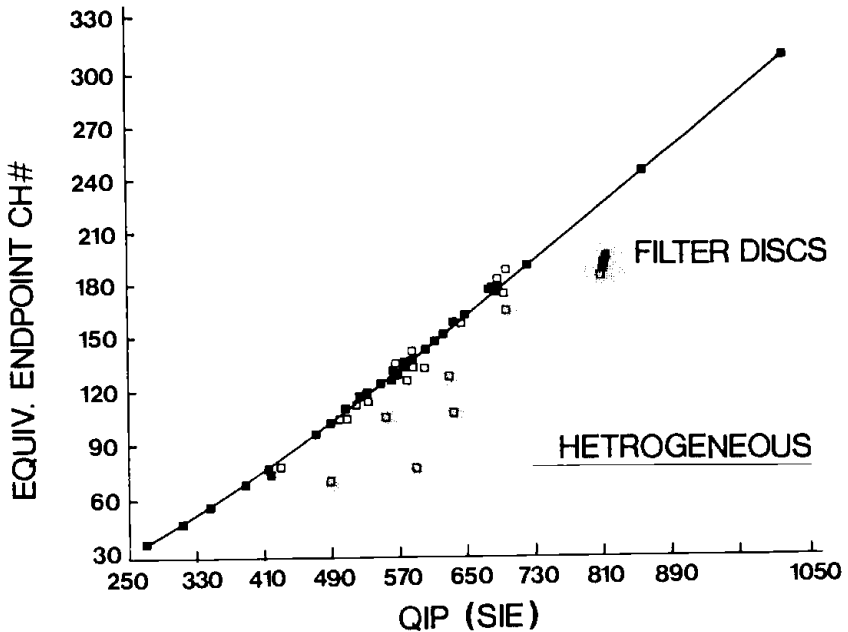


Figure 3. ^{14}C endpoint vs QIP (SIE) emulsifier cocktails

Table 1. Radionuclide Endpoint Determination Homogeneous Counting Solutions*

Radionuclide	SIE	Endpoint Channel
^3H	553 \pm 0.85%	16.64 \pm 1.04%
^{14}C	548 \pm 1.81%	121.84 \pm 3.71%

* Cocktails: Toluene scintillator (PPO, Me₂POPOP)
 Instagel®
 Monophase 40®
 Pico-Fluor 30®

Table 2. Carbon-14 Endpoint Determination in Emulsifier Cocktail (Instagel®)

^{14}C cpm	SIE	%H ₂ O	Counts/Channel	Endpoint Channel
150,200,250 2500, 25000	719 \pm 0.14%	1.5%	8.4 - 1212	184.9 \pm 1.39%
150-25000	607 \pm 0.12%	12.2%	10.4 - 1582	144.60 \pm 1.13%
150-25000	621 \pm 0.15%	16.0%	11.19 - 1633	129.70 \pm 1.68%
150-25000	442 \pm 0.15%	48.9%	16.80 - 2267	89.53 \pm 4.52%

energy of unquenched ^{125}I was reported as approximately 80 KeV⁴. The ^{125}I radionuclide endpoint, as a function of SIE, generates a curve which lies between that already shown for tritium and ^{14}C (Figure 1). Similarly, the curve for phosphorous-32 lies above the ^{14}C curve. In previous experiments using ^{63}Ni as the radionuclide of interest, a similar curve which lies between ^{125}I and tritium was obtained. Thus, each radionuclide produces similar correlation curves unique to the maximum endpoint energy and spectral compression.

