^{67}Ga: A NOVEL INTERNAL STANDARD FOR LSC

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ABSTRACT

The liquid scintillation spectrum of ^{67}Ga exhibits two monoenergetic peaks that correspond to the average energies of ^{3}H and ^{14}C. This interesting feature prompted experiments designed to use ^{67}Ga as an internal standard for either ^{3}H or ^{14}C or dual-labelled samples. A parameter based upon the ratio of the measured activity of the two ^{67}Ga peaks was chosen to reflect the degree of sample quenching. As an example, a dual-labelled experiment was performed and the equations relating the counting efficiency to the ^{67}Ga ratio are presented. The main advantages of this technique include the ability to recover the sample after the ^{67}Ga has decayed, and the elimination of accurate pipetting of the internal standards as only the ratio of activity between the two peaks need to be considered.

INTRODUCTION

The internal standard technique is one of the oldest methods for monitoring sample quenching in liquid scintillation (LS) counting[1-3]. Briefly described, this method involves counting the sample, adding a known quantity of activity of the same radionuclide, and then recounting the sample[4]. The counting efficiency can then be determined and the absolute activity (dpm) calculated.

The LS spectrum of ^{67}Ga (figure 1) is characterized by two major peaks corresponding to 8 keV Auger electrons and conversion electrons from the 93 keV level (Table 1)[5]. These two peaks are similar in energy to the average beta energy of ^{3}H (5.7 keV) and ^{14}C (49 keV)[6]. This similarity between the lower energy peak of ^{67}Ga and the ^{3}H beta spectrum and the higher energy peak of ^{67}Ga and the ^{14}C beta spectrum prompted a study to use ^{67}Ga as an internal standard for ^{3}H and ^{14}C. The contribution of the Compton electrons to this spectrum is discussed elsewhere[7].

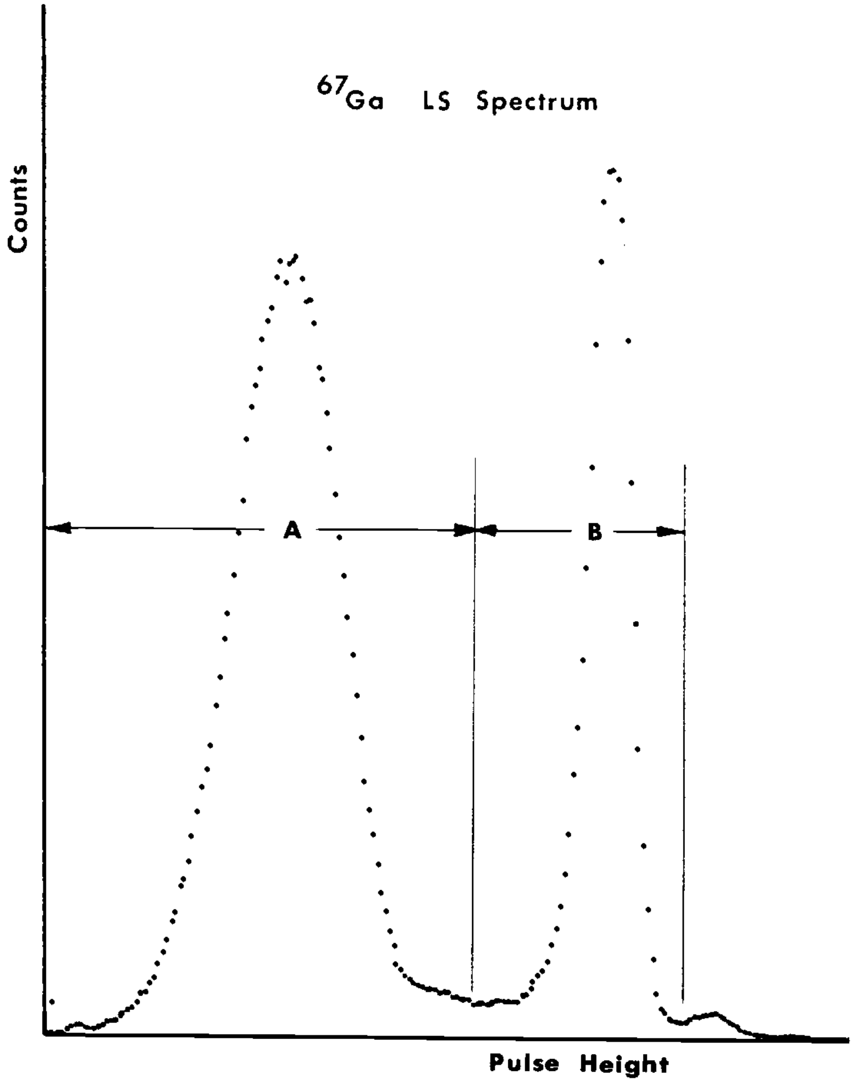

Figure 1. The LS spectrum of ^{67}Ga. Also shown are the two regions, A and B, used to calculate the ^{67}Ga ratio (A/B).

