

A SLOPE-RATIO DESIGN FOR ACCELERATED STORAGE TESTS

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An experimental design is presented for predicting the "expiry" dates of pharmaceutical preparations at various storage temperatures on the basis of the degradation rates observed in short time studies at elevated temperatures. The design is based on the observation that a suitable metameter of potency falls off linearly with time at a rate depending on the absolute temperature, in accordance with the Arrhenius equation. It is particularly applicable when relatively imprecise assay methods have to be employed. The design is so constructed to involve the minimum number of time-temperature combinations necessary to check the validity of the assumptions made in applying the Arrhenius equation, and also to provide a precise estimate of known error for the expiry date.

IN a series of papers Garrett, (1956a, b); Garrett and Carper, (1955) and McLeod, Pelletier and Campbell (1958) have described how, for a range of vitamins in various pharmaceutical preparations, expiry dates at storage temperatures may be quantitatively predicted on the basis of short term degradation studies at elevated temperatures. Briefly the method is based on the observation that a suitable metameter of the potency (usually the potency itself or its logarithm) falls off, at any chosen temperature, linearly with time; further, the different rates of degradation of the potency (or its metameter) obtained at different temperatures are related to these temperatures in accordance with Arrhenius' equation, that is, the logarithms of the degradation rates vary linearly with reciprocal absolute temperature. As a result of these relationships the degradation rate at storage temperature can be calculated from the experimentally determined degradation rates at elevated temperatures. These rates, being much faster, require only a short time for their determination.

However, the techniques advocated by these authors are not wholly suitable where imprecise analytical methods, such as microbiological assay, have to be used. In this context we are not concerned so much to explore the nature of the mathematical model to which the results conform, as to determine the expiry date with the highest possible precision by the assay method employed, using a model assumed to hold on the basis of pilot or published work. Observations not related to this objective should preferably be kept to the minimum required to ensure that the assumed model does hold for the preparation under study. The large number of unreplicated sampling times for each temperature (McLeod, Pelletier and Campbell (1958) recommend at least 8) is a disadvantage, as it detracts from the precision of the final estimate. Moreover it provides no objective test for the linearity of the degradation curves, since there is no independent check of the experimental error against which to test the failure of the observed points to fall exactly on a straight line. In addition, no use is made of the error of the determined degradation

constants either in fitting them to the Arrhenius equation or in calculating the error of the final estimate of expiry date. Instead, a second statistical analysis is performed on the results of the first, that is, on the logarithms of the estimated degradation constants (which in any event are generally of different precision), and their experimental error is determined by their failure to fit exactly the Arrhenius equation. Thus, unless many different temperatures are used, the final limits for the expiry date are unnecessarily widened, owing to the restricted degrees of freedom.

The purpose of this paper is therefore to show how, by a careful choice of experimental design, temperatures and times, these difficulties may be overcome. Provided only that an approximate provisional estimate,

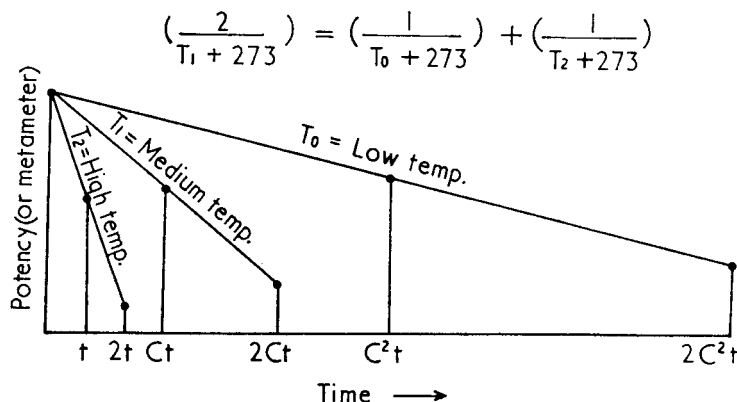


FIG. 1. The experimental design.

either from published or pilot work, of the temperature-decomposition rate relationship is available, a precise determination with known error of the storage life can be obtained from the procedure given in this paper.

