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OPTIMIZATION OF EXPERIMENTAL DESIGNS FOR TWO CASES IN ELEVATED TEMPERATURE STABILITY STUDIES

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16.1 Introduction

Quick, reliable estimates of the stability of many drugs and candidate product-formulations are needed in the early stages of drug development (1). In order to expedite the gathering of information, elevated temperature stability studies, with a limited number of assays, are usually performed. Analysis of the data assumes pseudo-first order kinetics and temperature activation in accordance with the Arrhenius equation. Although studies of this type have been used in the pharmaceutical industry for many years, they have not received universal acceptance because of instances of inaccurate results (2,3). Among the possible causes for erroneous stability prediction are large assay and sample variability coupled with both the inadequate number of assays usually available for this work and the use of non-optimal designs.

It is obvious that optimization of the stability study variables would be useful in getting the best stability estimates for the least cost. What is needed is general rules in keeping with the high volume, approximate and preliminary nature of the studies rather than a method for detailed optimization based on every particular of each study. We have developed and will

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present a few such rules drawn from generalizations of many individual optimizations for two special cases.

16.2 Theory

In an elevated temperature stability study, samples of a drug are stored at two or more elevated temperatures which cause gradual loss of drug potency at convenient rates. At intervals, samples are removed and the remaining drug is measured. The measured rate of loss of the drug at each temperature is expressed by an experimentally determined constant in a suitable rate equation.

16.2.1 Notation

Symbols

C: concentration of drug at a specified time, expressed as mg/ml, percentage of label claim, etc.

C_0: concentration at t = 0, where t is time

CV: coefficient of variation, equal to standard deviation divided by mean expressed in percent

E: energy of activation for the degradation reaction, expressed in thousands of calories/mole

F: frequency factor or Arrhenius constant

K: first order rate constant at the chosen base temperature, expressed as fractional loss per month (For predictive purposes, the base temperature should be the shelf temperature.)

k: first order rate constant at any other temperature

M: time that samples are held at a specified temperature, expressed in months

N: -- with a temperature subscript -- number of assays at that temperature

N: total number of assays, equal to the sum of the Ns at all temperatures

P: proportion of assays allotted to samples held at the indicated temperature

$$Q_S = X_L / X_H$$

$$Q_J = Q_S k_L M_L / (k_H M_H)$$

T: temperature in degrees C.

t: time

$$X_L = 1/(T_L + 273) - 1/(T_B + 273)$$

$$X_H = 1/(T_H + 273) - 1/(T_B + 273)$$

$$X_B = 1/(T_B + 273) - 1/(T_B + 273) = 0$$

Subscripts

A: assay plus sample variation, as in CV_A

B: base (shelf) temperature of Arrhenius study here equal to room temperature (23 degrees), but may be any other desired reference value

H: high temperature of Arrhenius study

H: H Rule for apportioning assays (The context will tell the reader which is the appropriate meaning for H.)

I: initial time, equal to starting point of Arrhenius study

J: J Rule, statistical in nature, for apportioning assays in an Arrhenius study

K: rate constant at base (shelf) temperature, as in CV_K

L: low temperature of Arrhenius study

S: conventional Statistical Rule for apportioning assays in a linear situation

Superscript

*: denotes optimum value

16.2.2 Equations

We used the so-called first order equation, Eq. (1,2), for our work because it fits a large majority of our historical data and the data reported in the pharmaceutical literature.

$$\text{loss rate} = -dC/dt = kC \quad (1)$$

$$C = C_0 e^{-kt} \quad (2)$$

Given two or more rate constants for drug potency loss at elevated temperatures, the conventional Arrhenius relation (3A) may be used to obtain the room temperature rate constant, which may then be employed in Eq. (2) to calculate the potency at any time (4).

$$k = Fe^{-E/(R(T + 273))} \quad (3A)$$

This was rewritten in a more efficient, convenient form (3B) which actually was used for the calculations.

$$k = Ke^{-EX/R} \quad (3B)$$

Equations (2) and (3B) may be combined to give Eq. (4)[a].

$$C = C_0 e^{-Kte^{-EX/R}} \quad (4)$$

16.3 Case One: Initial Potency Known (Two Sampling Points Used)

16.3.1 Conditions

We selected the following conditions in order to establish a relatively simple system for initial study. The first five conditions are critical; the others have been chosen arbitrarily, without loss of generality[b].

1. Temperature is the only factor in this study affecting the rate of degradation. E values of 10,20, (common in practice), and 30 were used.
2. The Arrhenius relationship is followed in the region of interest. There are only two elevated temperatures in the study: T_L and T_H,

[a] This has been used in our one-step-calculation, non-linear, least-squares computer program since 1972.

[b] It must be noted that the simple designs shown here must be augumented in practice by the inclusion of more times and temperatures to allow the statistical testingof the validity for each product/formulation of the reaction order and the monophasic nature of the Arrhenius relationship. Such more complicated designs are being investigated.

e.g., 37 and 70. No intermediate temperatures are used because the most efficient estimation of the heat of activation precludes any, given the validity of the Arrhenius relationship in the study region.

