Stability Charts

Design and Application to Accelerated Stability Testing of Pharmaceuticals

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The design of a nomographic chart to facilitate the analysis of stability data obtained in accelerated testing at elevated temperatures is described. The effects of errors on predictions made using the chart are discussed, particularly those arising from faulty assays, timing, and temperature control. An optimized stability testing program, based on the selection of elevated temperatures at equal intervals on a $1/T$ scale and on a predetermined assay schedule, is outlined for use with the chart.

THE APPLICATIONS of kinetics and drug stability data to the prediction of long-term stability of pharmaceuticals have been reviewed by Guttman (1) and Conine (2). The most soundly based predictive techniques are founded upon the validity of the Arrhenius equation and involve curve-fitting operations carried out graphically or through least mean squares calculations. At present, these techniques still do not have widespread application in the pharmaceutical industry, primarily because of economic considerations. In most instances, limited data must be utilized to make predictions. Furthermore, available assay data may indicate considerably less than 10% degradation. In these circumstances curve-fitting techniques become difficult to apply with a reasonable degree of confidence.

This report describes a nomographic representation of the Arrhenius equation for computing directly from assay data estimates of the shelf-life of pharmaceutical products. The nomograph (or stability chart) shows the relationship between room temperature stability, heat of activation, and the specific rate of degradation at two elevated temperatures.

This report considers the design of a stability chart, its application to stability prediction in situations where minimum data must be analyzed, and its applications to the analysis of errors which contribute to uncertainty in that prediction. These errors include those inherent in the measurement of stability data and those arising from lack of knowledge of reaction type. The errors are not peculiar to the use of this chart but must be considered in all methods of stability data analysis. In addition, an optimized stability testing program, based on a precalculated assay schedule, for use with the chart is outlined. This program has important advantages in theory and practice over the arbitrary programs now in common use. The most important advantage of the stability chart is that it affords a direct graphic presentation of the confidence limits of the results without the need for additional calculations.

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THEORY

To facilitate the design of a stability chart, the following conventions and definitions have been adopted:

- t = Sampling time measured in months. A month is defined as 30 days.
- t_{90}^{τ} = Time in months required for a product to be reduced to 90% of its initial potency when stored at a temperature of $\tau°$.
- t_{90}^{25} = The shelf-life or room temperature (25°) stability.
- F = Fraction of initial potency of undegraded reactant remaining at time t.

If we assume first-order degradation, then

$$K_1^{\tau} = \frac{0.1054}{t_{90}^{\tau}} = \frac{-\ln F}{t} \qquad \text{(Eq. 1)}$$

where K_1^{τ} is the specific first-order rate constant at $\tau°$. The mathematical formulation of the stability chart for a first-order process may be derived by combining Eq. 1 with the Arrhenius equation to eliminate all unknowns but the desired estimate of shelf-life. Consider that a degradation is carried out at constant temperatures, $T_1 = \tau_1 + 273°$ and $T_2 = \tau_2 + 273°$, where $T_2 > T_1$. The specific rates, $K_1^{\tau_1}$ and $K_1^{\tau_2}$, are related to the specific rate K_1^{25} at $T_0 = 298°$ by the Arrhenius equation, *i.e.*,

$$\ln \frac{K_1^{\tau_1}}{K_1^{25}} = B(1/T_0 - 1/T_1) \qquad \text{(Eq. 2a)}$$

and

$$\ln \frac{K_1^{\tau_2}}{K_1^{25}} = B(1/T_0 - 1/T_2) \qquad \text{(Eq. 2b)}$$

where $B = \Delta H_a/R$. ΔH_a, the heat of activation, is assumed to be independent of temperature. Eliminating B and removing the logarithmic operator, one can readily demonstrate that[1]

[1] Application of equations of the form of Eqs. 3 and 4 in this report to calculations of shelf-life have been described by Tootill (3).

