CHAPTER 27

The Standardization of ^{35}S Methionine by Liquid Scintillation Efficiency Tracing with ^{3}H

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INTRODUCTION

^{35}S ($T_{1/2}$ = 87.44 days) decays by beta-particle emission of maximum energy 166.74 keV and average energy 48.60 keV.[1] ^{35}S has been long used for labeling organic compounds for *in vitro* measurements.[2] It is now increasingly used for labeling sulfur-containing amino acids methionine (Met) and cystine (Cys) for protein sequencing studies, as well as in following the progress of amino acid incorporation during protein synthesis. Further potential applications of ^{35}S labeled compounds are in studies of the interactions of sulfur-containing carcinogens and mutagens (e.g., sulfur mustards, methyl methane sulfonate, ethicnine, etc.) with DNA, particularly with respect to the nature of the binding of such compounds to the macromolecule. Because of the low energy of the beta particles, ^{35}S is usually assayed by liquid scintillation counting.[3] Very few papers are available on the standardization of ^{35}S. Bryant et al., used the method of $4\pi\beta$-γ efficiency tracing with ^{60}Co.[4]

In previous work we have shown that efficiency tracing with ^{3}H can be used to standardize pure beta-particle emitters of low energy, e.g., nickel ^{63}Ni and ^{241}Pl,[5,6] and intermediate energy, e.g., ^{14}C.[5,6] These measurements were made, hwoever, under low quenching conditions. The present work was undertaken to see if the method could be used for ^{35}S labeled amino acids under high quench conditions such as are routinely encountered in assaying biological samples.

The basic principle of the efficiency tracing technique is to explicitly account for the beta-particle spectra of the radionuclide to be assayed (^{35}S) and the standard nuclide (^{3}H). The counting efficiency for a liquid scintillation system with two phototubes operating in coincidence, ϵ_c, is given by Coursey et al.[6]

$$\epsilon = \left\{ \int_{0}^{E_{\beta_{max}}} P(Z,E) \times [1 - \exp(-E\eta Q(E)W(E))]^2 \, dE \right\} \times \left\{ \int_{0}^{E_{\beta_{max}}} P(Z,E) \, dE \right\} \quad (1)$$

where P(Z,E) dE is the Fermi distribution function,
η is the figure of merit, photoelectrons per keV,
Q(E) is the ionization quenching function to account for the differences in light yield for electrons as a function of energy,[7] and
W(E) is a wall-loss function, taken here as unity for these low-energy β particles.

The liquid scintillation counter is the first efficiency calibrated with ^3H standards and the optimum value for the $\eta Q(E)$ term in Equation 1 is obtained. The system may then be used to standardize for activity any other radionuclide for which the Fermi spectrum is known.

EXPERIMENTAL

Materials and Methods

^{35}S labeled Met (5 mCi/0.5 mL (185 MBq/0.5 mL)) was obtained from Nordion (Ottawa, Canada).* A carrier solution was prepared for all dilutions of the ^{35}S consisting of 10 m*M* beta-mercaptoethanol, 50-m*M* N-[tris (hydroxymethyl) methyl] glycine (tricine), and 0.1 m*M* stable Met. The ^3H water was a dilution of NIST Standard Reference Material (SRM) 4927C. Each scintillation vial contained 10 mL Beckman Readysolv HPb scintillator in a polyconeseal glass vial (Kimble). Two sets of identical vials were prepared; one for the ^{35}S and one for the ^3H standards. For each set, chloroform was added in 50 μL increments (0 to 200 μL) to simulate the quenching expected in biological samples.

The radionuclide samples (27 to 82 mg) were added gravimetrically to the vials containing scintillation cocktail.

Equipment

Measurements were made with a Beckman LS7800 liquid scintillation counter on line to a Charles River Data Systems supermicro data aquisition system.[8] Data were downloaded to an IBM XT PC for processing. Efficiencies

*Mention of commercial products does not imply recommendation or endorsement by the National Institute of Standards, nor does it imply that the products identified are necessarily the best available for the purpose.

and spectra were computed using the program EFFY2 implemented on the NIST PC by Eduardo García-Toraño in 1988.[9]

RESULTS AND DISCUSSION

The samples were measured over a period of 30 days. The ^{35}S-methionine proved to be very stable (less than 0.1% change), while for the ^{3}H samples, the count rate decreased by approximately 0.16% per day.[5] The tritiated water separates from the scintillator with time, while the ^{35}S labeled Met is apparently in the organic phase.

