

A Flexible FORTRAN IV Program for Scintillation Counter Data Processing with Optional Features for User's Specific Requirements

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INTRODUCTION

Nowadays most users of scintillation counters expect automatic processing of raw data in order to calculate the absolute activity in their samples and possibly to perform further analysis of results. The techniques which have been described include:

1. simple production of counts per unit time within a calculator in the counter (for example, continuously during counting as in the Packard 3375, which also computes the standard deviation of the counts, or at the end of counting as in the Nuclear Chicago Mk. II);
2. 'on-line' computation by either a small computer built into the counter (as in the Intertechnique SL40) or by a desk-top calculator such as the Olivetti Programma 101, or Diel calculator as used with the Tracerlab Corumatic 2000;
3. transfer of data to a large computer, followed by processing the data as a batch-job, either off-line or by direct access via an on-line time sharing terminal.

The main advantages of the large computer are that the data can be readily stored and edited, and, because of the speed and storage capacity of the machine, more sophisticated and extensive processing of the data can be performed.

The kinds of data reduction which users require depend upon the type of experiments they perform and, in any environment where there are a number of users of one machine, there may be a wide variety of demands. Descriptions of a number of programs, written for large computers have appeared in the literature. Most have been written in high level languages such as FORTRAN or ALGOL, and thus can be used with only minor modification at most computer installations. Many of these programs have been cited in the review by Spratt,¹ other and more recent examples are those of Johnson et al.;² Barber and Bourne;³ Stanley;⁴ Assailly et al.;⁵ Ayrey and Evans;⁶ Deterding;⁷ Haissig and Schipper;⁸ Yund et al.;⁹ Cramer et al.;¹⁰ Hansen and Carroll;¹¹ Forker and Wycoff;¹² Boeckx et al.;¹³ O'Toole and Osburn.¹⁴ These programs have usually been written for a particular

application and with the needs of a few users in mind. They thus have the following disadvantages: (1) the format of the data expected by the program relates to one counter; (2) it is assumed that all of a given batch of data is to be processed in the same way; (3) the instructions concerning which samples are to be processed, methods of quench correction and degree of further data reduction are often not self-explanatory. The user is often required to provide instructions in a numerical, abbreviated form, a scheme which is not easy to remember nor provides a reliable system in a multi-user environment.

GENERAL FACILITIES OF THE PROGRAM

In order to overcome some of these disadvantages we have written a program in FORTRAN IV (for the IBM 360/370 G1 and H extended compilers and using list-directed READ routines), which provides very general facilities for processing scintillation counter data, according to simple self-explanatory instructions supplied by the user. The program which compiles in about 150K and runs in approximately 92K bytes of main store, performs the following functions:

1. reads data, from paper tape, cards, magnetic tape or disc, as required, in a format which is specified by the user;
2. translates users' instructions;
3. outputs a copy of instructions and raw data to facilitate data checking;
4. calculates and outputs counts per minute (c.p.m.) for each sample for each channel and also channels ratios if requested;
5. performs subtraction of background from samples. If determination of efficiency is by the External standard Channels Ratio (ECR) method (see below), quench corrected backgrounds may be determined by reference to a set of quenched backgrounds.^{15, 16} When the Sample Channels Ratio (SCR) method is used, quench corrected background subtraction is not used, but a mean background is used;
6. calculates disintegrations per minute (d.p.m.) for samples containing up to three isotopes. The following methods of computation can be selected:
 - i. counting efficiencies for each isotope, which are assumed constant for a set of samples, are supplied by the user;
 - ii. counting efficiencies, assumed constant for a set of samples, are computed from samples containing reference amounts of activity;
 - iii. quench correction for a set of samples is applied by SCR or ECR methods. In the SCR and ECR modes, the curves relating counting efficiency to channels ratio are described by second order polynomials. The coefficients of the polynomials may either be calculated by the program from sets of reference standards or supplied by the user. Optionally, quenched coefficients which have previously been computed or supplied may be normalised by reference to a 'reference' standard or standards.¹⁷ Using the ECR mode, d.p.m. for three mixed isotopes may be computed from counts in three channels. In the SCR mode, d.p.m. are calculated assuming no overspill of the lower energy isotope into the ratio channels used for the high energy isotope¹⁸⁻²⁰ and d.p.m. for a maximum of two mixed isotopes can be calculated;
7. d.p.m. for each isotope are corrected for decay during the time elapsed since the first sample began counting. The time elapsed during sample transfers, etc. in the sample changer is allowed for. Assumed values for individual counters are selected when the user specifies the format of the data input;
8. the mass of each labelled species in each sample is computed, if the specific activity of each isotope is provided;

