

## Chapter 8

# The Processing of Liquid Scintillation Spectrometer Data Using a Desk-Top Computing System

M. A. Williams and G. H. Cope

*Department of Human Biology and Anatomy,  
University of Sheffield, England.*

### INTRODUCTION

Liquid scintillation spectrometry is now widely used in biochemical and chemical laboratories for assaying soft  $\beta$ -emitters. Most modern spectrometers are sophisticated instruments utilising several channels and capable of automatically handling hundreds of samples and printing out the results. These instruments usually incorporate a means of estimating the degree of quenching, the most modern by means of the 'external standard-channels ratio' method. Some expensive new machines incorporate computation facilities that both determine and allow for the degree of quenching and can also relate the results to other variables such as sample weight or specific radioactivity. Some spectrometers can be linked directly, or via punched tape, to a large computer whilst others can be linked to desk-top computing systems. These latter systems have not to date proved entirely satisfactory. Limited programming steps and storage space has prevented both accurate expression of quench curves and the analysis of binary-labelled samples.

The work reported here is directed towards those workers with neither the money to purchase expensive machines with built-in computing facilities nor with regular access to a large computer. The system we have evolved uses a moderately priced desk-top system. Its use permits the owner of the more reasonably priced scintillation counter to have effective computing facilities for singly and doubly-labelled samples. Additionally he gains a desk-top computer for general laboratory work.

### METHODS

**Labelled materials.** 1,2- $^3\text{H}$ -n-hexadecane (specific radioactivity 2.45  $\mu\text{Ci/g}$ ) and 1- $^{14}\text{C}$ -n-hexadecane (specific radioactivity 0.97  $\mu\text{Ci/g}$ ) (The Radiochemical Centre, Amersham, Bucks, U.K.) were used as standards.

The livers from rats that had been injected intraperitoneally with Me-<sup>3</sup>H choline chloride and 2-<sup>14</sup>C -ethan-1-ol-2-amine hydrochloride were extracted with chloroform-methanol (2:1, v/v) and the extracts were broken into two phases with 0.2 vol. of 0.1 M KCl.<sup>1</sup> One phase contained <sup>3</sup>H- and <sup>14</sup>C-labelled phospholipids and the other water-soluble <sup>3</sup>H- and <sup>14</sup>C-labelled metabolic intermediates.<sup>2</sup> Samples of these phases were used as test solutions.

**Scintillation counting.** The scintillator solution consisted of 7 g of 2,5-diphenyloxazole (PPO), 150 mg of 1,4-bis-(5-phenyloxazol-2-yl) benzene (POPOP) and 50 g of naphthalene dissolved in 1 litre of dioxan-toluene (4: 1, v/v).

**External standard channels ratio method.** A Beckman LS 100 scintillation spectrometer fitted with a cesium-137 automated external-standard source was aligned by using n-hexadecane standards in scintillator. The upper discriminator of window I was set so that the uppermost portion of the tritium spectrum was excluded (window I: tritium efficiency 39.50%; carbon-14 efficiency, 8.70%). The lower discriminator of window II was set just above the tritium energy spectrum and the upper discriminator just above the top of the carbon-14 energy spectrum (window II: tritium efficiency, 0.00%; carbon-14 efficiency, 66.20%).

The external standard was set up according to manufacturer's instructions to record a value of about 12.50 for the tritium standard.

**Preparation of quench curves.** Separate tritium and carbon-14 standards were prepared in duplicate and their radioactivities were counted to a 2σ error better than 0.2%. The external-standard channels-ratio value for each standard was obtained before and after counting and the average was recorded. The vials were then opened and a single drop of chloroform (about 0.02 ml) was added to each, the vials were resealed and the chloroform was mixed in by gentle swirling. After the standards had been placed in the dark for