3. Some of the samples have been held for the full time, M_H, at the high temperature and the rest have been held for M_L, usually the same time, at the low temperature, e.g., four months. No samples are taken at intermediate times because the most efficient estimation of the rate constant at a temperature precludes any, given the apparent order of the reaction.
4. The initial potency is known accurately because of the conditions under which the samples are prepared. This would be typical of the preformulation phase of drug development.
5. The reaction follows first order kinetics with a rate constant of 0.002 per month (0.002 is not critical) at the base (shelf) temperature (T_B = 23 was used here). A drug with this loss rate would lose approximately 10% of its potency in four years at T_B.
6. There are N = 30 assays available for assaying the samples.
7. The assay plus sampling coefficient of variation is 1% at all concentrations. (This was not used as a typical value but for easy adjustment of N to allow for other CV_A values.)

16.3.2 Methods

One criterion for optimization was CV_K, the coefficient of variation of the estimated base (shelf) temperature rate constant K. Securing the best CV_K for a given expenditure of effort can be shown to be, with first order kinetics, equivalent to securing the best CV for "shelf-life" which, by conventional definition, is the length of time at the shelf temperature before the potency drops to 90%. We assessed the effect on CV_K of three rules for apportioning assays.

H Rule: $P_{L\ H} = 0.5$ (5)

S Rule: $P_{L\ S} = 1/(1 + Q_S)$ (6)

J Rule: $P_{L\ J} = 1/(1 + Q_J)$ (7)

The H Rule (or half rule or 50:50 rule) is a rule of thumb in common use. It puts half of the assays at each temperature, i.e., the same number of assays is done at each sampling point. The S Rule is an established rule for a linear relation between a dependent variable and an independent variable[c]. The J Rule was developed for this study. It is an extension of the S Rule to the more complicated Arrhenius case of two independent variables, time and temperature.

Originally, we expected to evaluate the three Rules by a Monte Carlo simulation method, i.e., by doing kinetic "experiments" on a computer. However, it proved possible to use a quicker method based upon linear approximations of the CV via Taylor series expansions of the complex time-temperature relation given by Eq. (4) (to be published elsewhere), the result of which is represented in Equation 8.

$$CV_K = (\text{constant})\ [(F_L/N_L) + (G_H/N_H)]^{0.5} \quad (8)$$

F_L is a function of parameters other than T_H; G_H similarly does not depend on T_L . F_L/N_L and G_H/N_H (which may be written as $G_H/(N - N_L)$) were found to be monotonic but opposed functions of N_L. This resulted in a minimum value of CV_K at some value of N_L.

16.3.3 Results

Figure 1 illustrates the change in CV_K as N_L changes and specifically shows that an optimum N_L (corresponding to the smallest CV_K) does exist for

[c]The S Rule provides that particular apportionment of assays between two values of the independent variable which minimizes the standard deviation of the dependent variable at a point which is linearly extrapolated from the two experimentally determined points, calculated by the least-squares method.

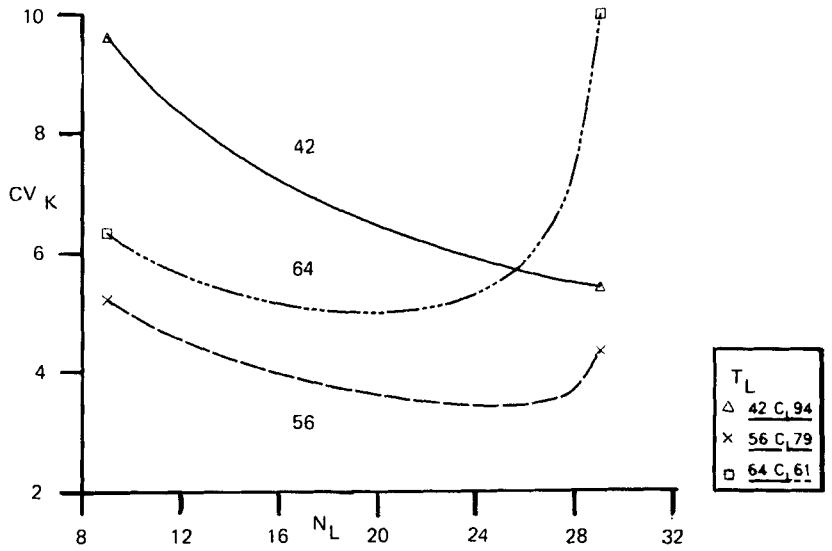

Figure 1. Effect of N_L on CV_K At Three T_L Values E=20, T_H=70, K=0.002, M=4, C_H=43

a given T_L and is not 15, the value given by the H Rule. Further it shows a T_L of 56 gives a smaller CV_K than can be obtained from the T_L curves for 42 and 64.

