Liquid-Scintillation-Spectrometer Data Processing by Desk-Top Computer

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A desk-top computing system has been programmed to store accurately the quench curves necessary for the calculation of total disintegrations/minute (d.p.m.) of samples containing either one or two radioactive isotopes. In producing d.p.m. values background counts are subtracted, and in binary-labelled samples the counts attributable to each radioactive isotope are separated. The programme also relates d.p.m. to the weight, volume and density of the sample. Each variable is easily recalled and adjustments can be made for different batches of samples without reprogramming. Equally easily changes of radioactive isotope, quenching agent, scintillator or window setting can be accommodated. Quadratic equations are used to express the quench curves. Counting efficiencies obtained when the coefficients in the quadratic equations are derived from three carefully chosen points on a quench curve are compared with those obtained when the coefficients are derived by the method of least squares. The results of both mathematical approximations are compared with the efficiencies read by the eye from graphs.

Liquid-scintillation spectrometry is now widely used in biochemical and chemical laboratories for the assay of isotopes that emit soft β -particles. Many spectrometers now incorporate multi-channel analysers, coincident photomultiplier circuitry, automatic sample transport and data print-out. To determine the efficiency of counting, and subsequently the total d.p.m. of a sample, the amount of sample quenching must first be measured. Modern instruments achieve this automatically by determining the ratio of the frequency of pulses detected in two portions of a radioactive isotope's pulse energy spectrum. Sample quenching causes a change in this distribution and hence a change in the ratio. A fair estimate of the efficiency of sample radioactivity counting can be determined from graphs plotted from the known counting efficiency of a series of quenched standard samples and their respective ratios. Ratios are obtained either from the photon energy spectrum induced by the sample isotope ('channels-ratio' method; Baillie, 1960; Bush, 1963) or from the photon energy spectrum induced in the scintillator by the Compton effect of an externally located γ -ray source ('external-standard channels-ratio' method; Hall & Weiser, 1966). This latter method is favoured by many manufacturers as it does not depend on the size of the sample radioactivity count (and concomitant

statistical counting errors) and as a single spectrum analysis will suffice for most of the commonly used radioactive isotopes.

A new generation of spectrometers is emerging with built-in computation facilities that will automatically and accurately determine the degree of sample quenching from curves stored as mathematical expressions, calculate d.p.m. values of single- or multiple-labelled samples and relate these to other variables such as sample size or specific radioactivity. Such calculations when performed by hand can be time-consuming. These machines are, however, very expensive, and in some instances their computation facilities cannot be used except for processing scintillation data. Some existing spectrometers may be linked on-line or via punched tape to a desk-top computing system. As applied to date, however, these have not been entirely satisfactory. Limited programming steps and storage space has prevented both the accurate expression of quench curves and the analysis of binary-labelled samples.

We report here the application of a commercially available desk-top system that will permit the accurate storage of the quench curves necessary for the calculation of total d.p.m. of samples containing either one or two radioactive isotopes. In producing d.p.m. values background counts are subtracted,

and in binary-labelled samples the counts attributable to each radioactive isotope are separated. The programme also relates d.p.m. to the weight, volume and density of the sample. Each variable is easily recalled and adjustments can be made for different batches of samples without reprogramming. Equally easily, changes of isotope, quenching agent, scintillator or window setting can be accommodated. The system described is versatile and relatively inexpensive and will fulfil, we believe, the data-processing function of the most sophisticated spectrometers currently available.

METHODS

Labelled materials. [1,2-³H]-*n*-Hexadecane (specific radioactivity 2.45 μ Ci/g) and [1-¹⁴C]-*n*-hexadecane (specific radioactivity 0.97 μ Ci/g) (The Radiochemical Centre, Amersham, Bucks., U.K.) were used as standards.

The livers from rats that had been injected intraperitoneally with [*Me*-³H]choline chloride and [2-¹⁴C]-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 (Folch, Lees & Sloane-Stanley, 1957). One phase contained ³H- and ¹⁴C-labelled phospholipids and the other water-soluble ³H- and ¹⁴C-labelled metabolic intermediates (Cope & Williams, 1969). 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).

A Beckman LS 100 scintillation spectrometer fitted with a ¹³⁷Cs 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 ³H spectrum was excluded (window I: ³H efficiency 39.50%; ¹⁴C efficiency, 8.70%). The lower discriminator of window II was set just above the ³H energy spectrum and the upper discriminator just above the top of the ¹⁴C energy spectrum (window II: ³H efficiency, 0.00%; ¹⁴C efficiency, 66.20%).

The external standard was set up according to manufacturer's instructions to record a value of about 12.50 for the ³H standard.

Preparation of quench curves. Separate ³H and ¹⁴C standards were prepared in duplicate and their radioactivities were counted to a 2 σ 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 5 min their radioactivities were re-counted with the same precision. This procedure was repeated until the recorded external-standard channels-ratio had fallen to 0.00 (about 180 drops). The ³H and ¹⁴C counting efficiencies in window I and the ¹⁴C counting efficiencies in window II were calculated after each drop of chloroform

Fig. 1. External-standard quench-ratio value plotted against counting efficiency for ³H and ¹⁴C standards (*n*-hexadecane in PPO-POPOP-naphthalene dissolved in dioxan-toluene) progressively quenched with chloroform. Curve 1 is of ¹⁴C efficiency in window I, curve 2 is of ¹⁴C efficiency in window II and curve 3 of ³H efficiency in window I (see the Methods section). The arrows indicate the sample points used for the three-point method.