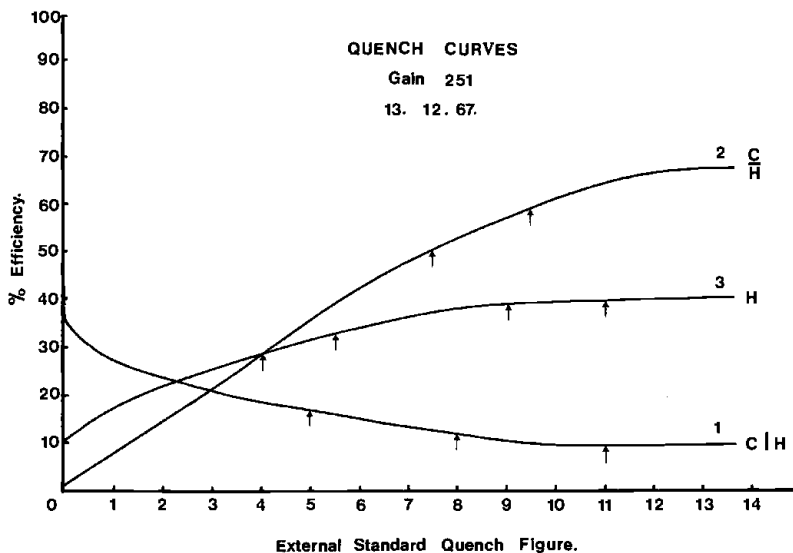


Fig. 1

5 min their radioactivities were recounted with the same precision. This procedure was repeated until the recorded external-standard channels-ratio had fallen to 0.00 (about 180 drops). The tritium and carbon-14 counting efficiencies in window I and the carbon-14 counting efficiencies in window II were calculated after each drop of chloroform had been added, and these were plotted against the respective average external-standard channels-ratio value (Fig. 1).

**Hand calculations.** The calculations necessary to separate the counts attributable to each radioactive isotope in binary-labelled samples and to determine d.p.m. per unit solvent volume are set out below. Counting efficiencies were read from graphs (Fig. 1) by applying the external-standard quench-ratio value printed out by the spectrometer. Subtraction of background counts:

$$(I)_p - k_1 = (I)_q \quad (1)$$

$$(II)_p - k_2 = (II)_q \quad (2)$$

Determination of carbon-14 counts in window I:

$$\frac{(II)_q \times y_1}{y_2} = R \quad (3)$$

Determination of tritium d.p.m.:

$$\frac{[(I)_q - R]}{y_3} \times k_3 = D_t \quad (4)$$

Determination of carbon-14 d.p.m.:

$$\frac{R + (II)_q \times k_3}{y_1 + y_2} = D_c \quad (5)$$

Tritium d.p.m. calculated per ml of solvent:

$$\frac{D_t \times k_4 \times v_2}{v_1} = \text{Tritium d.p.m./ml of solvent} \quad (6)$$

Carbon-14 d.p.m. calculated per ml of solvent:

$$\frac{D_c \times k_4 \times v_2}{v_1} = \text{Carbon-14 d.p.m./ml of solvent} \quad (7)$$

where  $(I)_p$  is c.p.m. in window I (printed out by spectrometer),  $(II)_p$  is c.p.m. in window II (printed out by spectrometer),  $(I)_q$  is c.p.m. in window I with background counts subtracted,  $(II)_q$  is c.p.m. in window II with background counts subtracted,  $k_1$  is background counts in window I,  $k_2$  is background counts in window II,  $k_3$  is 100,  $k_4$  is 1000 (factor converting d.p.m./mg into d.p.m./g),  $y_1$  is carbon-14 counting efficiency

in window I,  $y_2$  is carbon-14 counting efficiency in window II,  $y_3$  is tritium counting efficiency in window I,  $v_1$  is sample weight (mg),  $v_2$  is sample density, (g/ml)  $D_t$  is tritium d.p.m. (uncorrected for sample weight etc.),  $D_c$  is carbon-14 d.p.m. (uncorrected for sample weight etc.) and  $R$  is carbon-14 c.p.m. in window I.

**Programmed calculations.** An IME 86S desk-top calculator was used in conjunction with a programming unit (Muldivo Digicord DG 308) capable of storing 512 programme steps and an external data store (Muldivo MS 30) capable of storing 60 ten-digit values each with correct decimal point and sign. Values could also be summed into these stores. The results were printed out on an Imperial Data-log typewriter via a recoding output unit (Muldivo OP 207). Programming instructions can be entered manually through the calculator keyboard or inserted on eight-holed edge-punched cards. No special computer language is required.