The comparison of the T_L values is carried further in Figures 2-7 which show how CV_K varies with T_L for typical values of T_H, E and K. Note that the S Rule (Eq. 6) gives a different N_L value for each T_L; this is also true for the J Rule. It is apparent that use of the J Rule leads to the lowest value of CV_K at a given T_L, with the S Rule a close second. The effect of using the H Rule is seen to be a poorer CV_K, substantially so for most combinations of parameter values. For each curve there is one optimum T_L, designated as T^*_L, which gives a smaller CV_K than the others; the T^*_L obtained from the use of the J Rule is best, with that obtained by the S Rule close to it.

As T_H increases, the T^*_L also increases. The curves are fairly flat near the optimum, so that a slight deviation from T^*_L does not seriously

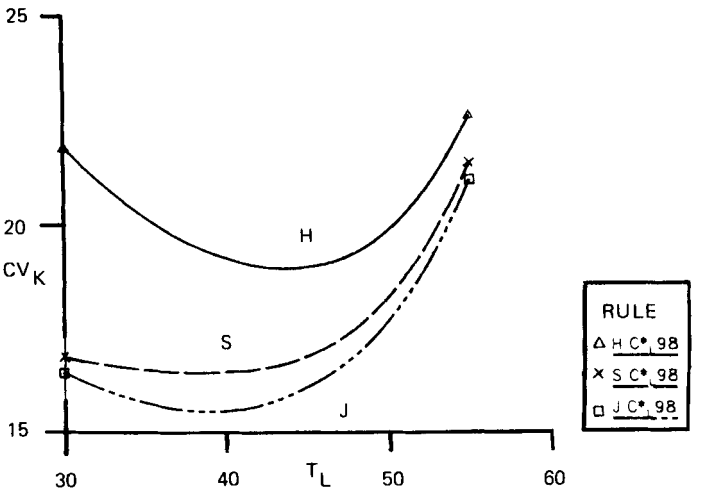

Figure 2. Effect of T_L on CV_K Using H, S and J Rules to Select N_L T_H=70, E=10, K=0.002, M=4, C_H=92.1

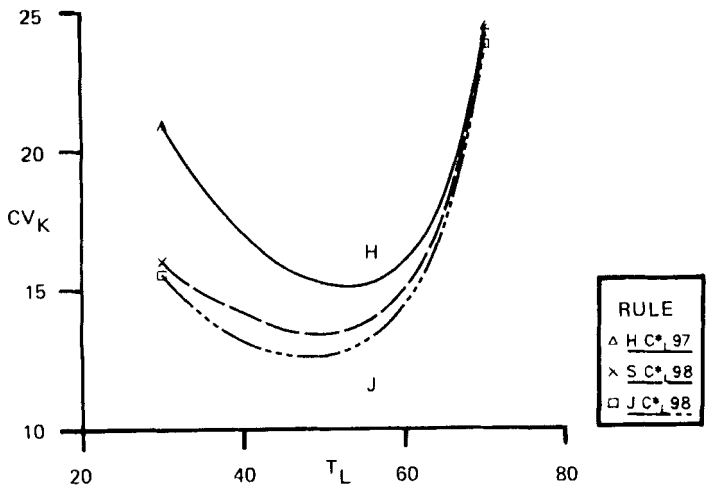

Figure 3. Effect of T_L on CV_K Using H, S and J Rules to Select N_L T_H=80, E=10, K=0.002, M=4, C_H=88.3

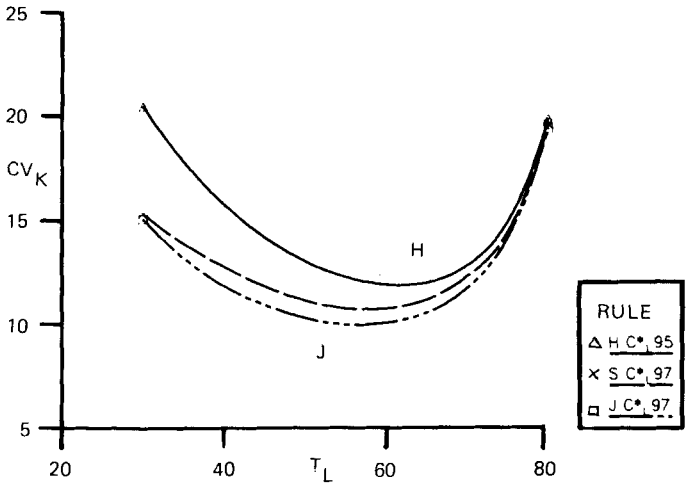

Figure 4. Effect of T_L on CV_K Using H, S and J Rules to Select N_I T_H=90, E=10, K=0.002, M=4, C_H=83.2