9. additional subroutines can be called to perform further arithmetical procedures according to individual requirements. The basic program does not have to be altered because further subroutines communicate with values already computed via 'common blocks' of the program.

FORMAT OF INSTRUCTIONS

The instructions allow the user to subdivide the data file into groups by sample pot number, each group being considered as one data set. Each data set may be processed in different ways according to the users' requirements by instructions punched on corresponding groups of cards, called instruction sets.

Each of the types of instruction card has as its first argument a name or an alias which reflects the nature of the instructions it contains. This name is then followed by further arguments, which provide information required for the instructions to be executed. The cards are as follows:

<u>Card</u>	<u>First Argument</u>	<u>Function of Instruction Card</u>
1	'*'	separation card between instruction sets.
2	'OPTIONS' 'O'	defines the counter used and format of data, for example 'NC1' defines 'one-line' output format from a Nuclear Chicago Mk. II counter.
3	'TITLE' 'T'	allows the addition of a title for the currently selected data set on the card following.
4	'ISOTOPES' 'I'	defines the isotopes present in the currently selected data set and the channel optimised for that isotope. Options for channels are 'A' 'B' 'C' and for isotope names '3H' '14C' '32P' '35S' '45CA' '125I' '131I'.
5	'SAMPLES' 'S'	defines the position (from the pot numbers on the scintillation counter output) of the currently selected data set.
6	'BACKGROUND' 'BG'	defines the position of background pots for the currently selected data set. If quenched background correction using ECR is required, the location of background pots is provided on the 'MODE' card, and this card is ignored.
7	'MODE' 'M'	defines the way in which d.p.m. are to be calculated for the currently selected data set. Permitted options which are specified by the second argument on this card are: 'READ' — reads the counting efficiencies of each isotope in each used channel; 'CALC' — calculates the counting efficiencies of each isotope in each used channel from specified pots containing reference material; 'SCR' — Sample Channels Ratio method; 'ECR' — External standard Channels Ratio method.
8	'REFERENCE' 'R'	this card is optional and allows 'normalisation' of quench correction coefficients by reference to a 'reference' standard.

9 'ADD' this card is optional and defines additional
 'A' subroutines provided by the user.

In order to process a single data set, one instruction set, consisting of cards 1—7 must be present, cards 8 and 9 are optional. To process several data sets or one data set in several ways, further instruction sets are provided. Only instruction cards which are to be changed are added; the other cards may be omitted, in which case the values of the arguments on these cards, which were defined for the previous data set, remain unchanged. Figure 1 shows an example of two instructions for the calculation of d.p.m. by the ECR and then SCR modes on one set of data, and Fig. 2 the output format for d. p. m.

Card Instruction

```

1  '*' /
2  'OPTIONS' 'NC1' /
3  'TITLE' /
   TEST RUN OF QUENCH SET PLUS SAMPLES
4  'ISOTOPES' '3H' 'A' '14C' 'B' /
5  'SAMPLES' 176 187 /
6  'BACKGROUND' /
7  'MODE' 'ECR' 'S' 234 8 10000 0, 218 8 10000 0, 250 8 /
8  '*' /
9  'M' 'SCR' 'B/C' 'S' 234 8 10000 0, 218 8 10000 0, 250 8 /
10 '*' /

```

The arguments on the 'MODE' cards (cards 7,9 in the example) signify:

- 'B/C' - ratio of channel B over channel C
- 'S' - quench coefficients to be computed from standards, then for each isotope
 - location of first pot containing standard (e.g. 234)
 - number of standards in quenched set (e.g. 8)
 - d.p.m. in standard (e.g. 10000)
 - time elapsed since standard was calibrated (e.g. 0)
 then for backgrounds
 - location of first pot containing background (e.g. 250)
 - number of backgrounds in quenched set (e.g. 8)

Fig. 1. An example of two instruction sets for processing a data set by the ECR and SCR modes.