### Mathematical expression of quench curves

Any discrepancy between hand-calculated and computer-calculated results originates from errors made in determining counting efficiencies from mathematical expressions. Sufficient programme steps are available to fit to cubic or quartic polynomials, but it was decided that the curves shown in Fig. 1 could be adequately expressed by quadratic equations ( $y = ax^2 + bx + c$ , where  $y$  is the counting efficiency,  $x$  the external-standard quench-ratio and  $a$ ,  $b$ , and  $c$  coefficients peculiar to each quench curve).

An entirely separate programme is used to determine the values of the coefficients  $a$ ,  $b$  and  $c$ , which are subsequently entered into the data store for use during the calculation of results (see below). Programmes have been devised to calculate these coefficients by two different methods. The first is a comparatively simple programme, which derives them from the co-ordinates of three representative points on each curve (three-point method). The second takes a larger and variable number of points and obtains a line of best fit by the method of least squares (least-squares method). These methods are described here and the results compared.

**Three-point method.** The co-ordinates of three representative points on a curve are entered at the beginning of the programme ( $x_1, y_1; x_2, y_2; x_3, y_3$ ). The programme then solves the following equations in sequence.

$$\frac{(y_3 - y_2)(x_2 - x_1) - (y_2 - y_1)(x_3 - x_2)}{(x_3 - x_2)(x_2 - x_1)(x_3 - x_1)} = a \quad (8)$$

$$\frac{(y_3 - y_2)(x_2^2 - x_1^2) - (y_2 - y_1)(x_3^2 - x_2^2)}{(x_3 - x_2)(x_2 - x_1)(x_3 - x_1)} = b \quad (9)$$

$$y_1 - ax_1^2 - bx_1 = c \quad (10)$$

These are derived from the equations:

$$ax_1^2 + bx_1 + c = y_1 \quad (11)$$

$$ax_2^2 + bx_2 + c = y_2 \quad (12)$$

$$ax_3^2 + bx_3 + c = y_3 \quad (13)$$

**Least-squares method.** The co-ordinates of a number of points on a curve are entered into a programme loop and values of  $\Sigma x$ ,  $\Sigma x^2$ ,  $\Sigma x^3$ ,  $\Sigma x^4$ ,  $\Sigma y$ ,  $\Sigma xy$ ,  $\Sigma x^2 y$  and  $n$  are accumulated. When all co-ordinates have been entered, the programme passes from the loop to solve the following equations in sequence:

$$\frac{[(\Sigma x^3)^2 - \Sigma x^4 \Sigma x^2] [\Sigma y \Sigma x^2 - \Sigma xy \Sigma x] + [\Sigma x^2 y \Sigma x^2 - \Sigma xy \Sigma x^3] [(\Sigma x^2)^2 - \Sigma x^3 \Sigma x]}{[(\Sigma x^2)^2 - \Sigma x^3 \Sigma x]^2 - [(\Sigma x)^2 - n \Sigma x^2] [(\Sigma x^3)^2 - \Sigma x^4 \Sigma x^2]} = c \quad (14)$$

$$\frac{[\Sigma y \Sigma x^2 - \Sigma xy \Sigma x] + c [(\Sigma x)^2 - n \Sigma x^2]}{[(\Sigma x^2)^2 - \Sigma x^3 \Sigma x]} = a \quad (15)$$

$$\frac{\Sigma xy - a \Sigma x^3 - c \Sigma x}{\Sigma x^2} = b \quad (16)$$

These were derived from the following equations, which satisfy the principle of least squares (the 'normal' equations):