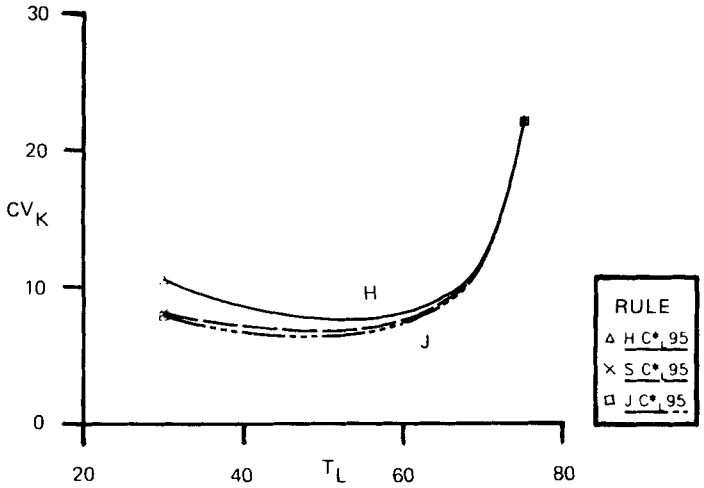

Figure 5. Effect of T_L on CV_K Using H, S and J Rules to Select N_I T_H=80, E=10, K=0.004, M=4, C_H=78.0

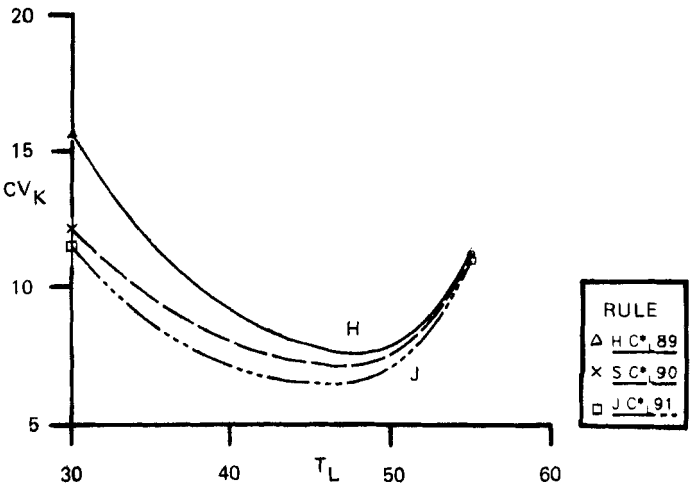

Figure 6. Effect of T_L on CV_K Using H, S and J Rules to Select N_L T_H=60, E=20, K=0.002, M=4, C_H=70.7

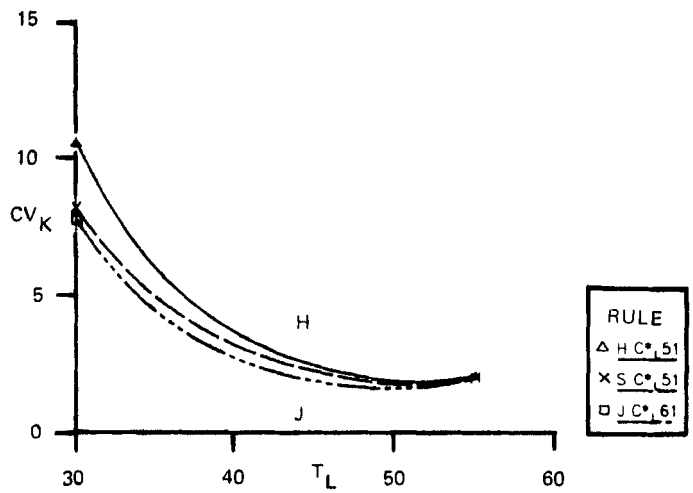

Figure 7. Effect of T_L on CV_K Using H, S and J Rules to Select N_L T_H=60, E=30, K=0.002, M=4, C_H=10.1

affect CV_K, and the near-T^*_L region becomes broader as K increases. At higher values of E, CV_K is smaller, and in addition the effect of the chosen assay apportionment Rule is less important. This is due in part to the progressive change from very little loss of potency during the experiment in Figure 2 to extensive loss in Figure 7. Note that C_L at $T^*_{L\ H}$ is less than C_L at $T^*_{L\ J}$ so a small C_L, indicating more significant degradation, is not the sole criterion; the extrapolation feature requires the balancing of a low C_L (obtainable at a higher T_L) with both a good (moderate) T_L for extrapolation to get K at T_B and a good apportionment of assays between T_L and T_H.

Summarizing Figures 2-7, it can be seen that the use of the H Rule of assay apportionment and T_L = 37, both common values, results in a large CV_K from which progressive improvement is possible. Changing to T^*_L as determined by the H Rule results in a smaller CV_K. However, the use of the J Rule and the T^*_L derived there from results in the smallest CV_K. All of this can be put in more concrete terms approximately as follows, averaged over the six figures. If the use of the H Rule and T_L = 37 requires N assays for a chosen value of CV_K:

(1) The use of the H Rule and $T_L = T^*_{L\ H}$ requires about 0.75N assays.

(2) The use of the J Rule and $T_L = T^*_{L\ J}$ requires about 0.40N assays.