TEST RUN OF QUENCH SET PLUS SAMPLES
 NO BG POTS SPECIFIED ON BG CARD: NO BG COUNTS HAVE BEEN SUBTRACTED,
 UNLESS BG POTS OR COEFFICIENTS HAVE BEEN DEFINED ON THE 'MODE' CARD,
 IN WHICH CASE A NOTE OF THESE APPEARS BELOW

BG COEFFICIENTS OBTAINED FROM 'MODE' 'ECR' CARD
 CHANNEL A - .355E+02 0.282E+02 -.366E+01
 CHANNEL B -.701E+01 0.754E+01 -.962E+00
 CHANNEL C 0.0 0.0 0.0

BG CPM OBTAINED FROM 'MODE' 'SCR' CARD ARE:
 CHAN A 18.14 CHAN B 7.56 CHAN C 2.89

THE THREE COEFFICIENTS OF THE QUENCH CURVE
 C.EFF = QC1 + QC2*RATIO + QC3*(RATIO) **2
 ARE GIVEN BELOW FOR EACH ISOTOPE IN EACH USED CHANNEL

3H	IN CHANNEL A	0.166E+02	0.892E+01	-0.959E+00
14C	IN CHANNEL A	0.283E+02	-0.744E+01	0.627E+00
3H	IN CHANNEL B	0.194E+01	-0.122E+01	0.194E+00
14C	IN CHANNEL B	-0.467E+02	0.518E+02	-0.572E+01

THE THREE COEFFICIENTS OF THE QUENCH CURVE
 C.EFF = QC1 + QC2*RATIO + QC3*(RATIO) **2
 ARE GIVEN BELOW FOR EACH ISOTOPE IN EACH USED CHANNEL

3H	IN CHANNEL A	0.221E+02	0.986E+01	-0.160E+01
14C	IN CHANNEL A	0.208E+02	-0.587E+01	0.472E+00
3H	IN CHANNEL B	0.199E+01	-0.190E+01	0.457E+00
14C	IN CHANNEL B	-0.401E+01	0.476E+02	-0.758E+01

TEST RUN OF QUENCH SET PLUS SAMPLES

DPM OF EACH ISOTOPE FOR THIS SAMPLE SET

SAMPLE NO.	POT NO.	DPM FOR 3H	DPM FOR 14C
1	176.	0.	9921.
2	177.	0.	9966.
3	178.	28.	9992.
4	179.	0.	10137.
5	180.	10218.	0.
6	181.	10232.	4.
7	182.	10209.	1.
8	183.	10190.	0.
9	184.	10219.	9733.
10	185.	10179.	9966.
11	186.	10161.	10037.
12	187.	10177.	10053.

TEST RUN OF QUENCH SET PLUS SAMPLES

DPM OF EACH ISOTOPE FOR THIS SAMPLE SET

SAMPLE NO.	POT NO.	DPM FOR 3H	DPM FOR 14C
1	176.	0.	9959.
2	177.	0.	9922.
3	178.	0.	9984.
4	179.	0.	10097.
5	180.	10077.	0.
6	181.	9892.	0.
7	182.	11890.	0.
8	183.	10917.	0.
9	184.	10057.	9798.
10	185.	10045.	9949.
11	186.	10029.	10006.
12	187.	10028.	10076.

Fig. 2. Output giving d.p.m. for the instruction set described in Fig. 1.