Design Recommended

The design recommended involves determining the potency initially and at each of 6 time-temperature combinations, and resembles the slope ratio assay discussed by Finney (1952). It is best illustrated graphically as in Fig. 1.

The experiment is carried out at three different temperatures T_0 , T_1 and T_2 , so chosen that their reciprocal absolute values are in arithmetical progression. Because of this, the slopes of the three decay lines b_0 , b_1 and b_2 will, if the Arrhenius relationship holds, be in geometric progression, the provisional estimate of the slope ratio (b_2/b_1 , or b_1/b_0) being C . The time scales at each temperature are also in geometric progression in the opposite direction, the maximum time being $2t$ at T_2 , $2Ct$ at T_1 and $2C^2t$ at T_0 . Thus, provided the provisional estimate of C is reasonably accurate, approximately the same amount of degradation will have taken place by the end of the experiment whatever the temperature. Samples

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are also withdrawn for assay at half these maximum times, t at T_2 , Ct at T_1 and C^2t at T_0 .

For a basic replication of $r > 1$, the contents of r containers of the substance under study are assayed at the beginning of the experiment. These containers should be identical to those in which the product will be normally stored. For each temperature there is a thermostatically controlled bath with $2r$ containers. At time t for T_2 , Ct for T_1 and C^2t for T_0 , r containers are withdrawn from each bath and their contents assayed, those of the remaining r containers being assayed at time $2t$ for T_2 , $2Ct$ for T_1 and $2C^2t$ for T_0 .

Although the time scales at the three temperatures are not the same, at any one temperature there are two times at which the contents of containers are assayed and this allows the linearity of the decay lines to be assessed by means of the "blanks" and "intersection" terms of the normal slope ratio assay described by Finney (1952).

Finally, the adjustment of the time scales in accordance with the provisional estimate of C , the constant characteristic of the substance under study, implies that each slope (b_0 , b_1 and b_2) is estimated with approximately equal precision. As will be shown later, this considerably simplifies both the subsequent statistical analysis and the test for conformity of the results with the Arrhenius model.

Factors Affecting Accuracy and Precision

It is clearly essential for the accuracy of the experiment that the temperatures be set accurately at the values chosen and that the thermostatic control mechanisms are capable of holding these temperatures precisely at these set values. The analytical method employed for assaying the withdrawn containers must also be accurate, if not precisely reproducible, and, to ensure that decomposition continues in any given container only for the time specified, these should be withdrawn at the specified time, chilled immediately and maintained at a low temperature until their contents can be assayed.

In theory, the most precise results will be obtained by setting the high temperature as high as possible and the low temperature as low as possible and by maintaining the containers at each temperature for that length of time which results in maximum decomposition at the second sampling time ($2C^2t$ at T_0 , $2Ct$ at T_1 and $2t$ at T_2). In practice the high temperature is limited by the presence of volatile constituents in the product and will normally be below 100° . The choice of low temperature is limited by the maximum length of time it is proposed to devote to the experiment, for too low a temperature would require an inordinately long time for appreciable decomposition. In practice it is also preferable to aim at 50 per cent decomposition at the second sampling time as this allows leeway for errors in the provisional estimates of the decomposition rates. Failure to make such allowance might result either in a final potency so low that the analytical method employed is no longer reliable, or even in total decomposition at some unknown time before withdrawal of the containers for assay.

Choice of Convenient Values for Temperatures and Times

The considerations outlined above lead to a choice of time and temperature combinations that will yield a result as precise as possible in the light of the total time to be devoted to the experiment, the assay method employed, the safety margin for errors in the provisional estimates and the nature of the product under study as indicated by published or pilot work. The times and temperatures so indicated, however, may be far from convenient, the temperatures all being recurring decimals and the times involving withdrawal for assay in the middle of the night or at week-ends.

Because of the leeway implied by aiming at 50 per cent decomposition, a limited adjustment of the combinations indicated above is possible. But any modified choice of combinations should be checked against the provisional estimates to ensure that there is no danger of total decomposition at any temperature before the end of the experiment and that the maximum time is still not longer than that allotted to the experiment.