(3) The results with the S Rule were sometimes intermediate to those in (1) and (2) above and sometimes the same as in (2).

In order to apply the J Rule as written, the values of E and K for the chemical reaction which is occuring must be known prior to the conduct of the study, but of course they are not. However, statistical analysis of our data for patterns has provided a way to use the J Rule without knowldege of E and K:

to a good approximation, 4/5 of the assays are always assigned to the optimum low temperature[d].

Rule 1. The HL Rule

$N_H:N_L = 1:4$

For additional views of the relationship between T_L and M,E, and T_H, the basic data are shown with different co-ordinates in Figure 8-13.

Henceforth only the results of applying the J Rule, which gives the optimum assay apportionment, are shown. Figures 8 and 9 show that at T^*_L, the value of CV_K after 4 months is approximately one-half as large as that after 2 months; this is independent of E. As expected, T^*_L is unaffected by M. Figures 10 and 11 illustrate the upward shift in T^*_L that occurs with an increase in E.

T_H should be chosen as high as possible consistent with not changing the mode of degradation, e.g., 55 to 100. Most container materials will withstand these temperatures; for ampoules the T_H can be higher. Figures 12 and 13 show how T^*_L varies with T_H at two values of E and give a clear picture of the improvement in CV_K provided by higher T_H values.

The foregoing data are summarized in Figure 14, which presents the dependence of T^*_L (from the J Rule) upon both T_H and E as data points, and in Figure 15, which shows the same relations as contours of T^*_L. (The contours were located by interpolation from data points.) The relations shown in Figure 15 can be further summarized approximately as follows:

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[d] If the coefficient of variation of the assay is not constant in the range used, a possibility endorsed by O.L. Davies (7), the proportion of assays to be done on the two-temperature sample is less than 4/5. Further work is needed on this point.

Rule 2. TL Rule

If $W = 0.85(T_H)$, then:

$$T^*_L \begin{cases} = W & \text{if } E = 30 \\ = (W - 5) & \text{if } E = 20 \\ = (W - 20) & \text{if } E = 10 \\ = T_B + 10 \text{ as the lower limit on } T^*_L \end{cases}$$

The number of assays needed to obtain a specified value of CV_K can be calculated. For example, if the assay plus sampling CV equals 1%, 2% or 3% with K equal to 0.002/month and E equal to 20 thousand calories/mole, a CV_K of 10% can be obtained as shown in Table I. The data in Tables I ane II are based on the nonlinear least-squares estimation program for Eq. (4). If the convention two-step Arrhenius calculation is used, the values of CV_K would be somewhat larger.

It is seen that for a 4-month study where T_H equals 60, 10 assays are needed if the assay CV is 1%; 8 assays --4/5 of the total-- are placed at T^*_L, which equals 46. If T_H is 70 and M is 4 months, only 3 assays are needed; after 4 monts, two samples --ca. 4/5-- held at T^*_L (equal to 54) and one 70 degree sample are assayed. This illustrates the value of setting T_H as high as possible. Note that keeping the low temperature sample at that temperature for an extra month permits an additional reduction in the number of assays needed; this comes from waiting for the potency of the low temperature samples to drop to a level more significantly different from the initial value.

Table I illustrates the fact that CV_K is many times the size of the CV_A, even with optimum study design. Table I also shows that the magnitude of a reliable Arrhenius study is strongly influenced by assay precision and/or sample variability. A corollary is that the use of a few assays with poor