DISCUSSION

General

We have found, in a multi-user environment, that the processing of scintillation counter data by computer is made much simpler and more reliable for most users by the provision of a program which allows input of instructions in a simple and self-explanatory format. A similar approach has recently been implemented in a program in PLAN4 for a 'pseudo-on-line' system using the ICL1900.²¹

Our program as it is presently implemented allows the choice between the two commonest methods of quench correction, SCR and ECR. The comparison of results produced by the two methods permits detection of inhomogeneity in sample preparation as suggested by Bush.²²

It would be desirable to add further subroutines to provide options for quench correction by the so-called parabolic curve-fitting channels ratio method,²³ and by the SCR method in the presence of overspill by the technique of Hansen and Carroll.¹¹ These routines could easily be added and would be invoked by new calling arguments on the 'MODE' card, e.g. 'PARA' and 'SCR1', without interference with the rest of the program. Implementation of the Hansen and Carroll techniques would also allow quench correction of backgrounds using SCR as suggested by Stanley,²⁴ again without alteration to other options.

Concerning error calculations

Scintillation counting finds most use in the measurement of isotopes used as markers of molecules in physical, chemical and biological experiments. The experimental errors associated with typical experiments derive from:

1. inherent variability in the experimental system, e.g. biological variation;
2. inaccuracies in conducting the experiment — e.g. pipetting, weighing, etc. up to and including the preparation of sample for scintillation counting;
3. error due to the random nature of radioactive decay — this is assumed to follow the Poisson distribution model, although will not do so if the counter is insufficiently stable — see Matthijssen and Goldzieher,²⁵ cited by Spratt;¹
4. errors due to machine instability, irregularity of sample vials and vial positioning;⁴
5. errors in performing quench correction by external standard due to irregularity of sample vials and positioning of external standard.⁴

It is obviously desirable to attempt an estimate of errors associated with the most significant error terms and as far as counting is concerned it is advisable to estimate both errors due to counting statistics for each sample and the error associated with fitting quench correction curves; the latter also being compounded of errors due to pipetting of the standards and counting statistics. It is probably most informative if these errors are presented separately as $\pm 95\%$ confidence limits and then an attempt is made to combine them to give an estimate of the overall 95% confidence limits for the counting process. In this way, the major source of error, viz. sample counting or curve fitting, can easily be identified. Furthermore, it is probably advisable to calculate the error which is apparent in repeated counting of each sample and to compare it with the theoretical counting statistics. In this way machine drift may be detected.⁷

Concerning errors in counting statistics

It is obviously straightforward to compute errors associated with the counts accumulated in each channel and to combine these with the errors in counting

the background in each channel, to give a net channel count rate error.^{1, 26}

Remembering that in combining errors, the following rules apply, where $\sigma^2(A)$ is the variance of A, and $\sigma(A)$ is the standard deviation of A.

If $C = A \pm B$
 then $\sigma^2(C) = \sigma^2(A) + \sigma^2(B)$ (1)

and $\sigma(C) = \sqrt{\sigma^2(A) + \sigma^2(B)}$

If $C = A \times B$ or $C = A/B$
 then $\frac{\sigma^2(C)}{C^2} = \frac{\sigma^2(A)}{A^2} + \frac{\sigma^2(B)}{B^2}$
 $\sigma^2(C) = C^2 \left[\frac{\sigma^2(A)}{A^2} + \frac{\sigma^2(B)}{B^2} \right]$ (2)

and $\sigma(C) = C \sqrt{\frac{\sigma^2(A)}{A^2} + \frac{\sigma^2(B)}{B^2}}$

Let sample gross count = S; background count = B; sample counting time = T_S ; background counting time = T_B ; sample gross count rate = SCPM; background count rate = BCPM; and let net count rate = NCPM.

$$\text{SCPM} = S/T_S \quad (3)$$

and $\text{BCPM} = B/T_B$ (4)

We wish to find the variance in the net count rate, and hence the standard deviation and 95% confidence interval.