For temperatures, it will normally suffice to select the nearest of the four sets set out below. They all consist of three temperatures whose reciprocal absolute values are in arithmetical progression and none involves a setting finer than 0.5°.

| | | | | |
|-----------|----|----|------|------|
| T_0 ° C | 39 | 42 | 52 | 60 |
| T_1 ° C | 63 | 63 | 64.5 | 69 |
| T_2 ° C | 91 | 87 | 78 | 78.5 |

The temperatures having thus been chosen, the provisional value of C can be determined from the provisional estimates. If C is expressed approximately as the improper fraction p/q where p and q are relatively prime integers, it can be seen that the times for maintaining the samples at the various temperatures will be as follows.

| Temperature | 1st Withdrawal | 2nd Withdrawal |
|-------------|----------------|----------------|
| T_0 | Kp^2 hr. | $2Kp^3$ hr. |
| T_1 | Kpq hr. | $2Kpq$ hr. |
| T_2 | Kq^2 hr. | $2Kq^3$ hr. |

If K is also an integer, these times will all be an integral number of hr.

However, only certain integral values for K will lead to values for these times such that the containers inserted in the constant temperature baths between 9 a.m. and 5 p.m. will be due for withdrawal also between 9 a.m. and 5 p.m. Such permissible values of K can be found as follows.

(1) From the product pq deduct the nearest multiple of 24 to yield a number $(pq - 24M)$, positive or negative, whose value, ignoring sign, lies in the range 0-12 inclusive.

(2) If either p^2 or q^2 (or both) should differ from a multiple of 24 by unity, the permissible values for K can be read from column one of Table I against the appropriate entry $\pm(pq - 24M)$.

(3) If neither p^2 nor q^2 should differ from a multiple of 24 by unity, the permissible values of K are read from column two of Table I.

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(4) For any permissible value of K, the values $24N \pm K$, where N is an integer, are also permissible.

These calculations ensure that each sampling time occurs within ± 8 hr. of an integral number of days after the insertion of the containers in the thermostatically controlled bath. Since 9 a.m. to 5 p.m. on the same day

TABLE I

| $\pm(pq - 24M)$ | Column one | Column two |
|-----------------|---------------|-------------|
| 0 | 1, 2, 3, 4, 8 | 3, 5, 8, 11 |
| 1 | 1, 2, 3, 4, 8 | |
| 2 | 1, 2, 4, 8 | |
| 3 | 8 | |
| 4 | 1, 2, 4, 8 | |
| 5 | 4, 8 | |
| 6 | 4, 8 | 8 |
| 7 | 3, 4, 8 | |
| 8 | 1, 2, 3, 4, 8 | |
| 9 | 3, 8 | |
| 10 | 2, 4, 8 | |
| 11 | 2, 4, 8 | |
| 12 | 2, 4, 8 | 8 |

is $+8$ hr. and 5 p.m. to 9 a.m. on the following day is 1 day $- 8$ hr., it is evident that the only remaining problem is to ensure that the integral number of days involved in each interval does not result in week-end work. This can always be achieved by expressing the number of days as w weeks $+ d$ days where d will be in the range ± 4 days. Table II gives the value of d for various combinations of insertion and withdrawal days. Thus, for example, if the first sampling time at a given temperature were

TABLE II

| | | Withdrawal from bath w weeks later, | | | | |
|---------------------|----|---------------------------------------|----|---------|----|----|
| | | M | Tu | on W | Th | F |
| Insert in bath, on: | M | 0 | +1 | +2 | +3 | +4 |
| | Tu | -1 | 0 | +1 | +2 | +3 |
| | W | -2 | -1 | 0 | +1 | +2 |
| | Th | -3 | -2 | -1 | 0 | +1 |
| | F | -4 | -3 | -2 | -1 | 0 |

280 hr. this would be expressed as 2 weeks $- 2$ days $- 8$ hr. The second sampling time is then 560 hr. which would be expressed as 3 weeks $+ 2$ days $+ 8$ hr. Thus the set of containers for the 280 hr. sampling time might be inserted in the bath at 5 p.m. on a Thursday and withdrawn at 9 a.m. on the Tuesday 12 days later. Those for the 560 hr. sampling time could be inserted at 9 a.m. on Monday and withdrawn at 5 p.m. on the Wednesday 23 days later.