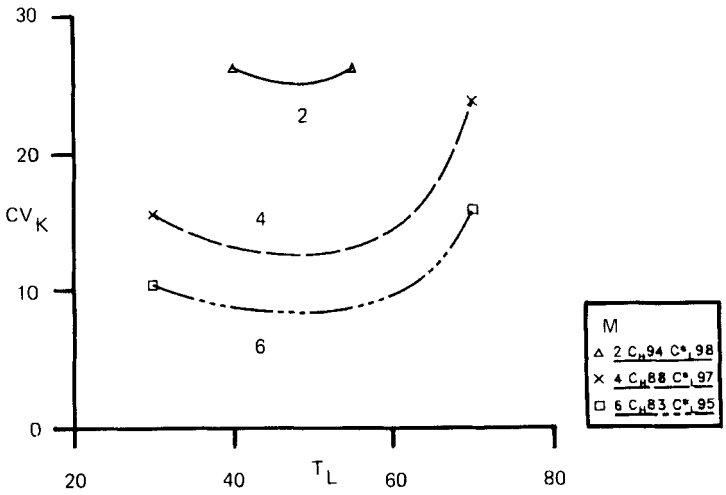

Figure 8. Effect of M on CV_K WITH N_L Selected by J Rule T_H=80, E=10, K=0.002

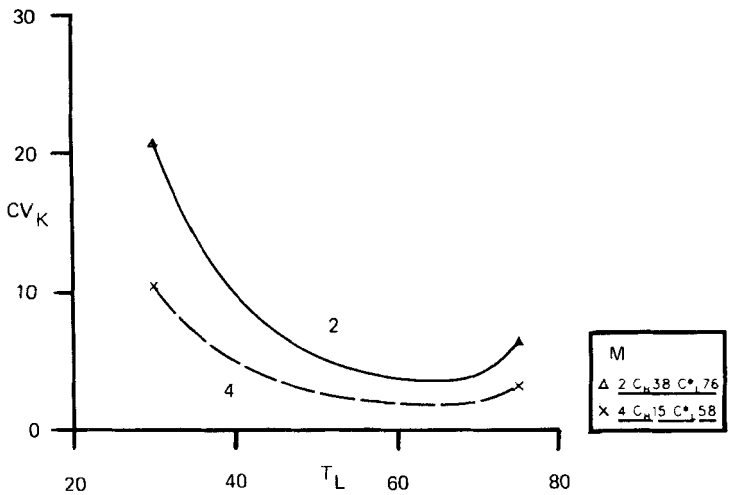

Figure 9. Effect of M on CV_K With N_L Selected by J Rule E=20, T_H=80, K=0.002

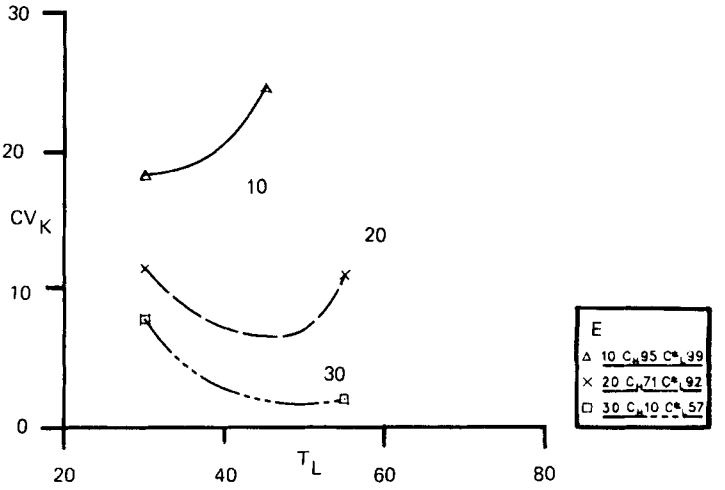

Figure 10. Effect of E on CV_K WITH N_L Selected by J Rule T_H=80, K=0.002, M=4

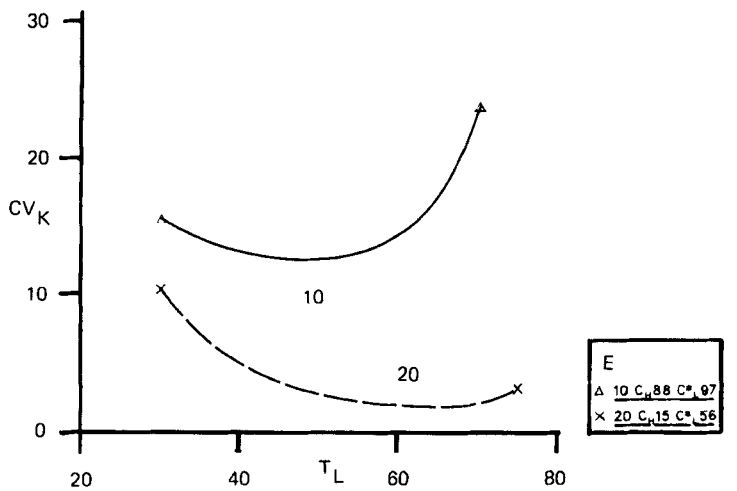

Figure 11. Effect of E on CV_K With N_L Selected by J Rule T_H=80, K=0.002, M=4

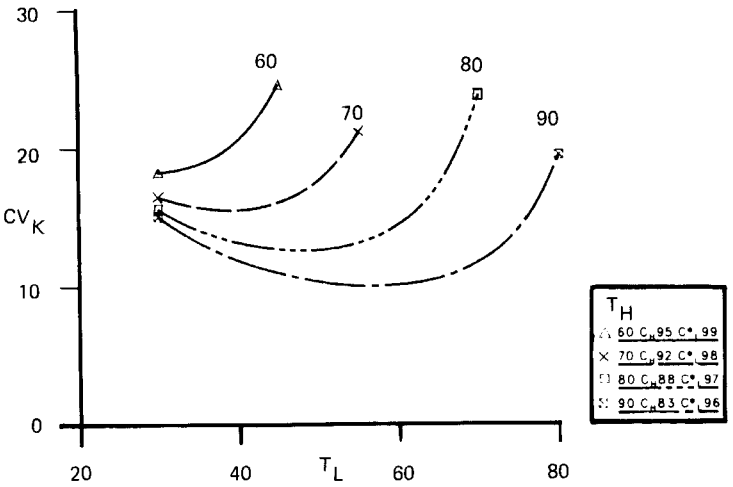

Figure 12. Effect of T_H on CV_K With N_L Selected by J Rule E=10, K=0.002, M=4

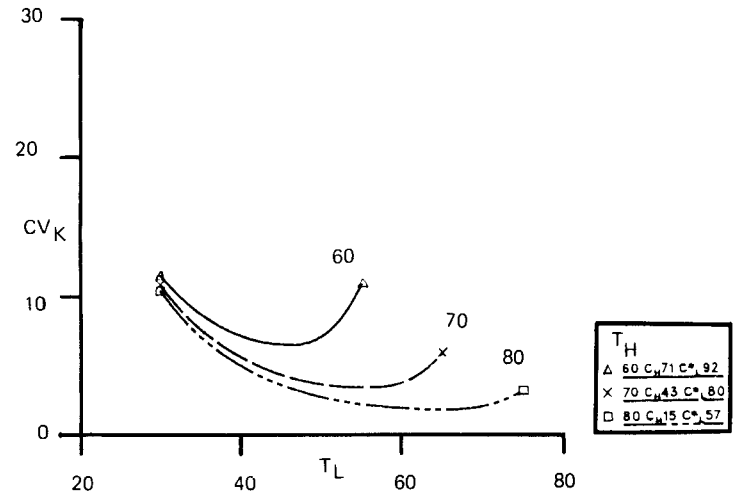

Figure 13. Effect of T_H on CV_K With N_L Selected by J Rule E=20, K=0.002, M=4

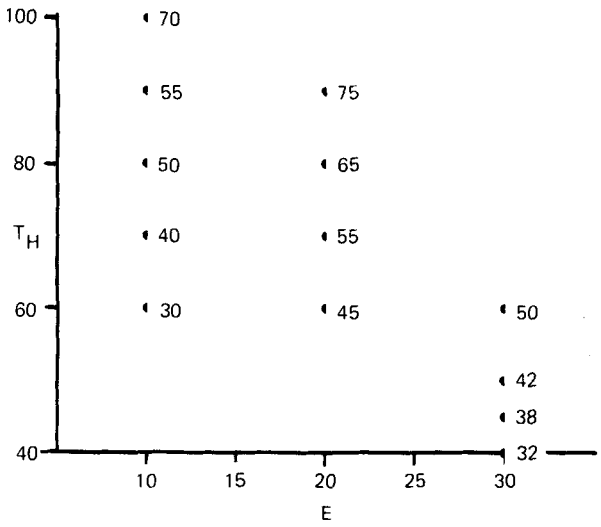

Figure 14. Optimum T_L Values versus T_H and E.

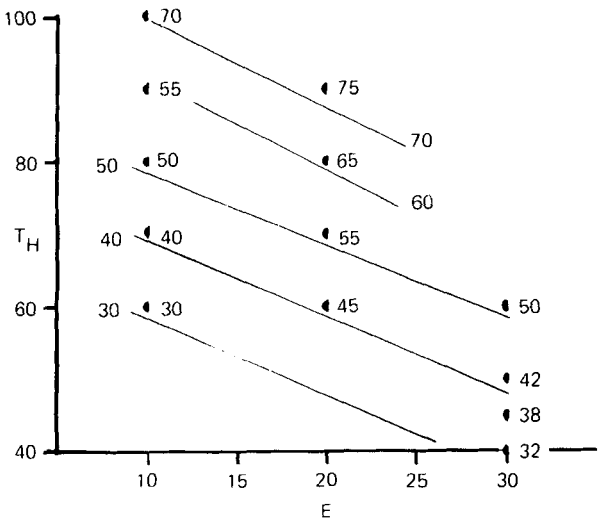

Figure 15. Optimum T_L Contours versus T_H and E.

Table I. Number of assays (and apportionment by the HL Rule) needed to estimate shelf life so that its CV equals 10% --

CASE 1: Initial Potency Known (Two sampling points).[a]

STUDY CONDITIONS				CV_A = 1%			CV_A = 2%			CV_A = 3%		
T_H	T^*_L	M_H	M_L	N	N_H	N_L	N	N_H	N_L	N	N_H	N_L
60	46	4	4	10	2	8	40	8	32	90	18	72
60	46	4	5	7	1	6	28	5	23	63	13	50
70	54	4	4	3	1	2	12	2	10	27	5	22
70	54	4	5	2	1	1	9	2	7	19	4	15

[a] K = 0.002 and E = 20.

Calculation note: The values of N in the CV_A = 1% column were obtained from an equation yielding non-integer values which were rounded off for reporting. An unrounded N value when multiplied by the square of any other CV_A gives the N needed in that case IF NO OTHER FACTORS SUCH AS T_H CHANGE.

precision, an non-optimal design, and/or samples with great variability can lead to spurious quantitative predictions and misleading conclusions; the only usefulness of such a study would be the identification of a grossly unstable product.