Now $\text{NCPM} = \text{SCPM} - \text{BCPM}$

from (3) and (4) $\text{NCPM} = S/T_S - B/T_B$

Using (1) to combine variances

$$\sigma^2(\text{NCPM}) = \sigma^2(S/T_S) + \sigma^2(B/T_B) \quad (5)$$

Now $\sigma^2(S/T_S)$ is simplified by combining variances as in (2).

Thus $\sigma^2(S/T_S) = (S/T_S)^2 \left[\frac{\sigma^2(S)}{S^2} + \frac{\sigma^2(T)}{T^2} \right]$

but $\frac{\sigma^2(T)}{T^2} \ll \frac{\sigma^2(S)}{S^2}$ because the counter time is sufficiently precise. Therefore

$$\begin{aligned} \sigma^2(S/T_S) &= (S/T_S)^2 \left(\frac{\sigma^2(S)}{S^2} \right) \\ &= \frac{\sigma^2(S)}{T_S^2} \end{aligned}$$

Likewise $\sigma^2(B/T_B) = \frac{\sigma^2(B)}{T_B^2}$

Substitute in Eqn. (5). Hence

$$\sigma^2(\text{NCPM}) = \frac{\sigma^2(S)}{T_S^2} + \frac{\sigma^2(B)}{T_B^2}$$

For the Poisson distribution, $\sigma^2(S) = S$, $\sigma^2(B) = B$. Therefore

$$\begin{aligned}\sigma^2(\text{NCPM}) &= \frac{S}{T_S^2} + \frac{B}{T_B^2} \\ &= \frac{\text{SCPM}}{T_S} + \frac{\text{BCPM}}{T_B}\end{aligned}$$

Hence also $\sigma(\text{NCPM})$ and \pm the 95% confidence limit (CI) where $\text{CI} = 1.96 \sigma(\text{NCPM})$. In practice the 2σ error is often used, being the 95.45% confidence limit, which is slightly more conservative.

Concerning the error in interpolating a quench correction curve

Determination of d. p. m. by ECR and SCR involves quench correction curves, which are often represented by a polynomial relating counting efficiency to channels ratio. The errors associated with interpolation of such a curve arise from counting statistics for the standards and also because, whatever the order of the polynomial, it may not represent the real behaviour of the machine. This is so even within the range of standards, let alone in extrapolated regions beyond. Some authors consider that other functions, for example logarithmic, give a better representation of machine performance.^{6,7} We will consider the error associated with fitting a second order polynomial although the treatment is general,²⁷ allowing handling of high order polynomials, and is performed in the program by an IBM supplied subroutine.

$$\text{Let} \quad E = a_0 + a_1 R + a_2 (R)^2 \quad (6)$$

where E is the efficiency and R is the ECR.

Assume we have n standards and thus produce n values of E and R given by e_n and r_n . The coefficients of this curve are generated by the method of least squares, i. e. minimising the sum of squares of the observed values ($= e$) from the expected values ($= E$) predicted by Eqn. (6). That is, we choose the values of a_i

$$\text{which minimise} \quad Q = \sum_{i=1}^n (e_i - E_i)^2$$

$$\text{which is} \quad Q = \sum_{i=1}^n (e_i - a_0 - a_1 r_i - a_2 r_i^2)^2$$

The values of a_1 , a_2 are given by the solution of the set of simultaneous equations

$$a_1 C_{11} + a_2 C_{12} = D_{e1} \quad (7)$$

$$a_2 C_{21} + a_2 C_{22} = D_{e2} \quad (8)$$

where

$$C_{11} = \sum_{i=1}^n (r_i - \bar{r}) (r_i - \bar{r})$$

$$C_{12} = \sum_{i=1}^n (r_i - \bar{r}) (r_i^2 - \bar{r}^2)$$

$$C_{21} = \sum_{i=1}^n (r_i^2 - \bar{r}^2) (r_i - \bar{r})$$

$$C_{22} = \sum_{i=1}^n (r_i^2 - \bar{r}^2) (r_i^2 - \bar{r}^2)$$

$$D_{e1} = \sum_{i=1}^n (e_i - \bar{e}) (r_i - \bar{r})$$

$$D_{e2} = \sum_{i=1}^n (e_i - \bar{e}) (r_i^2 - \bar{r}^2)$$

and

$$a_0 = \bar{e} - a_1 \bar{r} - a_2 \bar{r}^2 \quad (9)$$

Now in any subsequent determination of E from a single value of R we may determine an error in E from