Application

Choice of experimental conditions. A certain vitamin B₁₂ preparation with an initial potency of about 1,000 u/ml. was used. The potency was determined microbiologically with *Escherichia coli* M200 by the method of Harrison, Lees and Wood (1951), and in preliminary trials this was found graphically to fall about 0.217 u/ml./hr. at 51.5° and 1.96 u/ml./hr.

at 70°. It was proposed that the maximum time to be devoted to the examination of the product at elevated temperatures should be about 4 weeks (720 hr.). Graphical interpolation on a logarithmic plot of the above provisional degradation rates against reciprocal absolute temperatures showed that a rate of 0.694 u/ml./hr. (i.e. to give half the potency 1,000 u/ml. in 720 hr.) was likely to be achieved at a temperature of 61°. It was, therefore, decided to investigate the possibilities of using the final set of temperatures tabulated in the preceding section. The graph gave the provisional degradation rates:

| Temperature ° C | Provisional Rate |
|-----------------|------------------|
| 60 | 0.615 u/ml./hr. |
| 69 | 1.751 u/ml./hr. |
| 78.5 | 4.985 u/ml./hr. |

From this, the provisional value of $C = 2.8$. As an approximation this was taken to be $8/3 = 2.66667$, i.e. $p = 8$, $q = 3$.

TABLE III

| Containers put in bath | Containers withdrawn | Time |
|-------------------------|-------------------------|---------|
| Wed. 21st Sept. 9 a.m. | Tues. 4th Oct. 5 p.m. | 320 hr. |
| Wed. 21st Sept. 5 p.m. | Tues. 18th Oct. 9 a.m. | 640 hr. |
| Wed. 28th Sept. 5 p.m. | Mon. 3rd Oct. 5 p.m. | 120 hr. |
| Tues. 27th Sept. 3 p.m. | Fri. 7th Oct. 3 p.m. | 240 hr. |
| Tues. 18th Oct. 5 p.m. | Thurs. 20th Oct. 2 p.m. | 45 hr. |
| Fri. 7th Oct. 4 p.m. | Tues. 11th Oct. 10 a.m. | 90 hr. |

Since neither 8^2 nor 3^2 differs from a multiple of 24 by unity, the value $(3 \times 8 - 24) = 0$ was read off column two in Table I and the values $K = 3, 5, 8, 11$ found permissible. Taking $K = 5$, the times at the various temperatures were calculated as follows.

| Temperature ° C | 1st Withdrawal | 2nd Withdrawal |
|-----------------|------------------|-------------------|
| 60 | $Kp^2 = 320$ hr. | $2Kp^2 = 640$ hr. |
| 69 | $Kpq = 120$ hr. | $2Kpq = 240$ hr. |
| 78.5 | $Kq^2 = 45$ hr. | $2Kq^2 = 90$ hr. |

These times were achieved according to the schedule in Table III.

Results. At the end of the experiment the triplicate results given in Table IV were obtained ($r = 3$). The various figures $Z = 2,821$, etc., are used in subsequent calculations explained below.

Calculation of treatment effects and error. The first stage in the calculation involves summing the replicate results for each time-temperature combination. Thus:

$$Z = 987 + 864 + 970 = 2,821,$$

$$L_0 = 724 + 702 + 769 = 2,195,$$

$$M_0 = 447 + 585 + 638 = 1,670, \text{ etc.}$$

These totals are entered in the appropriate column and are then summed to yield the grand total:

$$G = Z + L_0 + M_0 + L_1 + M_1 + L_2 + M_2 = 13,952.$$

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The total variation among results is given by the sum of the squares of all individual results from which is subtracted $G^2/7r$. Thus:
 $987^2 + 864^2 \dots + 506^2 - 13,952^2/21 = 452,575$ for $(7r - 1) = 20$ degrees of freedom.