$$a \Sigma x^2 + b \Sigma x + nc = \Sigma y \quad (17)$$

$$a \Sigma x^3 + b \Sigma x^2 + c \Sigma x = \Sigma xy \quad (18)$$

$$a \Sigma x^4 + b \Sigma x^3 + c \Sigma x^2 = \Sigma x^2 y \quad (19)$$

**Calculation of results.** The computer is programmed to follow the same sequence as that shown for the hand calculations. Sample number, c.p.m. in two channels ( $I_p$  and  $II_p$ ) and the external-standard quench-ratio value are entered at the beginning of the programme and are first printed out as a permanent record. At the points in the programme where the three counting efficiencies are required ( $y_1$ ,  $y_2$  and  $y_3$ ) the programme uses the appropriate coefficients and the external-standard quench-ratio value entered at the beginning of the programme to solve a quadratic equation. The nine coefficients ( $a$ ,  $b$  and  $c$  for each curve), together with all constants, variables and intermediate results (shown above under 'hand calculations') are contained within the data store and are recalled into the calculations by the programming unit when required. Any value within the data store can be recalled between programme runs and altered if necessary. In this way, without reprogramming, the coefficients for the quench curves can be altered (thus changing the shape of the quench curves). Similarly, variables such as sample density or volume can be altered to accommodate different batches of samples. Finally, the programme prints out d.p.m. per unit solvent volume for each radioactive isotope, working registers are cleared and the machine returns to the start of the programme.

## RESULTS

**Accuracy of the quadratic equations in expressing the quench curves.** The quench curves shown in Fig. 1 are not parabolic and thus cannot be fitted exactly by a quadratic

equation. Over much of their length, however, they are approximately so, the least parabolic portions being at each end of the curves. In our experience most samples fall within the external-standard quench-ratio range 3.00 to 11.00, and curves that accurately predict within this range, at the expense of the extremities, are generally to be preferred.

**Three-point method.** By selecting the three points away from the extremities it was possible to obtain a curve of acceptable fit. Table 1(a) compares efficiencies read from graphs by the eye with those calculated by the three-point method (arrows on the curves shown in Fig. 1 mark the points selected). It shows that computed efficiencies deviate from graph-read efficiencies by less than 0.5% efficiency over the range 3.50 to 11.50. Beyond these limits larger deviations occur. A better fit for the extremities of the curves could be obtained by selecting points spaced wider apart. Invariably, however, this resulted in less accurate reproduction of the central portion of the curve.

**Least-squares method.** Naturally this method produces a curve of best fit. If the coordinate values of points along the whole length of a curve are used then overall best fit is obtained. Table 1(b) shows that efficiencies derived by this method deviate from the graph-read values by less than 1.60% efficiency over the whole range shown. Deviations, however, over the middle of the quench-ratio range (3.50 to 11.50) are sometimes larger than by the three-point method (*cf.* Tables 1(a) and 1(b)). By sampling within the limits set for the three-point method (between the left-hand and right-hand arrows on each curve in Fig. 1) a closer fit over the more important middle portion of the quench curve is achieved. Table 1(c) compares the efficiencies obtained by sampling over the restricted range with those read from graphs. Now, a slightly better fit over the middle of the external-standard quench-ratio range occurs (*cf.* Tables 1(a) and 1(c)).

The improvement of fit by the least-squares method is relatively slight, and whichever procedure is used a decision must be made as to which portion of the curve must be most accurately predicted.

**Computing system used to analyse radioactive hepatic phospholipids.** Weighed samples (100 to 700 mg) of upper or lower phase of extracts of rat liver containing both tritium and carbon-14 isotopes were counted for radioactivity. Table 2 shows a selection of results obtained after computer simulation of the curves compared with those obtained by reading counting efficiencies by the eye from graphs of the type shown in Fig. 1. To minimise graph reading errors by the eye, a maximum and a minimum counting efficiency were taken for each external-standard quench ratio-value and a mean value was obtained by averaging the d.p.m./ml of solvent obtained for each efficiency. (Only the minimum efficiency is quoted in Table 2.)