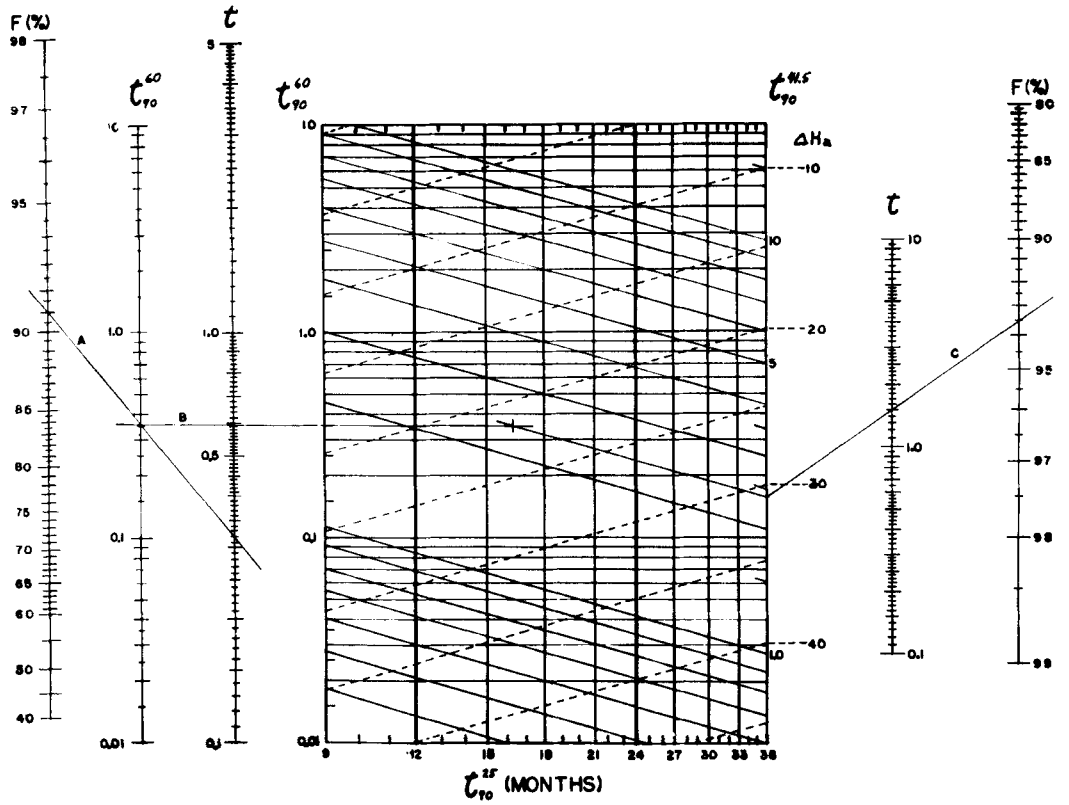

Fig. 1.—Stability chart constructed for a first-order degradation and the elevated temperatures 60° and 41.5°.

$$K_1^{25} = K_1^{T1}\left(\frac{K_1^T}{K_1^{T2}}\right)^{\alpha} \qquad \text{(Eq. 3)}$$

where

$$\alpha = \frac{T_2}{T_0}\left(\frac{T_1 - T_0}{T_2 - T_1}\right) \qquad \text{(Eq. 4)}$$

Equation 3 may be expressed alternately in terms of t_{90}^T values, *i.e.*,

$$t_{90}^{25} = t_{90}^{T1}\left(\frac{t_{90}^{T1}}{t_{90}^{T2}}\right)^{\alpha} \qquad \text{(Eq. 5a)}$$

Equation 5a then is put into logarithmic form and rearranged to

$$\log t_{90}^{T2} = \frac{\alpha + 1}{\alpha}\log t_{90}^{T1} - \frac{1}{\alpha}\log t_{90}^{25} \qquad \text{(Eq. 5b)}$$

A stability chart for any combination of two elevated temperatures can be constructed best by plotting t_{90}^{T2} as a function of t_{90}^{25} on log scales while holding t_{90}^{T1} constant. The slopes of the family of straight lines obtained are functions of α, and the intercepts are a function of α and t_{90}^{T1}. If $\alpha = 1.0$, considerable simplification in the design of the chart is possible. Equation 5b becomes

$$\log t_{90}^{T2} = 2\log t_{90}^{T1} - \log t_{90}^{25} \qquad \text{(Eq. 5c)}$$

The intercepts are now a function of only t_{90}^{T1}.