The variation due to treatments (i.e. time-temperature combinations) is given by:
 $(Z^2 + L_0^2 + M_0^2 + L_1^2 + M_1^2 + L_2^2 + M_2^2)/r - G^2/7r = 412,641$ for 6 degrees of freedom.

TABLE IV

| Time hr. | Temp. °C | Replicate results | | | | |
|------------|--------------|----------------------------|--|--|--|-------------------|
| 0 | — | 987 864 970 | Z = 2,821 | | | |
| 320 640 | 60 60 | 724 702 769 447 585 638 | L ₀ = 2,195 M ₀ = 1,670 | P ₀ = 2,720 Q ₀ = 5,535 | 1 = 1·000,000 | |
| 120 240 | 69 69 | 668 735 701 549 462 522 | L ₁ = 2,104 M ₁ = 1,533 | P ₁ = 2,675 Q ₁ = 5,170 | x = 1·058,000 | 1 = 1·000 |
| 45 90 | 78·5 78·5 | 724 683 652 531 533 506 | L ₂ = 2,059 M ₂ = 1,570 | P ₂ = 2,548 Q ₂ = 5,199 | x ² = 1·119,364 | 2x = 2·116 |
| | | | G = 13,952 | ΣP = 7,943 | 3(1 + x + x ²) = 9·532,092 | 3(1 + 2x) = 9·348 |
| | | | H = 66,144 | ΣQ = 15,904 | | |

The variation due to error is obtained by difference
 $452,575 - 412,641 = 39,934$ for $7(r - 1) = 14$ degrees of freedom.
 The analysis so far is summarised :

| Source of variation | D.F. | Squares | Variance |
|-------------------------|------|---------|----------|
| Total variation | 20 | 452,575 | |
| Treatments | 6 | 412,641 | |
| Error | 14 | 39,934 | 2,852 |

The variance, obtained by dividing the squares by the corresponding number of degrees of freedom, need only be evaluated for the error term.
Subdivision of treatment effects. The treatment effects calculated above are then further subdivided into terms relevant to the linearity of the decay curves and to the rates of decay observed.

The former terms are derivable from the P values at each temperature where $P = 2L - M$. The “intersection” term for 2 degrees of freedom is given by $\Sigma P^2/5r - (\Sigma P)^2/15r = 1,061$ and represents the variation due to the failure of the three decay curves, when continued back to zero time, to meet at a common point. The “blanks” term is obtained from $(\Sigma P - 3Z)^2/24r = 3,756$ for 1 degree of freedom and represents the variation due to the failure of the average of the three intersection points to coincide with the average initial potency.

The terms last mentioned are derivable from the Q values at each temperature where $Q = L + 2M$. The values of the slopes of the decay lines, each expressed on its own time scale, i.e. per $C^2t = 320$ hr. at $T_0 = 60^\circ C$, per $Ct = 120$ hr. at $T_1 = 69^\circ C$ and per $t = 45$ hr. at

$T_2 = 78.5^\circ \text{C}$, will not be expected to be the same unless the provisional value of C happens to correspond with the true value. The variation due to differences between these slopes is given by $\Sigma Q^2/5r - (\Sigma Q)^2/15r = 5,488$. The variation due to the average decay recorded during the experiment is given by $(9G - 7\Sigma Q)^2/168r = 402,336$.

This stage of the analysis is summarised:

| Source of variation | D.F. | Squares | Variance |
|---------------------------|------|---------|----------|
| Intersections | 2 | 1,061 | 530 |
| Blanks | 1 | 3,756 | 3,756 |
| Slope differences | 2 | 5,488 | |
| Mean slope | 1 | 402,336 | |
| Treatments | 6 | 412,641 | |

It will be observed that these terms sum to the squares previously calculated for the variation due to treatments. The variance terms for intersections and blanks should be obtained by dividing the corresponding squares by 2 and 1 degrees of freedom, respectively. If neither is significant when compared with the error variance, it may be assumed that, within experimental error, the three decay curves are linear, as required by the model on which the experiment is based.