Figure 1 shows the Fermi spectra of ^{35}S (Figure 1a) and ^{3}H(Figure 1b). The measured LS spectra for the two nuclides for low quenching (Horrocks' H# around 70) and high quenching (H# around 150) are shown in Figures 1c and 1d for the ^{35}S and ^{3}H, respectively. The low quenching samples were used to standardize the ^{35}S by the efficiency tracing technique.

Figure 2 shows the relationship between the figure of merit, the quench parameter H#, and the counting efficiencies of ^{3}H and ^{35}S. Although the ^{3}H efficiency decreases with quenching from 49 to 24%, the ^{35}S efficiency only

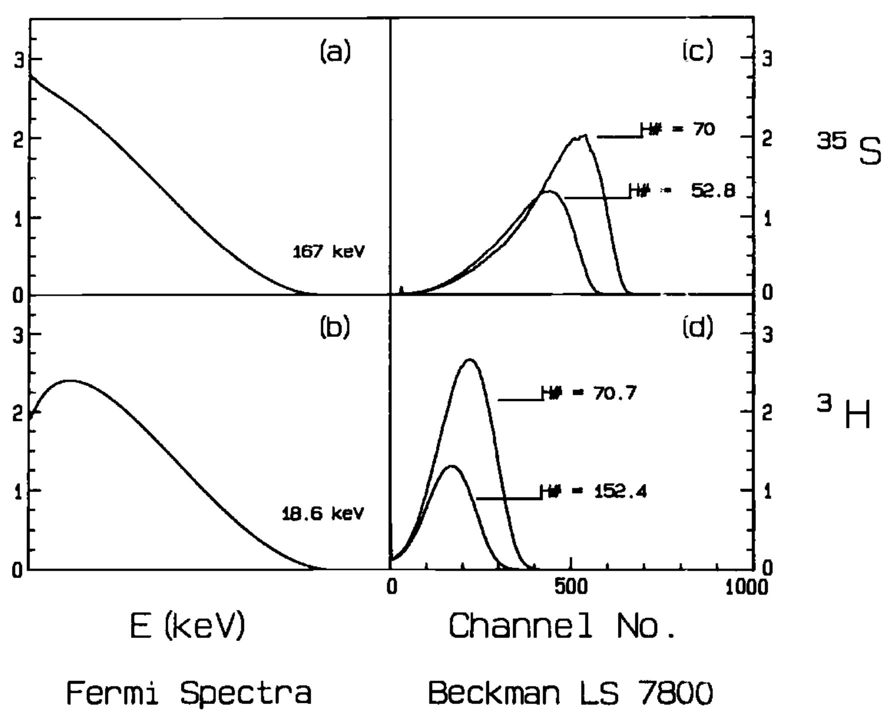

Figure 1. Beta particle spectra and liquid scintillation spectra for ^{35}S and ^{3}H. The experimental spectra was obtained on a system with a logarithmic amplifier.