It appears from an examination of stability studies in the literature (4, 5) that the selection of elevated temperatures has been somewhat arbitrary. The maximum temperature selected generally is determined by considerations of the effect of high temperature on products which may cause changes not occurring at room temperature. Values of 60° or 70° are typical. Intermediate temperatures are selected at roughly equal intervals between room and the maximum temperature. A study of Eqs. 2a and 2b suggests, however, that there is an optimal means for selecting intermediate temperatures. These should be chosen so that they are spaced at equal intervals on a $1/T$ scale[2] rather than on a T scale. This in fact corresponds to the condition that $\alpha = 1$.

Figure 1 shows an example of a stability chart constructed according to Eq. 5c. The main body of the chart consists of three superimposed logarithmic grids. The horizontal or t_{90}^{T2} grid and the vertical or t_{90}^{25} grid are drawn first to conform to the ranges desired. In the example shown, these are 0.01 to 10 and 9 to 36 months, respectively. The position and scale of the t_{90}^{T1} grid, shown in the figure by the downward sloping solid lines, then is established using Eq. 5c to calculate t_{90}^{T1} values for known values of t_{90}^{T2} and t_{90}^{25}. Nomographic scales are appended to the body of the chart to allow its direct application without the necessity of first computing t_{90}^T values from assay data. The scales

[2] Tootill's (3) slope–ratio design is based on the spacing of storage temperatures so that their reciprocal values are in arithmetical progression.

shown represent the graphical solution of Eq. 1, in which assay data are expressed as per cent of initial potency, and assay or sampling time are expressed in months.

An important feature of the chart is the linear grid, indicated by the upward sloping dashed lines. These lines represent constant heats of activation marked off at 5 Kcal./mole intervals. The position of this ΔH_a grid on the chart depends on the specific pair of elevated temperatures chosen. The grid illustrated in Fig. 1 was calculated using Eq. 2a or 2b for the temperatures $\tau_2 = 60°$ and $\tau_1 = 41.5°$. No difficulties are introduced if another temperature pair is used, as only the ΔH_a grid must be displaced in a vertical sense. For example, the position of the 20 Kcal./mole line, when $\tau_2 = 70°$ and $\tau_1 = 46°$, will correspond to the 25 Kcal. line on the 60°/41.5 chart. Although this particular chart could be applied to any accelerated stability study carried out at elevated temperatures for which $\alpha = 1$, the labeling of the ΔH_a grid defines the specific temperatures for which the chart is intended.[3]

The method of using the nomograph also is illustrated in Fig. 1. Lines A and C are drawn between points representing the per cents of initial potency and sampling times. For the example shown, these were 91 and 93.5% and 0.32 and 1.5 months at 60° and 41.5°, respectively. Line B, representing the computed t_{90}^{60} value, is drawn parallel to the horizontal axis onto the chart proper. The intersection of line C with the right-hand vertical axis of the chart represents the computed $t_{90}^{41.5}$ value. The intersection of a line drawn from this point parallel to the $t_{90}^{41.5}$ grid with line B indicates the predicted shelf-life—about 16 months.

DISCUSSION

Assay Scheduling.—In setting up a stability testing program, first one must decide the time at which the first sample for analysis should be withdrawn. Assay schedules like temperature programs appear to have been established arbitrarily. Tootill (3), however, describes the design of a schedule based on provisional knowledge of the degradation rate. Kennon (7) has pointed out that they arise naturally from a consideration of reference reaction paths. He has tabulated the values of heats of activation reported for many drugs. On the basis of this tabulation, one would expect that the heat of activation for degradation in pharmaceutical systems would not be lower than 10 Kcal./mole and, on the average, would be about 20 Kcal./mole. Times required to observe 10% loss in potency at the storage temperature have been computed for reference reactions having heats of activation of 10, 20, and 30 Kcal./mole and a room temperature stability of 12 months. These values are listed in Table I. The t_{90}^{25} value of 1 year was selected to keep the storage times within a reasonable range.