Although percentage deviations are quoted in Table 2, this does not necessarily imply that the computed results are less accurate, especially when the deviations are small. The accuracy of the hand calculations is limited by the precision with which the curves are read by eye (say  $\pm 0.1\%$  efficiency), whereas the simulations are worked to several places of decimals. The final deviations in samples containing two radioactive isotopes are the result of the interaction of errors on each of the three curves used to separate tritium counts from carbon-14 counts in window I. In some instances errors for each curve have cancelled each other out, whereas in others they have become accumulated. In samples containing only one radioactive isotope the deviation is a direct function of the difference between a real and a simulated curve. The deviations quoted here for binary-labelled

Table 1. Counting efficiencies read from graphs by the eye compared with those computed from quadratic equations.<sup>d</sup>

External standard quench-ratio value	Carbon-14 counting efficiency in window I			Carbon-14 counting efficiency in window II			Tritium counting efficiency in window I		
	Graph value <sup>a</sup>	Computed value	Deviation	Graph value <sup>a</sup>	Computed value	Deviation	Graph value <sup>a</sup>	Computed value	Deviation
<b>1(a) Three-point method</b>									
0.50	30.70	26.54	4.16-	4.00	3.45	0.55-	14.00	15.23	1.23
1.50	25.00	23.93	1.07-	10.50	6.26	4.24-	19.10	19.63	0.53
2.50	22.00	21.52	0.48-	17.00	15.25	1.75-	23.10	23.41	0.31
3.50	19.40	19.29	0.11-	24.00	23.53	0.47-	26.40	26.79	0.39
4.50	17.20	17.25	0.05	30.80	31.09	0.29	29.40	29.75	0.35
5.50	15.40	15.40	0.00	37.50	37.94	0.44	32.30	32.30	0.00
6.50	13.90	13.74	0.16-	43.70	44.08	0.38	34.70	34.44	0.26-
7.50	12.30	12.27	0.03-	49.50	49.50	0.00	36.50	36.17	0.33-
8.50	10.80	10.98	0.18	54.20	54.21	0.01	37.70	37.49	0.21-
9.50	9.50	9.89	0.39	58.20	58.20	0.00	38.40	38.40	0.00
10.50	8.80	8.98	0.18	61.60	61.48	0.12-	38.90	38.90	0.00
11.50	8.60	8.27	0.33-	64.20	64.04	0.16-	39.20	38.99	0.21-
12.50	8.70	7.74	0.96-	66.20	65.89	0.31-	39.50	38.67	0.83-
<b>1(b) Least squares method (best overall fit<sup>b</sup>)</b>									
0.50	30.70	29.36	1.34-	4.00	2.47	1.53-	14.00	14.41	0.41
1.50	25.00	25.90	0.90	10.50	10.50	0.00	19.10	18.84	0.26-
2.50	22.00	22.75	0.75	17.00	18.06	1.00	23.10	22.84	0.27-
3.50	19.40	19.92	0.52	24.00	25.13	1.13	26.40	26.40	0.00
4.50	17.20	17.40	0.20	30.80	31.72	0.92	29.40	29.53	0.13
5.50	15.40	15.21	0.19-	37.50	37.83	0.33	32.30	32.23	0.07-
6.50	13.90	13.33	0.57-	43.70	43.47	0.23-	34.70	34.50	0.20-
7.50	12.30	11.78	0.52-	49.50	48.62	0.88-	36.50	36.34	0.16-
8.50	10.80	10.54	0.26-	54.20	53.29	0.91-	37.70	37.74	0.04
9.50	9.50	9.62	0.12	58.20	57.49	0.71-	38.40	38.71	0.31
10.50	8.80	9.01	0.21	61.60	61.20	0.40-	38.90	39.25	0.35
11.50	8.60	8.73	0.13	64.20	64.93	0.73	39.20	39.36	0.16
12.50	8.70	8.76	0.06	66.20	67.18	0.98	39.50	39.03	0.47-
<b>1(c) Least-squares method (restricted sample range<sup>c</sup>)</b>									
0.50	30.70	27.20	3.50-	4.00	2.71-	6.71-	14.00	14.07	0.07
1.50	25.00	24.44	0.56-	10.50	6.65	3.85-	19.10	18.67	0.43-
2.50	22.00	21.88	0.12-	17.00	15.37	1.63-	23.10	22.80	0.30-
3.50	19.40	19.53	0.13	24.00	23.44	0.56-	26.40	26.46	0.06
4.50	17.20	17.38	0.18	30.80	30.86	0.06	29.40	29.65	0.25
5.50	15.40	15.44	0.04	37.50	37.63	0.13	32.30	32.37	0.07
6.50	13.90	13.71	0.19-	43.70	43.75	0.05	34.70	34.62	0.08-
7.50	12.30	12.18	0.12-	49.50	49.22	0.28-	36.50	36.40	0.10-
8.50	10.80	10.86	0.06	54.20	54.05	0.15-	37.70	37.71	0.01
9.50	9.50	9.74	0.24	58.20	58.22	0.02	38.40	38.55	0.15
10.50	8.80	8.83	0.03	61.60	61.75	0.15	38.90	38.72	0.02
11.50	8.60	8.12	0.48-	64.20	64.62	0.42	39.20	38.81	0.39-
12.50	8.70	7.62	1.08-	66.20	66.85	0.65	39.50	38.24	1.26-