In aqueous samples with SIE values between 450 and 700, and ^{125}I activity of 2500 cpm to 150,000 cpm, statistically accurate endpoint

determinations were measured using this technique. Heterogeneous samples such as filter discs were rapidly identified as shown in Figure 4. After several days the same samples, containing ^{125}I (as sodium iodide) on filter discs, were observed to become more homogeneous. The longer the interval between counting, the closer the data points fall to the true solution relationship. Iodine-125 is known to be a reactive anion which can undergo halide exchange and iodinate solvent or fluors, producing intimate radionuclide dissolution which results in true solution counting performance. The measured endpoints in moderately quenched samples of ^{125}I with approximately 2500 cpm to 150,000 cpm, were reproducible within 2 sigma % (Table 3). Iodine-125 samples ranging in activity between 120 cpm and 220 cpm contained between 1200 - 2200 total counts distributed over the entire pulse spectrum. For these samples, insufficient counting time yielded a nonstatistical accumulated pulse height spectrum of ^{125}I . The same samples were recounted allowing a similar statistical collection of the pulse height spectrum as in higher count rate samples, 0.5% 2 sigma. The measured endpoint for an ^{125}I sample with a count rate of 110 cpm and similar quench level as samples shown in Table 3 (564) was 55.49 channels. Collecting a statistically precise pulse height spectrum identifies this data point as that of an homogeneous solution.

Table 3. Iodine-125 Endpoint Determination in Emulsifier-type Cocktails containing 30% v/v water.

^{125}I cpm 0-80 channels	SIE	Counts/channel	Endpoint Channel
2461 ± 0.64%	546 ± 0.91%	445.00	51.79 ± 0.07%
26240 ± 0.25%	542 ± 0.13%	2964.00	49.60 ± 0.86%
139312 ± 0.25%	536 ± 0.26%	2984.00	50.55 ± 0.42%

Freshly prepared samples of ^{32}P (2500 cpm - 150,000 cpm) generated homogeneous solutions under recommended conditions with emulsifier

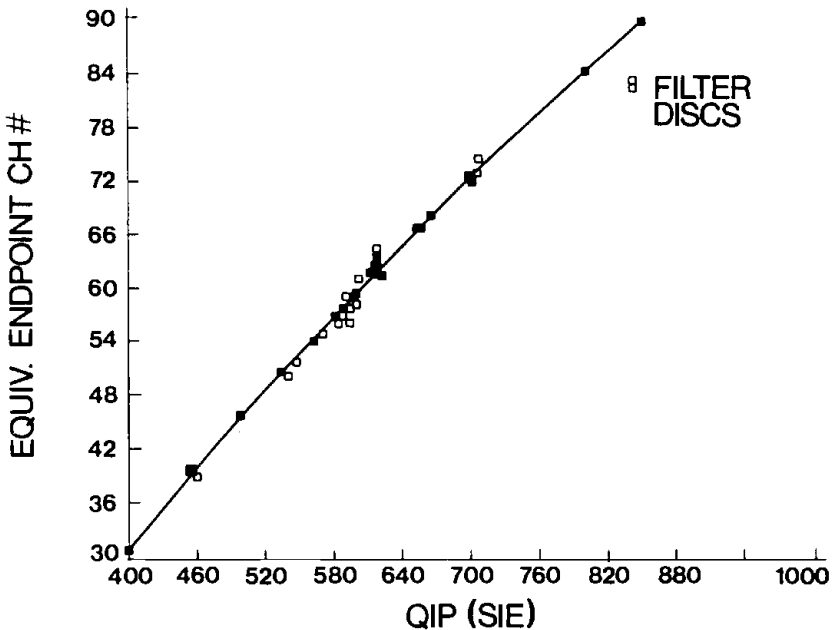


Figure 4. ^{125}I endpoint vs QIP (SIE). Emulsifier cocktails 1.5 . 48.9% aqueous, 2500 - 150000 cpm

cocktails (Figure 5). At some aqueous concentrations in emulsifier cocktails in which both tritium and ^{14}C were identified as heterogeneous mixtures, ^{32}P labelled samples were performing as homogenous solutions. This did not occur in samples with gross phase separation or adsorption effects. The measured equivalent endpoint in moderately quenched samples of ^{32}P in emulsifier-type cocktails was reproducible (Table 4). In low activity samples of ^{32}P (100 cpm to 250 cpm) a reproducible endpoint determination was not observed upon count time termination (10 minutes). When these samples were assayed, based on a statistical accumulation of counts as in higher activity samples (approximately 0.5 2 sigma %), accurate results were obtained. In a sample which contained approximately 83 cpm ^{32}P (6.94% 2 sigma) the percent difference in the measured endpoint energy as compared to a ^{32}P sample containing approximately 160,000 cpm (0.5% 2 sigma) was 10%. By counting this same sample to 0.35% 2 sigma, the percent difference was reduced to 1.51%. This was within the allowed statistical variation for the endpoint determination.