As a sample is quenched, the monoenergetic beta spectrum of ^{67}Ga is expected to behave differently than the broad beta spectrum of ^{3}H and ^{14}C; the lower energy component of a broad spectrum being more effected by quenching. Several methods using different aspects of the ^{67}Ga spectrum were tested to discover that which proved to be the most acceptable quench monitor.

Table 1: Prominent decay modes for liquid scintillation detection techniques

	decay mode	mean number/ disintegration	mean energy (keV)	
^{67}Ga	Auger e^-			
	KLL	0.514	7.5	
	KLX	0.14	8.5	
	LMM	1.73	0.9	
	conversion e^-			
	K	0.25	83.6	93 keV level
	L	0.037	92.2	
	K	0.025	174.8	184.5 keV level

Half-life = 77.9 hours

Data from J. Nucl. Med. 11: Supp. 4, 1970.

EXPERIMENTAL

All the quantitative and pulse height data were obtained from a Beckman 9000 LS counter interfaced to a Northern Scientific multichannel analyzer (MCA) and a Digital Equipment Corporation PDP 11/05 minicomputer[8]. Standard 20 ml LS vials (Kimble, Toledo, OH, USA) containing 15 ml of Merit (Isolab Incorporated, Akron, OH, USA) were used for all measurements. Tritiated-n-hexadecane, ^{14}C-n-hexadecane (Amersham, Oakville, ONT, Canada) and ^{67}Ga-citrate (Frosst, Kirkland, ONT, Canada) were used to provide the appropriately labelled samples. Chemical quenching was accomplished using reagent grade nitromethane (Fisher, Scientific, Edmonton, AB, Canada).

A series of vials containing known quantities of either ^{3}H, ^{14}C or ^{67}Ga were prepared and their corresponding pulse height spectra were measured with the MCA. Chemically quenched standards were prepared by pipetting increasing quantities from 0 µl to 400 µl of nitromethane into the appropriate vials and pulse height spectra and total counts were obtained for all levels of quenching.

To test the applicability of using ^{67}Ga as an internal standard the following dual-labelled experiment was performed:

1. A set of ^{3}H, ^{14}C and ^{67}Ga quenched standards were prepared.
2. The standards were counted and the counting efficiency for all samples and the ^{14}C spillover correction factor for each ^{3}H sample were recorded as well as the ^{67}Ga ratio (described below) for the ^{67}Ga samples.
3. The mathematical relationship between the ^{67}Ga ratio and the ^{3}H and ^{14}C counting efficiencies was calculated.
4. The unknown sample was counted, its spectrum (a) recorded and an aliquot of ^{67}Ga was added and the combined spectrum (b) recorded.
5. The difference between the spectra (b-a) recorded in the previous step will yield the ^{67}Ga spectrum from which the ^{67}Ga ratio can be obtained.
6. The counting efficiency for this sample was determined using the equations obtained in step 3.

RESULTS

The LS spectrum of ^{3}H, ^{14}C and ^{67}Ga spectra for unquenched, moderately quenched and highly quenched samples are shown in figure 2 and represent samples quenched with 0 μl, 150 μl and 400 μl of nitromethane respectively. These figures demonstrate the similarity between the range of pulse heights covered by ^{67}Ga when compared to ^{3}H and ^{14}C.

Several observations can be made from these figures. The high energy peak of ^{67}Ga (peak B) is located above the ^{3}H spectrum except at high levels of quenching (^{3}H counting efficiency < 10%). During quenching, the low energy peak of ^{67}Ga (peak A) and the ^{3}H spectrum are both shifted toward smaller pulse heights and counts are lost from the spectrum. The relative counting efficiencies (100% for an unquenched sample) of ^{3}H and peak A of ^{67}Ga shown in Table 2 reflect this behavior. As expected peak B of ^{67}Ga was resistant to quenching and although this peak was shifted to lower pulse heights as quenching was increased, the total number of counts remained approximately constant. This resistance was most probably due to the absence of a low energy component in the monoenergetic beta spectrum. This can be compared to the broad spectrum of ^{14}C in which the counting efficiency varied from 91.5% for an unquenched sample to

