Quenching Curves: Solutions by Second-Order Polynomial Regression

George H. Barrows, Ellis Samols, and Bill Becker

Veterans Administration Hospital and the University of Louisville,
School of Medicine, Louisville, Kentucky

This paper describes an inexpensive practical approach for the automated computation of quench corrections in beta-scintillation experiments, using a table-top calculator. This approach applies a second-order fit, $ax^2 + bx + c = y$, where x is the channel A/B ratio for known quench standards and y describes efficiency. There is excellent agreement between measured values and the polynomial expression for most experimental A/B ratios.

J Nucl Med 17: 1017–1018, 1976

In beta-scintillation detection, interference within the scintillation fluid decreases the apparent activity of the sample. Chemical interference with scintillation emission (chemical quench) and optical absorption of scintillation (optical quench) decrease the efficiency of counting. To measure the radioactivity in a liquid-scintillation experiment accurately, corrections must be made for the quenching present. A shift in the pulse-height spectrum of a beta-emitter causes a change in the integral counting efficiency of a liquid-scintillation sample (*1*). By comparing the ratio of high- and low-energy measurements with known amounts of quench, an index of counting efficiency can be made (*2*). Calibration curves obtained by this "channels ratio" method are independent of the quenching medium for ^{3}H and ^{14}C over most counting ranges (*1,3*).

Usually the counting efficiency is relatively constant within any set of experiments. However, variations among samples or the presence of colored pigments (such as carotene or bilirubin) may introduce substantial counting error. Thus, quench correction should be employed in every beta-scintillation experiment (*2*).

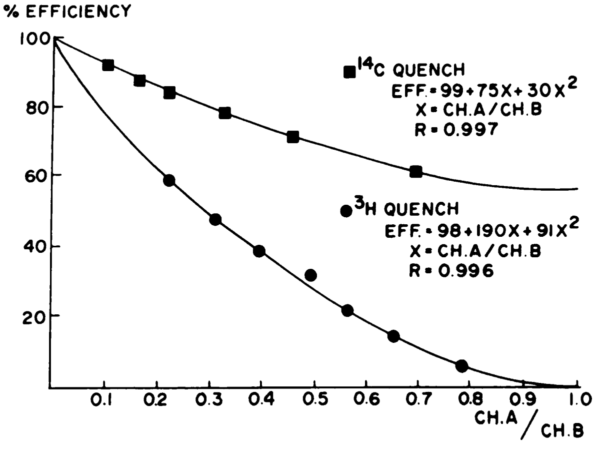

FIG. 1. Quench curves for ^{3}H and ^{14}C.

We have found that the channel A/B ratios obtained from quench standards fall along a second-order polynomial curve described by the general formula $ax^2 + bx + c = y$, where x is the A/B ratio and y is the efficiency. Standard second-order polynomial regression may be carried out on channel A/B ratios found for standard samples, thus yielding the coefficients for this polynomial. The A/B ratios for ^{3}H and ^{14}C standards, using a Searle liquid-scintillation counter, are shown in Fig. 1. These experimental values for A/B and efficiency are fitted by the corresponding polynomial curves for ^{3}H and ^{14}C. The correlation coefficient is 0.996 for tritium and 0.997 for carbon-14. This suggests excellent agreement between the measured values and the polynomial expression. However, A/B ratios above 0.9 and below 0.1 are not well defined by this experiment and should not be substituted into the polynomial to calculate efficiency. Since each liquid-scintillation system will have different characteristics, the polynomial constants a, b, and c must be calculated for each counter.

The second-order polynomial is readily incorporated into a table-top calculator. Thereafter, efficiency and disintegrations per minute can be calculated for any beta-scintillation experiment by entering channel A and channel B counts. A simple program written for the Hewlett-Packard 9830-A calculator may be obtained from the authors. The quenching curve must be calculated for each counter and for each emitter. The program should be constructed to reject A/B ratios outside the range of values described by the quenching curve. Table 1 shows the two channel counts,

---

Received Jan. 13, 1976; revision accepted June 2, 1976.

For reprints contact: Ellis Samols, Nuclear Medicine Service, V.A. Hospital, Louisville, KY 40202.