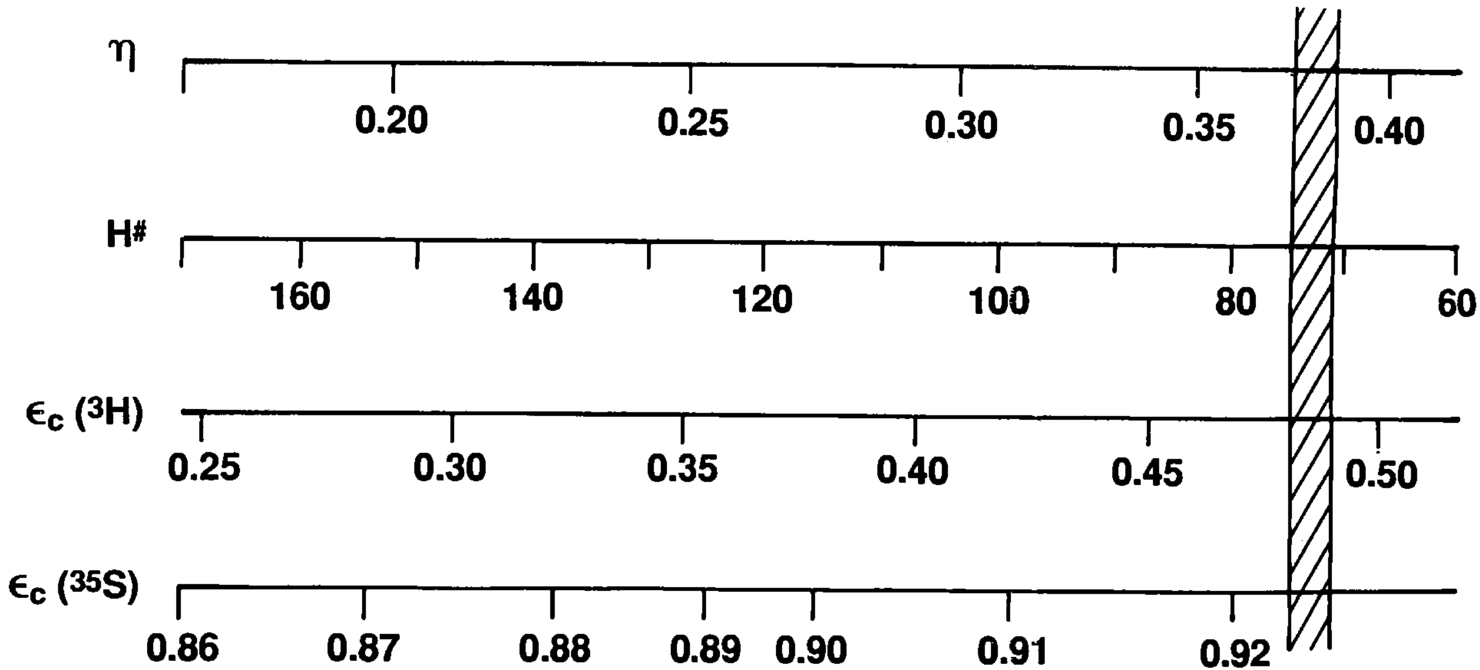

Figure 2. Relationships between the figure of merit (η), the quench paremeter (H#), and the two-phototube coincidence counting efficiencies for ^{3}H and ^{35}S. The shaded area is the unquenched region used for the most accurate standardization of the ^{35}S. This nomograph is strictly valid for only one scintillator and counting system.

Table 1. Results for Nordion ^{35}S Methionine as of 1200 EST February 24, 1989 (uncertainties are given as one standard deviation)

Vial No.	H#	ϵ_C	Predicted Activity (Bq/mg)
1	71.3	0.9256	69.43
2	70.5	0.9261	69.15
3	71.4	0.9255	69.14
4	72.8	0.9245	69.34
5	71.6	0.9253	69.23
			69.26 ± 0.12
6	104.7	0.9028	69.50
7	118.1	0.8940	69.81
8	131.4	0.8854	69.51
9	141.0	0.8793	69.45
10	152.8	0.8719	69.25
11	171.3	0.8604	68.75
			69.38 ± 0.36

drops from 92 to 87% over the same chloroform concentration range. The predicted values of the activity for all samples are shown in Table 1. The average for the quenched samples, 69.38 ± 0.36, compares favorably with the value obtained for the unquenched samples 69.26 ± 0.12 Bg/mg (uncertainties are one standard deviation). The estimated uncertainty in the ^{35}S activity concentration, which includes systematic as well as random components, is given in Table 2.

Since this standardization method can be extended to high quenching for ^{35}S, it should work as well for ^{14}C and ^{32}P. Figure 3 shows computed counting efficiency for all three radionuclides and ^{3}H as a function of quenching. Using the "*measured ^{3}H efficiency*" one can compute the efficiency of any of the other three to within 1 to 2%.

Table 2. Estimated Uncertainties in the Standardization of ^{35}S Methionine

	Percent (%)
a) Liquid scintillation measurements	0.18
b) ^{3}H reference beta-particle standard	0.21
c) Quenching in the liquid scintillation measurements	0.05
d) Source preparation	0.10
e) Uncertainty in efficiency curve fit	0.07
f) Scintillator stability	0.05
g) Uncertainty in numerical spectra integration	0.05
h) Dead time	0.03
Combined in quadrature	0.32
Overall uncertainty (×3)	1.0