$$\begin{aligned} \sigma^2(E) &= \sigma^2(\bar{e}) + \frac{(E - \bar{e})^2}{\bar{r}^2} \sigma^2(a_1) \\ &\quad + (E^2 - \bar{e}^2) \sigma^2(a_2) \\ &\quad + 2 (E - \bar{e}) (E^2 - \bar{e}^2) \text{CoV} (a_1 a_2) \end{aligned} \quad (10)$$

where

$$\sigma^2(\bar{e}) = \frac{s^2}{n} \quad (11)$$

and s^2 is the residual variation of the regression.

Fortunately in solving Eqns. (7) and (8) for a_1 and a_2 if the matrix

$$D = \begin{vmatrix} C_{11} & C_{12} \\ C_{21} & C_{22} \end{vmatrix}$$

is inverted to give

$$1/D = \begin{vmatrix} C^{11} & C^{12} \\ C^{21} & C^{22} \end{vmatrix}$$

then the terms C^{zz} are proportional to the variances and covariances of the coefficients a_1, a_2 as follows:

$$\begin{aligned} \sigma^2(a_1) &= s^2 C^{11} \\ \sigma^2(a_2) &= s^2 C^{22} \\ \text{CoV}(a_1, a_2) &= s^2 C^{12} \\ &= s^2 C^{21} \end{aligned}$$

Hence from Eqns. (10) and (11)

$$\begin{aligned} \sigma^2(E) &= \frac{s^2}{n} + (E - \bar{e})^2 s^2 C^{11} \\ &\quad + (E^2 - \bar{e}^2) s^2 C^{22} \\ &\quad + 2(E - \bar{e})(E^2 - \bar{e}^2) s^2 C^{12} \end{aligned} \quad (12)$$

In the program, this error calculation is performed for each determination of counting efficiency and the 95% confidence interval (CI) calculated from

$$CI = t_{n-2} \sigma(E)$$

where the value of t , Student's t for $n - 2$ degrees of freedom, is calculated by the program.

Concerning the combination of errors of counting statistics and interpolation of quench correction curves

Single isotope case. In this case

$$DPM = \frac{NCPM}{E}$$

Thus

$$\sigma^2(DPM) = (DPM)^2 \left[\frac{\sigma^2(NCPM)}{(NCPM)^2} + \frac{\sigma^2(E)}{(E)^2} \right]$$

This case is straightforward, but notice that if the net background count rate is determined from a quench corrected background, then $\sigma^2(BCPM)$ is determined as in Eqn. (12) and

$$\sigma^2(NCPM) = \frac{SCPM}{T_S} + \sigma^2(BCPM)$$

Double isotope case. In this case the combination of errors by the methods already discussed becomes more complicated. Assume the ECR method is used and that in counting the sample overspill occurs from the low energy sample channel, A, to the high energy sample channel, B. Let E_{ij} be the counting efficiency for isotope i in channel j . Then

$$(\text{CPM}_A) = E_{1A} (\text{DPM}_1) + E_{2A} (\text{DPM}_2)$$

$$(\text{CPM}_B) = E_{1B} (\text{DPM}_1) + E_{2B} (\text{DPM}_2)$$

hence

$$\text{DPM}_1 = \frac{E_{2B}(\text{CPM}_A) - E_{2A} (\text{CPM}_B)}{E_{1A} E_{2B} - E_{2A} E_{1B}} \quad (13)$$

$$\text{DPM}_2 = \frac{E_{1B} (\text{CPM}_A) - E_{1A} (\text{CPM}_B)}{E_{2A} E_{1B} - E_{2B} E_{1A}} \quad (14)$$

The values of $\sigma^2(E_{ij})$ are computed for the four quench correction curves as usual for each sample and the net count rate errors for CPM_A and CPM_B are also known. Hence by the use of Eqns. (1) and (2) for combinations of errors, $\sigma^2(\text{DPM}_1)$ and $\sigma^2(\text{DPM}_2)$ could be computed.