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 ¹⁴C counts in window I:

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

Determination of ³H d.p.m.:

$$\frac{(I_q - R)}{y_3} \times k_3 = D_t \quad (4)$$

Determination of ¹⁴C d.p.m.:

$$\frac{II_q \times k_3}{y_2} = D_c \quad (5)$$

³H d.p.m. calculated per ml of solvent:

$$\frac{D_t \times k_4 \times v_2}{v_1} = {}^3\text{H d.p.m./ml of solvent} \quad (6)$$

¹⁴C d.p.m. calculated per ml of solvent:

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

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 spectro-

Fig. 2. Block diagram illustrating the arrangement of the desk-top calculator with its programming unit and typewriter print-out. Programmes can be fed in via the calculator keyboard (IME 86S) or via eight-holed edge-punched cards or punched tape.

meter), I_a is c.p.m. in window I with background counts subtracted, II_a 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 ^{14}C counting efficiency in window I, y_2 is ^{14}C counting efficiency in window II, y_3 is ^3H counting efficiency in window I, v_1 is sample weight (mg), v_2 is sample density (g/ml), D_1 is ^3H d.p.m. (uncorrected for sample weight etc.), D_2 is ^{14}C d.p.m. (uncorrected for sample weight etc.) and R is ^{14}C 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 Datalog typewriter via a recoding output unit (Muldivo OP 207). The arrangement of units is shown diagrammatically in Fig. 2. 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.

(a) 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:

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

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

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

(b) Least-squares method. The co-ordinates of a number of points on a curve are entered into a programme loop and

values of Σx , Σx^2 , Σx^3 , Σx^4 , Σy , Σxy , Σx^2y and n are accumulated. When all co-ordinates have been entered, the programme passes from the loop to solve the following equations in sequence:

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

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

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

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

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

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

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

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. However, over much of their length 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 3.00–11.00, 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 3.50–11.50. 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 co-ordinate 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. However, deviations over the middle of the quench-ratio range (3.50–11.50) are sometimes larger than by the three-point method (cf. Tables 1a and 1b). 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 1a and 1c).

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 (100–700mg) of upper or lower phase of extracts of rat liver containing both ^3H and ^{14}C 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 minimize 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.)

Table 1. *Counting efficiencies read from graphs by the eye compared with those computed from quadratic equations*

External-standard quench- ratio value	¹⁴ C counting efficiency in window I			¹⁴ C counting efficiency in window II			³ H counting efficiency in window I		
	Graph value*	Computed value	Deviation	Graph value*	Computed value	Deviation	Graph value*	Computed value	Deviation
1(a) Three-point method									
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—
1(b) Least squares method (best overall fit†)									
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—
1(c) Least-squares method (restricted sample range‡)									
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	7.82	1.08—	66.20	66.85	0.65	39.50	38.24	1.26—

* Values read by eye from the graphs shown in Fig. 1.

† Computed from co-ordinate points along the whole length of each curve.

‡ Computed from co-ordinate points within the limits set by the left-hand and right-hand arrows on each curve in Fig. 1.

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 ³H counts from ¹⁴C 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 deviation quoted here for binary-labelled 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.

DISCUSSION

The IME desk-top computing system described is used in our laboratory to deal with various numerical problems and not solely to process scintillation data. For this reason we have not used it on-line with the spectrometer, although this is

Table 2. *Comparison of results obtained by 'hand calculation' and by desk-top computation*

The results are for samples of upper or lower phase from a chloroform-methanol extraction of rat liver containing ^3H - and ^{14}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 1b) was used.

Sample no.	External-standard quench-ratio value	Minimum counting efficiencies used in 'hand calculations'			'Hand-calculated' results		Deviations* by three-point method		Deviations* by least-mean-squares method	
		^{14}C counting efficiency in window I	^{14}C counting efficiency in window II	^3H counting efficiency in window I	$10^{-3} \times ^{14}\text{C}$ d.p.m./ml of solvent	$10^{-3} \times ^3\text{H}$ d.p.m./ml of solvent	^{14}C deviation	^3H deviation	^{14}C deviation	^3H 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

* 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.

technically possible. On-line use would be wasteful, since computation time is considerably less than counting time, particularly when minimal statistical errors are required.

Quench curves are often constructed by drawing a parabola of best fit through about six or eight points derived from a series of quenched standards supplied by spectrometer manufacturers. Obviously such curves can be perfectly predicted and may give the impression that any calculations based on these curves will carry no error. However, in our experience quench curves are seldom parabolic, and it is necessary to determine many more than six to eight points before constructing a curve.

Besides quench curves for chloroform-quenched [^3H]- and [^{14}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. However, sufficient space remains within the data store and programming unit for the decay calculations to be included if required.

We have also ascertained that the computing system is equally applicable to channels-ratio data.

A series of samples was counted in a Nuclear-Chicago model 6801 spectrometer and curves were constructed. The ^{14}C quench curve obtained by channels ratio could be accurately simulated over much of its length by quadratic equations. A slight modification was made to the programme that calculates the results. First, a channels-ratio value is determined by dividing the sample radioactivity counts in one channel by those in another. This value is then used in a similar way to the external-standard channels-ratio value, and the programme proceeds as described above.

It is envisaged that the system described here would be most useful where the radioactivities of large numbers of related binary-labelled samples are to be counted, for example column chromatography or sucrose-density-gradient fractions. Equally well, a large number of single-labelled samples can be processed.

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