Attribution of the slopes to the Arrhenius equation. The values of the slopes at each temperature b_0 , b_1 and b_2 , each in terms of its own time scale, may be obtained as shown below.

$$\begin{aligned}
 B_0 &= 40rb_0 = H - 8Q_0 = 21,864 \text{ therefore } b_0 = 182.2 \text{ u/ml./320 hr.} \\
 B_1 &= 40rb_1 = H - 8Q_1 = 24,784 \text{ therefore } b_1 = 206.5 \text{ u/ml./120 hr.} \\
 B_2 &= 40rb_2 = H - 8Q_2 = 24,552 \text{ therefore } b_2 = 204.6 \text{ u/ml./45 hr.} \\
 &\text{where } H = 15G - 9\Sigma Q = 66,144.
 \end{aligned}$$

If the true value of the slope ratio is Cx , whereas the provisional value chosen for the experiment is C , then the ratio of b_1/b_0 and b_2/b_1 should be x . In practice, even if the Arrhenius model holds good, these two slope ratios will not be exactly the same owing to experimental error. The least squares value for x can, however, be obtained to an accuracy of about 0.002 from the nomogram. The values $B_0/B_1 = 0.882$ and $B_2/B_1 = 0.991$ are calculated and the nomogram is read, yielding $x = 1.058$.

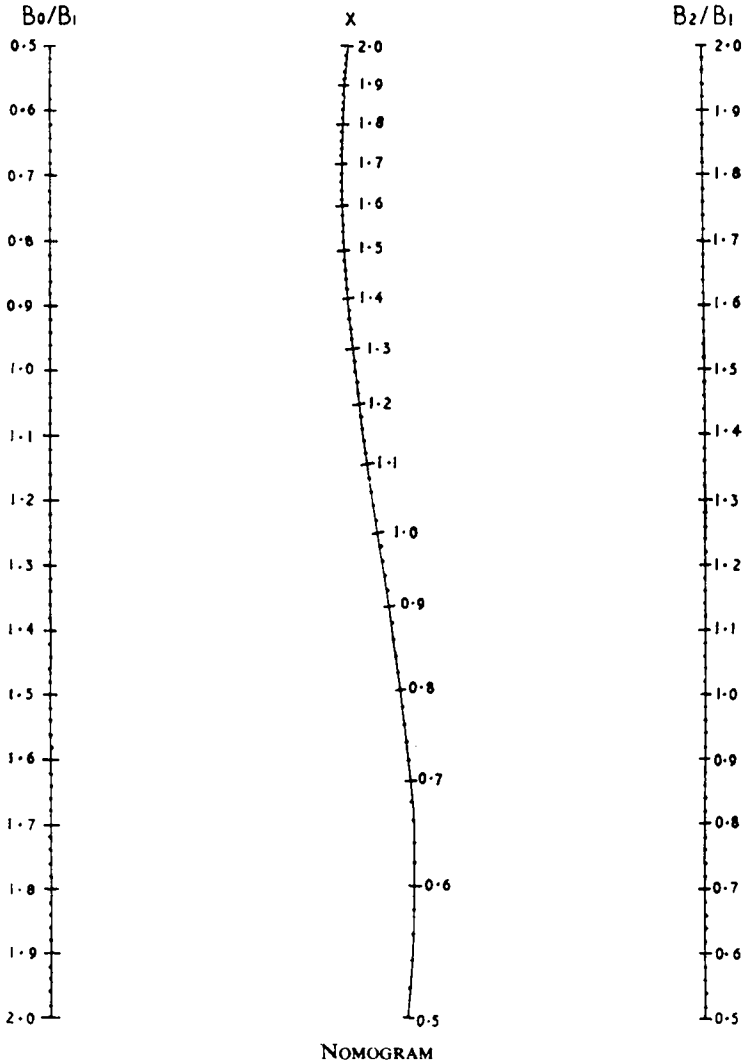
From this value of x is column containing 1 against Q_0 , x against Q_1 and x^2 against Q_2 is entered, three times the sum of this column, i.e. $3(1 + x + x^2)$ being entered at the foot of the column. Similarly, a second column containing 1 against Q_1 and $2x$ against Q_2 , together with three times its sum, i.e. $3(1 + 2x)$ at the foot, is also constructed. The corresponding total used for the Q column is not ΣQ but G the grand total of all results:

| | | |
|-------|------------------|-------------|
| Q_0 | 1 | |
| Q_1 | x | |
| Q_2 | x^2 | |
| G | $3(1 + x + x^2)$ | $3(1 + 2x)$ |

By utilising only the entries involving x the following F functions may readily be calculated.