<sup>a</sup> Values read by eye from the graphs shown in Fig. 1.

<sup>b</sup> Computed from co-ordinate points along the whole length of each curve.

<sup>c</sup> Computed from co-ordinate points within the limits set by the left-hand and right-hand arrows on each curve in Fig. 1.

<sup>d</sup> With permission from *Biochem. J.* 118, 379 (1970).

Table 2. Comparison of results<sup>b</sup> obtained by 'hand calculation' and by desk-top computation<sup>c</sup>.

Sample no.	External standard quench-ratio value	Minimum counting efficiencies used in 'hand calculations'			'Hand-calculated' results		Deviations <sup>a</sup> by three-point method		Deviations <sup>a</sup> by least-mean-squares method	
		<sup>14</sup> C counting efficiency in window I	<sup>14</sup> C counting efficiency in window II	<sup>3</sup> H counting efficiency in window I	<sup>14</sup> C d.p.m./ml of solvent	<sup>3</sup> H d.p.m./ml of solvent	<sup>14</sup> C deviation	<sup>3</sup> H deviation	<sup>14</sup> C deviation	<sup>3</sup> H deviation
1	12.08	8.70	65.90	39.40	14.33	362.74	1.30	2.11	0.44-	2.10
2	11.50	8.80	64.90	39.40	39.45	967.67	1.57	1.43	0.50	1.42
3	10.57	8.90	62.00	38.90	90.59	5646.90	0.65	0.10-	0.50	0.10-
4	9.49	9.60	58.10	38.50	43.55	1387.13	0.03-	0.07-	0.65	0.08-
5	8.48	10.80	54.20	37.70	45.24	1451.25	0.16	0.16	1.34	0.15
6	7.59	12.10	50.00	36.70	1712.23	2482.75	0.01	0.65	1.38	0.30
7	6.51	13.90	43.80	34.70	4.88	14.82	1.04-	0.90	0.16	0.64
8	5.77	15.10	39.70	33.00	4.69	14.04	0.28-	0.57	0.49	0.26
9	4.39	17.70	30.40	29.30	3.50	8.37	0.31-	0.54	1.63-	0.27
10	3.66	19.20	25.50	26.80	956.43	9877.37	1.29	0.13-	2.32-	0.08-
11	2.51	22.10	17.30	23.10	462.92	3644.65	9.35	0.76	2.71-	1.77
12	1.47	25.30	10.60	19.30	441.32	3614.15	59.87	4.42-	8.91	3.11
13	0.65	29.40	5.20	15.00	499.39	3881.33	576.34-	142.47	71.95	14.32

a Difference between d.p.m./ml obtained by the three-point method or the least-mean squares method and the 'hand calculations' expressed as a percentage of the hand-calculated value.

b The results are for samples of upper or lower phase from a chloroform-methanol extraction of rat liver containing <sup>3</sup>H- and <sup>14</sup>C-labelled phospholipids or their water-soluble metabolic intermediates.

For the desk-top computation the least-mean-squares method giving overall best fit (as shown in Table 1(b)) was used.

c With permission from *Biochem. J.* 118, 379 (1970).

samples are from cases where both radioactive isotopes have been counted to a 2σ error better than 0.2% but have been considered as absolute values for the present purpose. If one isotope is counted to a smaller degree of precision than the other then obviously the errors incurred may be considerably increased.