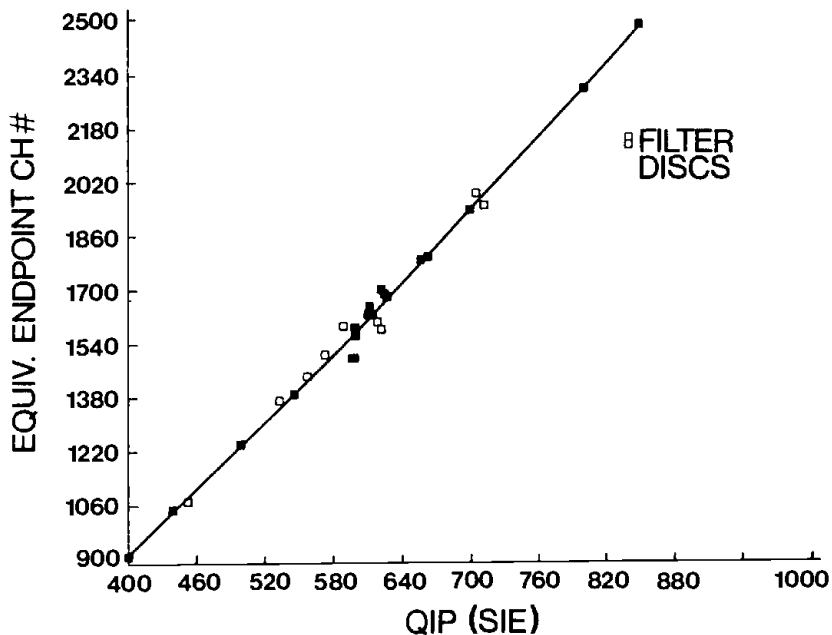


Figure 5. ^{32}P endpoint vs QIP (SIE). Emulsifier cocktail 1.5 ~ 48.9% aqueous, 2500 - 150000 cpm.

Table 4. Phosphorous-32 Endpoint Determination in Emulsifier Type Cocktails containing 30% v/v water.

^{32}P cpm 0-2400 channels	SIE	Counts/channel	Endpoint Channel
2887 ± 0.59%	545	22.56	1431.4
30488 ± 0.25%	548	124.80	1393.4
118736 ± 0.25%	556	121.20	1441.5

In dual labelled samples containing tritium and ^{14}C , the equivalent endpoint of the ^{14}C contribution was determined and related to SIE in homogeneous solutions. Homogeneous dual labelled samples containing aqueous soluble tritium and ^{14}C in moderately quenched

emulsifier-type cocktails were assayed. The average equivalent ^{14}C endpoints were determined in dual labelled samples containing 7.5% ^3H , 92.5% ^{14}C dpm to 96.6% ^3H , 3.4% ^{14}C dpm (Table 5).

Table 5. Tritium- ^{14}C Dual Label Endpoint Determination in Emulsifier Type Cocktails containing from 1.5% - 48.9% v/v water.