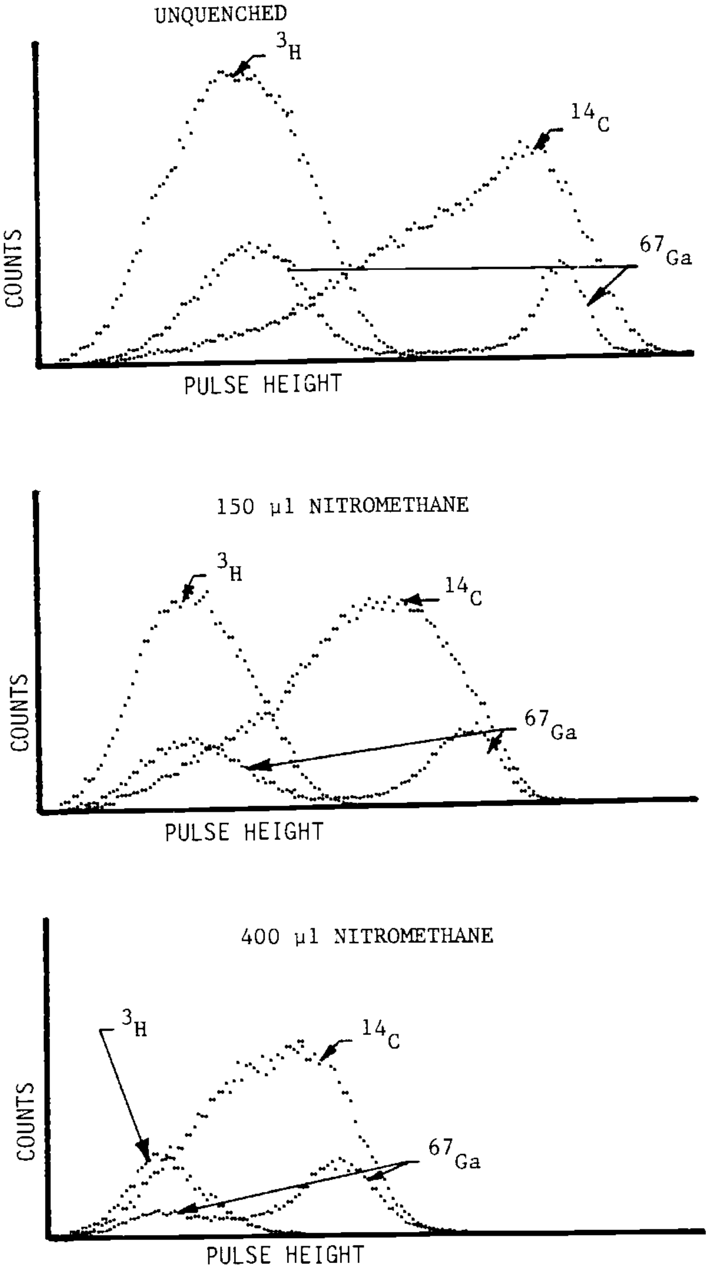

Figure 2. LS spectra of ^{3}H, ^{14}C and ^{67}Ga for different levels of chemical quench.

Table 2: The counting efficiencies of ^{3}H and ^{14}C and the ^{67}Ga ratio as a function of the amount of chemical quencher (nitromethane).

nitromethane (μl)	^{3}H relative efficiency	^{3}H absolute efficiency	^{67}Ga peak A relative efficiency	^{14}C absolute efficiency	^{67}Ga A/B
0	100.0%	48.85	100.0%	91.5%	2.57
80	89.1	43.5	82.7	89.0	2.07
150	62.1	30.3	55.8	85.3	1.29
250	35.0	17.1	34.0	78.1	0.72
400	14.8	7.2	16.6	65.3	0.37

65.3% for a sample quenched with 400 μl of nitromethane.

Table 2 contains a summary of these results. The number of events in peak A and peak B of ^{67}Ga were determined by selecting a point midway in the valley between the peaks, and then integrating the spectrum on either side of this point.

The quench correction data for a dual-labelled experiment are plotted in figure 3. A cubic equation was used to fit this data and produced the following equations relating the ^{67}Ga ratio 'G' to the counting efficiency E:

$$E = -0.045 + 0.33G - 0.051G^2 + 0.30G^3 \quad - 1$$

$$E = -0.46 + 0.65G - 0.34G^2 + 0.062G^3 \quad - 2$$

These equations introduced a deviation of less than 1% compared to the actual observed values.