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$$B_0 (9x^4 - 43x^3 + 27x^2 - 17x) - B_1 (17x^4 - 18x^3 + 18x - 17) + B_2 (17x^3 - 27x^2 + 43x - 9) = 0$$



From the row opposite Q_2 :

$$D = 175[1 + (2x)^2 + (x^2)^2] - 10[3(1 + x + x^2)]^2$$

$$= 175[1 + 2 \cdot 116^2 + 1 \cdot 119, 364^2] - 9 \cdot 532,092 \times 95 \cdot 320,920 = 269 \cdot 217, 78$$

From the final column:

$$F_1 = \frac{35[1 + (2x)^2] - [3(1 + 2x)]^2}{D}$$

$$= \frac{35[1 + 2 \cdot 116^2] - 9 \cdot 348^2}{269 \cdot 217, 78} = 0 \cdot 387, 514, 7$$

From the corresponding entries of the final two columns:

$$F_2 = - \frac{35[x + (x^2 \times 2x)] - [3(1 + 2x) \times 3(1 + x + x^2)]}{D}$$

$$= - \frac{35[1 \cdot 058 + 2 \cdot 116 \times 1 \cdot 119, 364] - 9 \cdot 348 \times 9 \cdot 532, 092}{269 \cdot 217, 78}$$

$$= - 0 \cdot 114, 495, 0$$

From the penultimate column:

$$F_3 = \frac{35[1 + x^2 + (x^2)^2] - [3(1 + x + x^2)]^2}{D}$$

$$= \frac{35[1 + 1 \cdot 058^2 + 1 \cdot 119, 364^2] - 9 \cdot 532, 092^2}{269 \cdot 217, 78}$$

$$= 0 \cdot 100, 926, 1$$

By operating on the Q column the following R functions may be obtained:

$$R_1 = \frac{1}{7}G \times 3(1 + x + x^2) - (Q_0 + xQ_1 + x^2Q_2)$$

$$= \frac{13,952 \times 9 \cdot 532, 092}{7} - (5535 + 5170 \times 1 \cdot 058 + 5199 \times 1 \cdot 119, 364)$$

$$= 2174 \cdot 388$$

$$R_2 = \frac{1}{7}G \times 3(1 + 2x) - (Q_1 + 2xQ_2)$$

$$= \frac{13,952 \times 9 \cdot 348}{7} - (5,170 + 5199 \times 2 \cdot 116)$$

$$= 2,460 \cdot 815$$

With the aid of these functions we may now calculate the least squares value, β_0 , of the slope at $T_0 = 60^\circ$, assuming the Arrhenius model to hold, and the correction, $r\beta_0\Delta x$, to be applied to the nomographic value of x (which, as mentioned above, may be in error by about 0.002).

$$r\beta_0 = F_1R_1 + F_2R_2 = 0 \cdot 387, 514, 7 \times 2,174 \cdot 388 - 0 \cdot 114, 495, 0 \times 2,460 \cdot 815$$

$$= 560 \cdot 856 \text{ whence } \beta_0 = 186 \cdot 95 \text{ u/ml./320 hr.}$$

$$r\beta_0\Delta x = F_2R_1 + F_3R_2 = - 0 \cdot 114, 495, 0 \times 2,174 \cdot 388 + 0 \cdot 100, 926, 1 \times 2,460 \cdot 815$$

$$= - 0 \cdot 596 \text{ whence } \Delta x = - \frac{0 \cdot 596}{560 \cdot 856} = - 0 \cdot 00106$$