^3H dpm	^{14}C dpm	SIE	Endpoint Channel
1074	728,1393,2817 5445,7835,13217	634 ± 1.24%	149.2 ± 2.65%
2163	728 - 13217	637 ± 0.41%	147.2 ± 0.93%
4217	728 - 13217	635 ± 0.90%	145.2 ± 1.84%
8447	728 - 13217	638 ± 0.46%	146.3 ± 2.99%
12404	728 - 13217	642 ± 0.71%	145.2 ± 2.57%
20637	728 - 13217	636 ± 0.31%	140.9 ± 3.03%

Application of this technique in triple label analysis has been made using samples containing approximately 30,000 cpm each of tritium, ^{14}C and ^{36}Cl . The measured equivalent endpoint was determined using the more energetic radionuclide ^{36}Cl . The relationship established between radionuclide endpoint and SIE was similar to the relation for single label ^{36}Cl . This particular curve would lie between similar curves for ^{32}P and ^{14}C if plotted on the same graph, due to the maximum beta energy of ^{36}Cl . Further experiments are being conducted which will characterize the sensitivity of the measurement with different ratios of these radionuclides.

CONCLUSION

A liquid scintillation counting technique for determining sample homogeneity has been developed using a Packard Tri-Carb Spectralyzer Series Liquid Scintillation Spectrometer. The application and flexibility of this technique has been demonstrated using the most common external standard sources, ^{137}Cs , $^{133\text{m}}\text{Ba}$, and ^{226}Ra . This

technique can be extended to include the use of any appropriate external standard source providing the instrument of use has the ability to perform pulse height analysis on the accumulated energy spectrum for both the external standard and the sample which is used to determine the radionuclide(s) observed endpoint and external standard first moment.

Finally, a technique which monitors radionuclide dissolution can be used to rapidly screen liquid scintillation samples for heterogeneity which would otherwise produce misleading results. This technique now provides assurance of meaningful quench corrected results. Single and multiple radiolabelled analytes in either hydrophobic or hydrophilic scintillation solutions which result in nonstable counting samples were identified based on both the degree of cocktail quenching and radionuclide dissolution. True solution counting was determined by reference to a simple equation unique for the higher energy radionuclide of interest and external standard source for known homogeneous samples in all cocktails tested. This technique was not confined to a particular counting solution. All cocktails tested at a given degree of cocktail quench in homogeneous samples for a given radionuclide produce essentially the same observed endpoint. This technique was essentially independent of total solution volume over a wide operating range of approximately 10.0 mL to 20.0 mL.

The flexibility and sensitivity of this technique were demonstrated in samples of ^{14}C which contained approximately 150 - 25,000 cpm and a 1.5% aqueous load. A pulse spectrum distributed over a large energy range requires sufficient counting time to accumulate a statistically accurate pulse shape compared to a pulse spectrum which is only distributed over a few energies. As in the case of unquenched ^{14}C the average counts/channel were approximately 8.4. Statistical collection of counting data was necessary to achieve accurate radionuclide endpoint measurements in low activity samples. Increased counting times may require a means of background compensation.

In summary, this technique provides a unique application of the Tri-Carb Spectralyzer as a homogeneity monitor. This technique is applicable to the Packard system but also to any scintillation

instrument using an external standard as a quench indicating parameter and capable of spectral analysis. This technique is more universal than preceding methods which have previously been described^{1,2}

Liquid scintillation counting can now be applied as a more complete tool to quantify radioactivity with the expanded ability to determine true solution counting.

REFERENCES

1. E.T. Bush, "A Double Ratio Technique as an Aid to Selection of Sample Preparation Procedures in Liquid Scintillation Counting", J. Appl. Rad. Isotopes, 19, 447-452, 1968.
2. D.L. Horrocks and A.J. Kolb, "Instrumental Methods for Detecting some Common Problems in Liquid Scintillation Counting", Laboratory Practice, 30, 5, 1981.
3. L.J. Everett, J.G. Ring and D.C. Nguyen, "The Application of Spectral Analysis in Liquid Scintillation Counting" in "Liquid Scintillation Counting Recent Applications and Developments", editors Peng, Horrocks, Alpen, pp. 119-128, Academic Press, 1980.
4. S.J. De Felippis and L.J. Everett, "Dual Label Determination of Tritium and Iodine-125 by Liquid Scintillation Counting", Trans. Am. Nucl. Soc., 39, 21-22, 1981.