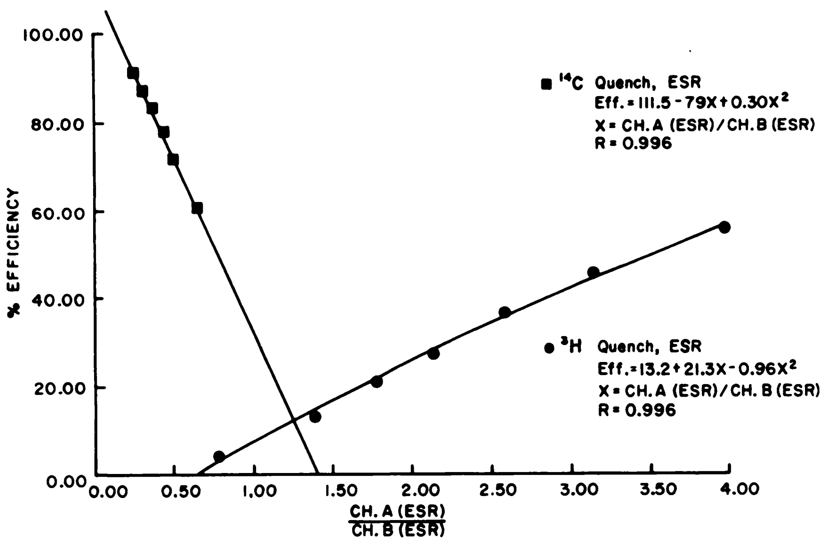

FIG. 2. Quench curve using external-standard ratio method.

TABLE 1. SAMPLE PRINTOUT FOR ALDOSTERONE RADIOIMMUNOASSAY

Single-channels-ratio quench correction for aldosterone (replicates in triplicate)

Tube	Channel A	Channel B	Corrected B	Efficiency
1	17,114	43,847	112,338	0.3903
2	18,154	45,807	115,582	0.3963
3	18,393	46,417	117,139	0.3963
Averaged cpm	1	—3	115,020	
4	436	1,295	3,846	0.3367
5	367	1,068	3,108	0.3436
6	517	1,507	4,393	0.3431
Averaged cpm	4	—6	3,782	

corrected counts, and efficiency for a ^{3}H-aldosterone experiment.

A similar approach is possible for external-standard corrections. Figure 2 shows quench curves for ^{3}H and ^{14}C standards using the external-standards method. As in the channels-ratio method, the polynomial for the quench curves can be used in a calculator program to correct for quench in each sample. The program should be modified so that the efficiency calculated by the polynomial from the external-channels count is used to correct the sample counts. As in channels-ratio method, each counting system must be fitted with its individual polynomial. The external-standards method is somewhat less accurate than the channels-ratio method for optically quenched samples (*4*). In addition, the use of different counts (channel A, channel B, channel A ESR [External Standard Ratio], channel B ESR, and standard counts) makes the computations more cumbersome. At low count rates, however, the external-standards ratio method is faster and more accurate, since the external standard provides a large number of counts per minute for the quench correction.

In summary, the use of a second-order polynomial curve fit for efficiency (y) and channel A/B ratio (x) is simple, practical, and inexpensive. The method is also adaptable to the external-standards ratio method. This approach appears to be generally applicable for automated quench correction. We have found it invaluable for automation of data analysis for radioimmunoassays employing liquid-scintillation counting.

REFERENCES

1. Baille LA: Determination of liquid scintillation counting efficiency by pulse height shift. *Int J Appl Radiat Isot* 8: 1–7, 1960

2. Hendee WR: *Radioactive Isotopes in Biological Research*. New York, Wiley, 1973, pp 208–212

3. Bush ET: General applicability of the channels ratio method of measuring liquid scintillation counting efficiencies. *Anal Chem* 35: 1024–1029, 1963

4. Noujaim A, Ediss C, Wiebe L: Precision of some quench correction methods in liquid scintillation counting. In *Organic Scintillations and Liquid Scintillation Counting*, Horrocks DL, Peng CT, eds. New York, Academic, 1971, pp 705–715