$$\text{whence } x = 1 \cdot 056, 94$$

The variation attributable to the Arrhenius model is given by

$$\frac{r\beta_0 \times R_1 + r\beta_0\Delta x \times R_2}{r} = \frac{560 \cdot 856 \times 2,174 \cdot 388 - 0 \cdot 596 \times 2,460 \cdot 815}{3}$$

$$= 406,017 \text{ for 2 degrees of freedom.}$$

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The total variation due to the 3 degrees of freedom for slopes, is obtained by adding that due to the 2 degrees of freedom for slope differences 5,488 to that for the single degree of freedom for mean slope 402,336, calculated under 'sub-division of treatment effects': this gives 407,824. The difference 407,824 - 406,017 = 1,807 for 1 degree of freedom represents the departure of the results from the Arrhenius model.

These findings are summarised :

| Source of variation | D.F. | Squares | Variance |
|-------------------------|------|---------|----------|
| Slopes | 3 | 407,824 | |
| Arrhenius model | 2 | 406,017 | |
| Deviation | 1 | 1,807 | 1,807 |

Only the variance for deviation from the Arrhenius model need be considered ; if this is not significant by comparison with the error variance, it may be taken to justify the assumption that the slopes are in geometric progression within experimental error.

Calculation of degradation rate at any specified temperature. For any specified temperature T the value y may be calculated as follows :

$$y = - \frac{(273 + T_1) (T_0 - T)}{(273 + T) (T_1 - T_0)}$$

Thus if T = 25° for T₀ = 60°, T₁ = 69°

$$y = - \frac{(273 + 69) (60 - 25)}{(273 + 25) (69 - 60)} = -4.4631$$

The slope β_y for this value of T may be calculated from

$$\log \beta_y = \log \beta_0 - 4.4631 \log Cx.$$

With β₀ = 186.95 u./ml./320 hr., C = 2.6667 and x = 1.056, 94, this yields β_y = 1.8334 u./ml./320 hr. at 25°.

The standard error of this estimate is given by

$$\text{s.e.} (\beta_y) = \sqrt{\frac{\text{error variance}}{r}} \times \frac{\beta_y}{\beta_0} \times \sqrt{F_1 + 2\left(\frac{y}{x}\right) F_2 + \left(\frac{y}{x}\right)^2 F_3}$$

which as y/x = -4.222, 65 is in the above case,

$$\text{s.e.} (\beta_y) = \pm \sqrt{\frac{2,852}{3}} \times \frac{1.8334}{186.952} \times$$

$$\begin{aligned} & \sqrt{0.387, 514, 7 + 2 \times 0.114, 495, 0 \times 4.222, 65 + 0.100, 926, 1 \times 4.222, 65^2} \\ & = \pm 30.833 \times 0.009, 806, 8 \times 1.775, 96 \\ & = \pm 0.5370 \end{aligned}$$

In practice we are concerned that the product should not decay faster than we have estimated, so that only the upper fiducial limit is of interest. This being so, the Students t value to be taken from the tables is that related to twice the probability value we wish to use. Thus for the 95 per cent upper fiducial limit we do not take the 5 per cent value but

rather the 10 per cent value of t for $7(r - 1)$ degrees of freedom. In the above example, for 14 degrees of freedom $t = 1.761$, hence the 95 per cent upper fiducial limit for the decay at 25° is $\beta_y + 1.761 \text{ s.e. } (\beta_y) = 1.8334 + 1.761 \times 0.5370 = 2.7791 \text{ u/ml./320 hr.}$ Hence if we require that the product lose not more than 20 per cent of its nominal initial value of 1,000 u/ml., i.e. not more than 200 u/ml., the maximum life for $P = 0.95$ at $25^\circ = 200/2.7791 \times 320 \text{ hr.} = 23,029 \text{ hr.}$ or about 32 months.

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The paper was presented by the AUTHOR. The following points were made in the discussion.

The method was designed for products that obeyed the Arrhenius equation, and included validity tests which showed up non-conforming preparations at an early stage of the calculations. Estimations of the degree of error permissible in the times of measurement and the temperature of the test were dependent upon the products to be tested. It was necessary to avoid very short times, and a statistician could make allowance for deviations from the required temperatures if such deviations were known.