**Use of the system for channels-ratio quench correction.** A series of samples was counted in a Nuclear Chicago Model 6801 spectrometer. The windows were set up using standards supplied by the manufacturer. Curves were constructed by plotting the ratio of counts in two of the channels against the counting efficiency. It was found that the system described above could simulate such curves with great precision for about 90% of their length. Only a slight modification in the programme used for calculating the results was necessary. First a channels ratio value was determined by dividing the sample activity in one channel by that in another. This value was then used in a similar way to the external standards channels-ratio value.

## GENERAL CONCLUSIONS

Besides quench curves for chloroform-quenched (<sup>3</sup>H)- and (<sup>14</sup>C)-n-hexadecane samples in a dioxan-based scintillator (the most applicable to the samples counted here), a number of other quench curves have been constructed. These account for a range of radioactive isotopes and window settings, colour and chemical quenching agents and a toluene-based scintillator. As all curves can be adequately expressed by quadratic equations, reprogramming is not required. A record is kept of the computed coefficients

*a*, *b* and *c* for each set of curves, and these are fed into the data store before a batch of samples is processed.

The programmes described here have been drawn up to deal with radioactive isotopes of relatively long half-lives and no account has been taken of isotopic decay. Sufficient space remains, however, within the data store and programming unit for the decay calculations to be included if required. Also, data may be automatically reworked for samples channels-ratio standardisation, d.p.m. estimates made by this means may then be compared with estimates made by the external standard channels ratio method. A tape reader is now available for this computing system allowing it to accept directly the output from certain scintillation counters.

## REFERENCES

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- 2 G. H. Cope and M. A. Williams, *J. Microscopy* **90**, 31 (1969).

## DISCUSSION

**T. L. Woods:** What is the average calculating time per sample (including data preparation time)?

**M. A. Williams:** For a reasonable number of samples (say 50), counted for 2 isotopes, it would average out at about 40 to 50 s each. This includes the two programs, the first to obtain the coefficients for the curves, the second to obtain d.p.m., subtract background, and relate d.p.m. to sample volume, density, weight, etc.

**J. A. B. Gibson:** The use of 180 points in establishing a quench curve is reasonable initially but there should be a simple relationship between curves. This should require a simple transformation of the variables (or axes) and hence only a limited number of points would be required for a repeat quenching curve.

**M. A. Williams:** Yes, it is sometimes quite possible to adjust the variables. However, at the start of a large batch of new material—every month or two—we prefer to check the curve. We feel that frequently people do not sufficiently define their quench curves, i.e. they do not use enough points.

**D. S. Glass:** What is the lowest efficiency you are prepared to work on for carbon-14? How important is it to calibrate at low efficiency levels?

**M. A. Williams:** We do not normally accept results from samples with total carbon-14 efficiencies below about 40%. Our first resort is to reprepare the sample. If this does not work we would consider doing some curve simulation, paying particular attention to the lower part (for double labelled samples). For single labelled materials one can always use an internal standard (one is not often driven to this!).

**D. Moore:** Have the calibration curves been produced for various quenching media as the shape of the curves will depend upon the material producing the quench?

**M. A. Williams:** Quench curves have been produced for a variety of chemical quenchers. The shapes of the curves vary, but in all cases simulation of the curves was feasible with this computing system.

**C. P. Bond:** With a similar instrument we find that for different counting systems, e.g. toluene as opposed to toluene/Triton X-100 (2:1), quench curves are entirely different—this may be due to the use of an emulsion system.

**J. R. Clapham:** What difficulties did you encounter due to the fact that data from the spectrometer is not on punch tape?

**M. A. Williams:** From our point of view the problems are small. We lose some time since we are tied to the computer during data processing. The time involved is about 30 to 40 min for 50 samples. To us this is not a serious matter.

**B. Scales:** Do your calibration curves vary in shape, depending on the scintillation mixture used?

**M. A. Williams:** Yes, they do. There are small changes in the general shape, together with changes in slope. Toluene based scintillators frequently give higher efficiencies than dioxan based ones. This, in itself, will alter the slope of the curves.