Assay schedules should be flexible; the result of the first assay should determine the schedule to be followed. A minimum assay schedule based upon a pattern illustrated by Table I is proposed in the event that flexibility is not feasible. Practical considerations which dictate that the estimate of shelf-life be arrived at in a reasonable period of time must be counterbalanced by the fact that no information may be available concerning the rapidity of the degradation. Whether an assay schedule is rigid or flexible, the time of first assay is most important. This was based on a 20 Kcal./mole reference reaction, which seems the best compromise between those degradations with low- and those degradations with high-temperature coefficients. Suggested samplings from the low-temperature oven were based on the 30, 20, and 10 Kcal./mole reference reactions taken in this order. The recommended pattern is shown in Table II for both 70°/46° and 60°/41.5° temperature pairs.

Analysis of Stability Data.—Using the stability chart, one can draw certain conclusions from the first assay. If the heat of activation for the degradation is known approximately, an immediate estimate of self-life can be read from the chart at the intersection of the ΔH_a line and the t_{90}^{72} line computed from the assay value.

In the absence of any definite knowledge of the heat of activation, certain deductions can still be made using the chart in conjunction with the information in Table I. For example, if the observed loss in potency in a sample stored at 60° for 10 days is greater than 10%, then either $\Delta H_a > 20$ Kcal./mole or the system has a room-temperature stability less than 12 months. On the other hand, if the loss in potency is less than 10%, then the shelf-life must be greater than 12 months or $\Delta H_a < 20$ Kcal./mole.

Subsequent assay values are most directly handled by considering all possible combinations of data at the two temperatures. To illustrate this point, stability data for a hypothetical product are shown in Table III. These data result in six separate

TABLE I.—t_{90}^{τ} VALUES IN MONTHS COMPUTED ASSUMING ROOM TEMPERATURE STABILITY OF 12 MONTHS

ΔH_a, Kcal./mole	Storage Temp. 41.5°	60°	46°	70°
10	5	2	4	1.3
20	2	0.35	1.3	0.14
30	0.84	0.06	0.43	0.016

TABLE II.—RECOMMENDED MINIMUM ASSAY SCHEDULE

Sample Storage Temp.	Sampling Time, Mo. 1	2	3
60°	0.35	0.84	2
41.5°	0.84	2	5
70°	0.14	0.43	1.3
46°	0.43	1.3	4

TABLE III.—STABILITY DATA CHARACTERIZING A HYPOTHETICAL PHARMACEUTICAL[a]

Temp.	Storage Time, Mo. 0.33	0.84	2.0	5.0
60°	94.5	88.5		
41.5°		97.5	94.5	87.2

[a] Data are presented as per cent of initial potency.

[3] A parallel-line nomograph can be constructed to provide both t_{90}^{25} information and ΔH_a information. The present form of the chart was chosen because it resulted in relatively better balanced t_{90}^{τ} scales, and it gave a two-dimensional perspective to the scattering of shelf-life estimates. To cover the same information as the stability chart, a parallel-line nomograph would require a t_{90}^{72} scale about 10 times the length of the t_{90}^{25} scale. The methods used to construct the chart are described in *Reference 6*.