From inspection of Eqns. (13) and (14), however, it is clear that the expression for $\sigma^2(\text{DPM})$ is arithmetically cumbersome and does not apparently have a general form. The computation would probably best be performed by the use of two sub-routines for combining errors. This has not so far been implemented.

General consideration. The computational untidiness associated with estimation of propagated errors could probably be overcome by the use of special techniques²⁸ such as (1) rounded interval arithmetic, (2) unnormalised arithmetic, (3) normalised floating point arithmetic with an index of significance, (4) automatic controlled precision arithmetic. Certainly some compilers have been written for rounded interval arithmetic in high level language such as Algol 60.²⁹ It is generally held, however, that measurement of propagation of errors by these techniques may yield excessively pessimistic estimates.

REFERENCES

- 1 J.L. Spratt in Liquid Scintillation Counting, Vol. 2, Heyden, London, 1972, p.245.
- 2 P. Johnson, P.A. Rising and J.T. Rising, in Liquid Scintillation Counting, Vol. 2, Heyden, London, 1972, p.267.
- 3 H.E. Barber and G.R. Bourne in Liquid Scintillation Counting, Vol. 2, Heyden, London, 1972, p.279.
- 4 P.E. Stanley in Liquid Scintillation Counting, Vol. 2, Heyden, London, 1972, p.285.
- 5 J. Assailly, C. Bader, J.-L. Funck-Brentano and D. Pevel, in Liquid Scintillation Counting, Vol. 2, Heyden, London, 1972, p.293.
- 6 G. Ayrey and K.L. Evans in Liquid Scintillation Counting, Vol. 2, Heyden, London, 1972, p.303.
- 7 J.H. Deterding in Liquid Scintillation Counting, Vol. 2, Heyden, London, 1972, p.313.
- 8 B.E. Haissig and A.L. Schipper, Analyt. Chem. 42, 1456 (1970).
- 9 M.A. Yund, E.W. Yund and F.C. Kafatos, Biochem. Biophys. Res. Commun. 43, 717 (1971).
- 10 C.F. Cramer, M. Nicholson, C. Moore and K. Teng, Intern. J. Applied Radiation and Isotopes 22, 17 (1971).
- 11 D.L. Hansen and C.O. Carroll, Intern. J. Applied Radiation and Isotopes 22, 677 (1971).
- 12 F.L. Forker and D. Wycoff, Anal. Biochem. 45, 107 (1972).
- 13 R.L. Boeckx, D.J. Protti and K. Dakshinamurti, Anal. Biochem. 53, 491 (1973).

- 14 J.J. O'Toole and J.O. Osburn, Intern. J. Applied Radiation and Isotopes 19 821 (1968).
- 15 B. Scales, Anal. Biochem. 5, 489 (1963).
- 16 P.D. Klein and W.J. Eisler, Analyt. Chem. 38, 1453 (1966).
- 17 B.E. Gordon, W.T. Shebs and R.U. Bonnar, J. Amer. Oil Chemists' Soc. 44, 711 (1967).
- 18 L.A. Baillie, Intern. J. Applied Radiation and Isotopes 8, 1 (1960).
- 19 E.T. Bush, Analyt. Chem. 35, 1024 (1963).
- 20 R.W. Hendler, Anal. Biochem. 7, 110 (1964).
- 21 B. Seaton, Biochemical Soc. Trans. 1, 517 (1973).
- 22 E.T. Bush, Intern. J. Applied Radiation and Isotopes 19, 447 (1968).
- 23 B.D. Caddock, P.T. Davies and J.H. Deterding, Intern. J. Applied Radiation and Isotopes 18, 209 (1967).
- 24 P.E. Stanley, this symposium, 'Fundamental Approaches for the Assessment of Chemical and Colour Quenching in Backgrounds and Samples', p. 65.
- 25 C. Matthijssen and J.W. Goldzieher, Anal. Biochem. 10, 401 (1965).
- 26 G.E.A. Wyld, in The Current Status of Liquid Scintillation Counting (ed. E.D. Bransome), Grune and Stratton, New York, 1970, p. 69.
- 27 O.L. Davies (ed.), Statistical Methods in Research and Production, 3rd Edn., Oliver and Boyd, London, 1967, p. 238ff.
- 28 E. Hansen (ed.), Topics in Interval Analysis, Clarendon Press, Oxford, 1969.
- 29 K. Nickel, in Topics in Interval Analysis (ed. E. Hanson), Clarendon Press, Oxford, 1969.