16.4 Case Two: Initial Potency to be Assayed (Three Sampling Points Used).

16.4.1 Conditions

When large numbers of samples are prepared during dosage form development, experience has shown that it is best to determine the initial potency by assay

Table II. Number of assays (and apportionment by the HIL Rule) needed to estimate shelf life so that its CV equals 10% -- CASE 2: Initial Potency assayed (Three sampling points).[a]

STUDY CONDITIONS				CV$_A$ = 1%				CV$_A$ = 2%				CV$_A$ = 3%			
T_H	T^*_L	M_H	M_L	N	N_H	N_I	N_L	N	N_H	N_I	N_L	N	N_H	N_I	N_L
60	46	4	4	14	2	5	7	54	9	18	27	120	20	40	60
70	54	4	4	5	1	2	2	18	3	6	9	41	7	14	20

[a] K = 0.002 and E = 20.

as well as those after storage at T_H and T_L. That is, there are now three experimental points among which the N assays must be apportioned.

Condition 4. above has been changed accordingly.

16.4.2 Methods

We have done some work on this case following the same methods as above.

16.4.3 Results

Using the J Rule, with K equal to 0.002 and E equal to 20, the approximate relation $N_H:N_I:N_L$ equals 1:2:3 appears to apply to the optimum assay apportionment. (Note that the subscripts and the numbers are both in ascending order.)

Rule 3. HIL Rule

$N_H:N_I:N_L$ = 1:2:3

The assay numbers and apportionment required to obtain a value of CV_K of 10% are given in Table II is approximately 50% greater than the corresponding value in Case 1.

16.5 Discussion

In more complicated, real-life cases, the accelerated stability study of an appropriate drug/formulation probably should be conducted in two or three stages. Many samples would be stored at many temperatures and some removed at various times to storage under non-degrading conditions, until most of them -- if not all -- are removed, to be assayed later as determined best at the successive stages of data analysis.

1. Some of these samples would not be chosen following Rules 1-3 above for a pilot, i.e., stage one, study to determine the approximate values for the energy of activation and the degradation rate at the shelf temperature.

2. This would be followed by the stage two study consisting of two parts. In the first part additional samples would be selected, from among those already available, according to the design outlined above. In the second part, samples for intermediate times and temperatures would be selected from those already available to allow checks on the order of the degradation reaction and the linear nature (5,6) of the Arrhenius relationship[e]. (Note: degradation of 20% or more at one of the temperatures is needed to distinguish first and zero orders.) The analysis of these data, in some cases, will validate the underlying assumptions (or lead to a more restricted region where they are valid) and provide a sufficiently precise estimate of shelf-life.

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[e] If the added time of taking a sample at a given temperature is M/2 (the best time to detect departure from first order kinetics) it causes no improvement in the CV of the rate constant at that temperature. If the added temperature is $0.5X_H + 0.5X_L$ (the best temperature to detect departure from linearity in Arrhenius relationship) it causes no improvement in the CV of E but it does improve CV_K. This improvement is not as great as that which would have been realized had the same number of additional assays been apportioned to T_L and T_H only. Thus these added points at intermediate time and temperature are mainly for validation and contribute less than proportionately to the precision of the shelf-life estimate IF THE VALIDITY IS SUBSTANTIATED. On the other hand, if the added assays cause a shift to other, more appropriate assumptions, the accuracy as well as the precision of the shelf-life estimate are greatly improved.

3. If the precision turns out to be insufficient, either because of large assay plus sampling variation or because the validity of the assumptions was found to hold in only a sub-region of the study, then at the third stage further samples would be selected (perhaps at a higher temperature, if only greater precision is needed, and if feasible). This would be done only if prospects were good and the costs justifiable, with samples selected, from among those already available, according to the design principles outlined above. This selection would be done using the latest information about the highest possible storage temperature, the estimate of the energy of activation, the corresponding optimum low temperature, and the resulting assay apportionment.

It is clear from Tables I and II that Arrhenius studies can require the analysis of many samples, if a moderate value of CV_K is needed, plus the samples (mentioned in the discussion) needed to check the validity of the assumptions. If the assay/sampling variation is large, an acceptable value for CV_K may not be reasonably attainable. In any situation, the rational use of these design principles will minimize the cost as well as help in the recognition of the inherent limitation of small studies and/or large sample/assay variation. It is expected that the shelf life predicted for a marketed product will be checked with shelf-temperature data at that time and before.

16.6 Summary

In two simple, first-order cases for a shelf storage temperature of 23 degrees C., the design of an elevated temperature stability study to determine the shelf life of a drug product has been optimized to minimize the coefficient of variation of the estimated shelf life by the use of three rules (the HL, TL, and HIL Rules) for choosing the optimum low temperature and apportioning the assays in an Arrhenius type study. The total number of assays needed to estimate the shelf-life with a coefficient of variation of 10% was indicated for a few situations. The adaptation of these design principles to more complex, realistic studies is discussed briefly.

According to the derived design the first step, following the choice of the high temperature using whatever kinetic information is available, consists of the optimal choice of the low temperature. This is followed by the optimal apportionment of the available assays to the low and high temperature samples, and to the zero time samples, if appropriate.

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