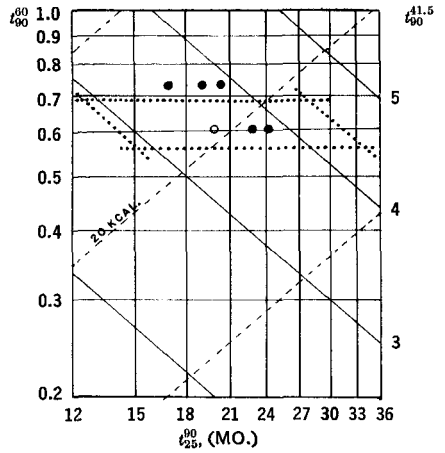

Fig. 2.—Section of a stability chart showing the results of analysis of data listed in Table III, assuming first-order degradation. Key: O, shelf-life estimate derived from the first samples withdrawn from the 60° and 41.5° ovens; ●, shelf-life estimates derived from subsequent samples.

estimates of shelf-life. These have been computed assuming first-order degradation. Their placement on the stability chart is shown in Fig. 2. The assays of the first samples withdrawn from the 60° and 41.5° ovens suggest a shelf-life of 20 months. All estimates taken together indicate a range of about 17–24 months.

Application of the chart to data of this sort is based upon the premise that all data are valid and may be used directly to calculate a rate constant. Scattering of the shelf-life estimates indicates failure of this premise and is, consequently, a measure of the uncertainty in the conclusions which can be drawn. The scatter is the result of errors in measurement and/or errors owing to an incorrect assumption of order of reaction. If there were no error, all data combinations should converge to the same point on the chart.

Effective use of the chart requires an analysis of the effect of possible errors on the results. This analysis has suggested that the proper shelf-life estimate derived using the chart is the mode, not the average, of the results. Since the specific concern is that a product should not degrade faster than predicted, only the lower limit of the range of estimates should be considered. Averaging the results would favor the upper limit of the range. Where limited data are analyzed, the suggestion is that the best estimate of shelf-life is the 3-month interval delineated on the chart in which most of the results fall. Therefore, Fig. 2 indicates a probable shelf-life of 18–21 months. This expression of the shelf-life includes errors introduced in locating the points on the chart (which is determined largely by the size of the chart), errors inherent in the data, and the fact that the chart does tend to magnify the actual scatter in shelf-life estimates. If a single estimate of shelf-life is desired, greater weight should be given to data obtained from the longest storage times and from assays closest to the indication of 10% loss in potency. In this case, Fig. 2 indicates a probable shelf-life of about 20 months.

Analysis of Errors Arising from Measurement of Stability Data.—Errors in measurement include those inherent in the assay, timing errors, and errors resulting from inexactness of temperature control. The nomographic feature of the chart enables one to determine readily the uncertainty in an estimate of shelf-life introduced by timing and assay errors. The dotted lines in Fig. 2 show the uncertainty (range: 12–34 months) in the estimate derived from the first samples when a ±0.5 potency error is assumed in both the 41.5° and 60° data in Table III. It is evident that error in the low-temperature sample contributes most to uncertainty in the estimate. This uncertainty would not be altered greatly even if there was no error in the 60° sample. The precision required in subsequent assays can be estimated from the first value. In the example shown in Fig. 2, a potency error of ±0.1 in the 0.84-month 41.5° value, assuming an error of ±0.5 in the 0.33-month 60° sample, is required to reduce the uncertainty in the shelf-life estimate to a range of 7 months. Requirements for precision would be less stringent the more degradation is induced in the sample, *i.e.*, at later storage times.

This sort of analysis demonstrates that sample replication is the best means of increasing confidence in shelf-life estimates (8). Simply increasing the number of sampling times will generally result in greater scatter of estimates.

Timing errors also vary, depending upon the length of storage time and the actual amount of degradation observed in a sample. In the case of the 0.84-month 41.5° sample, a 15–20% timing error is equivalent to an 0.5% assay error. Timing errors can, therefore, be neglected if reasonable care is taken in sampling.