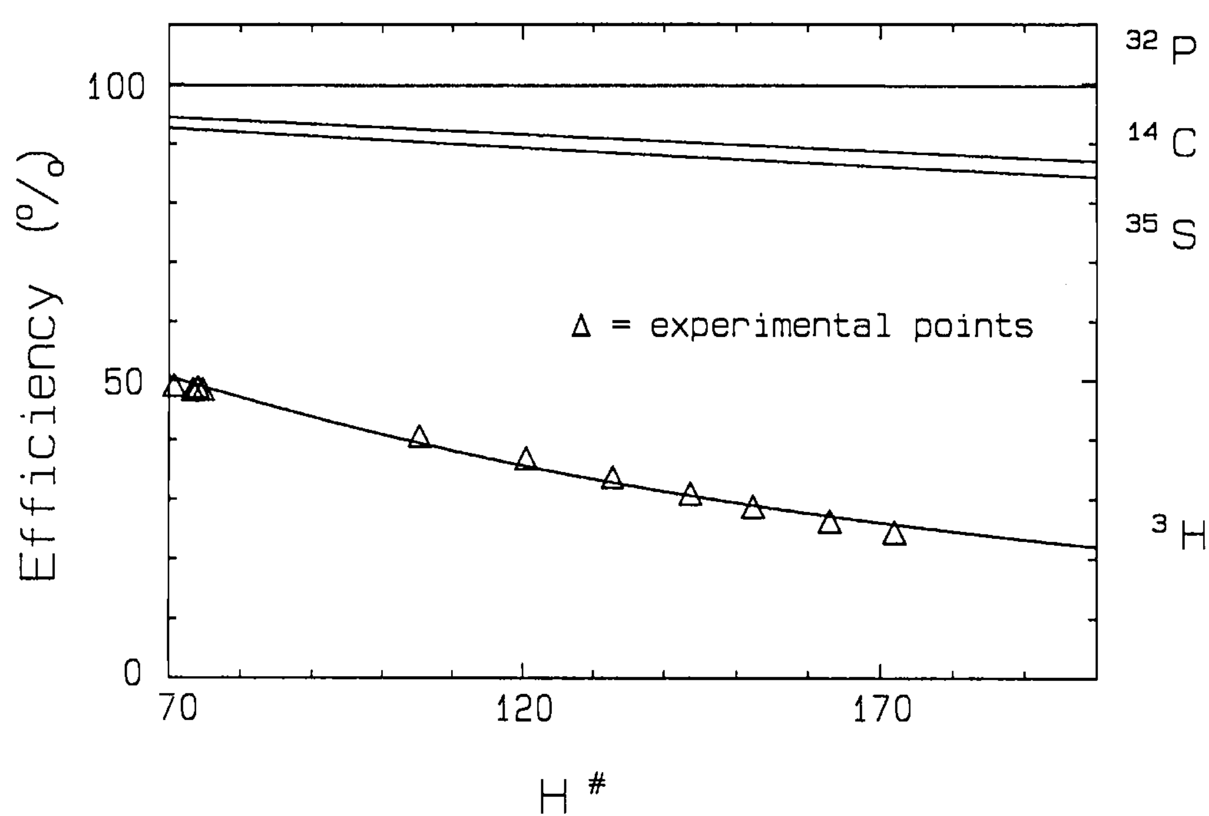

Figure 3. Counting efficiency as a function of quenching, with experimental points from this work. Curves computed using code EEFY2 (Grau Malonda and Garcia-Toraño (1985)).

CONCLUSIONS

^{35}S labeled methionine has been standardized by the method of $4\pi\beta$ liquid scintillation efficiency tracing with ^{3}H. The method appears to work well for chemically quenched samples up to at least H# 171. Since this program is now implemented on a personal computer, it should be possible to adapt most commercial liquid scintillation counters to directly compute ^{35}S activities, providing a set of ^{3}H quenched standards is available to establish a quench curve vs a quenching parameter.

REFERENCES

1. *Handbook of Radioactivity Measurements Procedure 58*, Second ed. (Bethesda, MD: National Council on Radiation Protection and Measurements, 1985).
2. Gordon, B.E., H.R. Lukens, and W. ten Hove. *Int. J. Appl. Radiat. Isotp. 12*: 145 (1961).
3. Horrocks, D.L. *Applications of Liquid Scintillation Counting*, (New York: Academic Press, 1974).
4. Bryant, J., D.G. Jones, and A. McNair. in "Standardization of Radionuclides," proceedings of a symposium, International Atomic Energy Agency, Vienna, (1967).
5. Coursey, B.M., L.L. Lucas, A. Grau Malonda, and E. García-Toraño. *Nucl. Instrum. Meth. Part A*. in press, (1989).
6. Coursey, B.M., W.B. Mann, A. Grau Malonda, E. García-Toraño, J.M. Los Arcos, J.A.B. Gibson, and D. Reher. *Int. J. Appl. Radiat. Isopt. 37*, 403 (1986).
7. Grau Malonda, A. and E. García-Toraño. *Int. J. Appl. Radiat. Isopt. 33*: 249 (1982).
8. Coursey, B.M., M.P. Unterweger, L.L. Lucas, and E. García-Toraño. *Trans. Amer. Nucl. Soc. 55*: 54 (1982).
9. Grau Malonda, A. and E. García-Toraño. *Comp. Phys. Comm. 36*:307 (1985).