DISCUSSION

An examination of the spectra of ^{3}H, ^{14}C and ^{67}Ga under chemical quenching revealed several possibilities for the use of ^{67}Ga as a novel internal standard. For tritiated samples, the low energy peak ranges of ^{14}C quenching and possibly peak B for higher quenching. It follows that samples labelled with both ^{3}H and ^{14}C could thus be corrected for sample quenching using the information contained in both

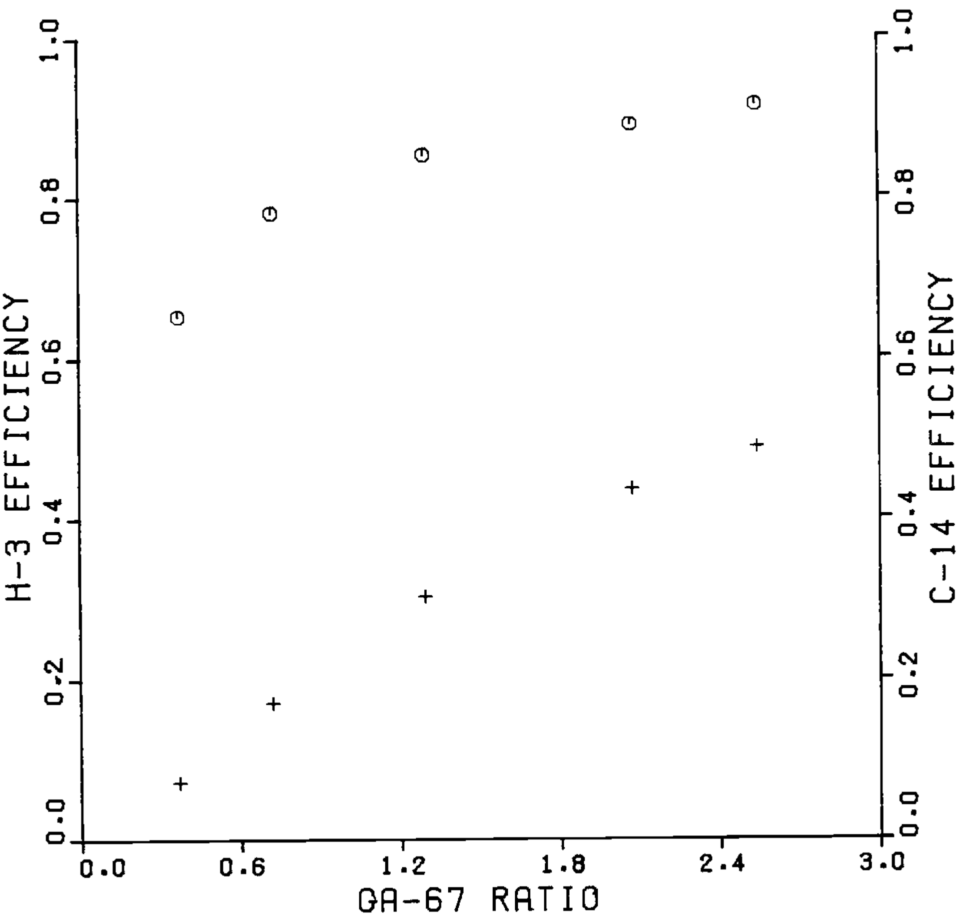

Figure 3. Quench Correction Curves Based on the ^{67}Ga internal standard ratio for ^{3}H (⊙) and ^{14}C (+).

the ^{67}Ga peaks, and lead to the possibility of using a single internal standard for a dual-labelled sample.

The criteria used in the selection of an appropriate method were that the technique be easy to perform and provide statistically acceptable results. The method that used the ratio of the activity in the two ^{67}Ga peaks was selected as this would eliminate the need for accurate pipetting of the internal standard. The technique required only that the activity in the ^{67}Ga peaks be sufficient to produce acceptable counting statistics. Either ^{3}H, ^{14}C or dual-labelled samples could be analyzed and corrected for sample quenching using the ^{67}Ga ratio.

Thus several of the disadvantages associated with the internal standard technique are eliminated with the introduction of ^{67}Ga as the internal standard. As an added benefit, the original sample can be recovered due to the short half-life of ^{67}Ga permitting the sample to be recounted to recheck its activity.

CONCLUSION

This novel technique of using ^{67}Ga as an internal standard for ^{3}H and ^{14}C permits recovery of the original sample and reduces the work load of an experimenter who wishes to use an internal standard.

Although this technique was tested on an atypical set-up involving an LS counter interfaced to an MCA and computer, the introduction of microprocessors into modern LS counters could significantly reduce the complexity of the instrumentation. Future LS counters may even be able to provide a researcher with a small, built-in MCA which would be interfaced to the internal microprocessor of the instrument. This would greatly facilitate this type of experiment for individuals who do not have access to this type of instrumentation.

The LS spectrum of ^{67}Ga, in addition to providing a technique for monitoring sample quenching, could also be used to energy calibrate an LS counter using its two monoenergetic peaks.

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