DISCUSSION

E.B. Mueller: Does your program average multiple countings of recycled samples?

D.E. Bowyer: Not at the moment but this option can be added and would, I think, be a desirable addition.

E.B. Mueller: I believe that the statistical variation in multiple countings is more important than the expected statistical error for a single counting.

D.E. Bowyer: I would not say more important in general, but certainly valuable for detecting erroneous counts due to machine malfunction, etc. during one counting period. The problem in everyday work in association with experimentalists, biologists at any rate, is persuading them to do repeated counting.

L.A. Wegner: Why do you restrict the polynomial fit to the second order? Using a modified Nuclear-Chicago program (which makes available fits of up to the order $n = 10$) the experience of our laboratory is: performing the fitting routinely for three different types of Liquid Scintillation Spectrometers, in most cases the 'best' polynomial fit (with a minimum n) is achieved with values of n : $-4 \leq n \leq 6$.

D.E. Bowyer: You must be aware that the results produced by using higher-degree fits may not be correct in the regions between the points used for fitting.

L.A. Wegner: There may arise difficulties of this kind. But if one eliminates fits with 'oscillating' behaviour and only permits the use of functions with monotonic behaviour (inside of the interval of approximation) the difficulties mentioned will not occur.

D.E. Bowyer: I agree that the use of higher order polynomials will probably give a mathematically better fit to the experimental points as judged by the residual

variation of the regression. The point at issue, however, is whether this computed curve is a better representation of the real behaviour of the machine. It is generally agreed that the oscillatory solution produced by a high order polynomial does not represent the real relationship between efficiency and, say, ECR. The problem is to find a balance between representation of machine behaviour and a mathematically defined best-fit, by least squares, to the experimental points. This, of course, depends upon the machine performance as determined, for example, by the vial variation, vial geometry and reproducibility of positioning of the external standard as discussed by Stanley {P.E. Stanley in Liquid Scintillation Counting, Vol. 2, (Eds. B. Scales, M.A. Crook and P. Johnson) Heyden and Son, London, 1972, p.285}. We believe that the counting conditions and range of quench correction standards should be chosen so that the quench correction curve shows, by graphing of repeated counts, neither maxima nor minima, thus avoiding the possibility of multiple solutions. The presence of a graphing routine for quench curves in a program is obviously very desirable for this reason. The usefulness of the computed curve as a representation of the machine behaviour may then be judged by testing for a significant difference between observed values produced by repeated (i.e. recycled) counting of standard(s) and the value predicted from the curve (F). This is simply achieved by an F test of the ratio of the variances of the curve fitting, $\sigma^2(E)$, to the variance associated with repeated counting, $\sigma^2(S)$. If there is no significant difference, then obviously the curve at that point is acceptable. If there is a significant difference, on the other hand, it would be necessary to compute a new curve, using a higher order polynomial, and to test at the point of interpolation again. We have not so far implemented this technique in our program, but it is obviously simple enough and assuming a reproducible, constant error for each counter, associated with repeated, recycled counting of a standard, the relevant values of $\sigma^2(S)$ for each machine could be invoked by the OPTIONS card which also defines data format and transfer times of the samples during sample changing.