Errors resulting from inexactness in temperature control can be estimated by substitution in Eq. 5a. Their magnitude depends on the heat of activation of the degradative process; they will be large if the heat of activation is large. Interpretation of these errors is based on the consideration that α differs from unity. The degree of difference will depend upon the difference in actual sample temperature and that presumed to be set in the oven. The

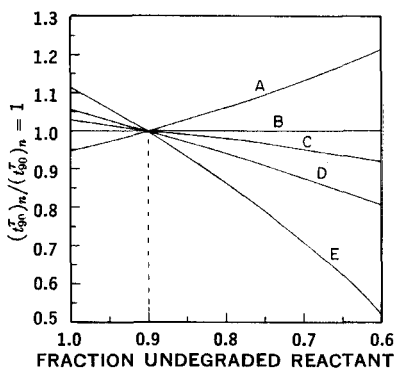

Fig. 3.—t_{90}^T values computed, assuming zero- and second-order kinetics, compared to the t_{90}^T value computed assuming first-order kinetics. Key: A, $n = 0$; B, $n = 1$; C, $n = 2, r = 2$; D, $n = 2, r = 1$; E, $n = 2, r = 0.5$. (n is the reaction order, and r is the ratio of initial potency of reactants in a second-order degradation.)

TABLE IV.—ADJUSTED SPECIFIC RATE CONSTANTS FOR DIFFERENT REACTION TYPES

Reaction Order n	Reaction Type	$K_n^{\tau a}$	$K_n^{\tau} \cdot t_{90}^{\tau}$
0	$A^b \rightarrow$ Products	$\frac{k_0^c}{C} = \frac{1 - F}{t}$	0.1
1	$A \rightarrow$ Products	$k_1^c = -\frac{\ln F}{t}$	0.1054
2	$A + B^d \rightarrow$ Products $C = (A)_0 = (B)_0/r$		
	$r = 1$	$k_2^c C = \frac{1 - F}{Ft}$	0.111
	$r \neq 1$	$k_2 C(1 - r) = -\frac{\ln\left(\frac{rF}{r - 1 + F}\right)}{t}$	$\ln\left(\frac{0.9r}{r - 0.1}\right)$

[a] K_n^{τ} is the specific rate constant at temperature, τ, adjusted for initial potency, C, of reactant and defined for order, n. [b] Reactant A is the drug, the stability of which is to be predicted. [c] k_0, k_1, k_2 are specific rate constants for zero-, first-, and second-order reactions. [d] Reactant B is the component of the pharmaceutical system, the degradation of which may not be followed.

following conditions lead to an α value greater than 1: (*a*) τ_2 is low and τ_1 is correct; (*b*) τ_1 is high and τ_2 is correct; (*c*) both τ_1 and τ_2 are high; (*d*) τ_2 is low and τ_1 is high. If the converse is true, α will be less than 1.0. When $\alpha < 1$, predicted stabilities will be greater than the actual stability; when $\alpha > 1$, predicted stabilities will be less than actual. Control of the low-temperature oven is somewhat more critical than that of the high-temperature oven. Those temperature errors leading to low α values may result in grossly optimistic estimates of shelf-life.[4]

Errors resulting from thermal lags in samples can be interpreted in terms of the effective time of storage at the oven temperature (9) which will be less than the actual sampling time or in terms of a lower actual storage temperature. This source of error, however, can be neglected owing to the long storage periods generally employed in pharmaceutical stability testing.

Analysis of Errors Arising from an Invalid Assumption of Order.—Scatter of shelf-life estimates, computed from assay values measured at different sampling times, may also be an indication of the invalidity of the first-order assumption. In a stability study, consideration of the actual reaction type is not necessary if t_{90}^{τ} values are measured directly (7). However, this requires an elaborate sampling schedule.

Figure 3 shows a comparison of relative rates of reaction computed in terms of t_{90}^{τ} values for zero- and some second-order processes compared to a first-order process. Table IV lists specific rate constants adjusted for initial potency for several reaction types. It is apparent that between 5 and 15% potency loss, computed t_{90}^{τ} values will be least sensitive to error arising from ignorance of reaction order. At less than 10% degradation, it usually is not possible, within the limits of experimental error involved in stability studies, to distinguish between first-, zero-, and simple second-order kinetics using curve-fitting techniques. Consequently, the assumption of first-order kinetics should result in minimum error in the absence of knowledge of the actual reaction type.

One can ascertain the validity of the first-order assumption through recalculation, assuming different orders, or correcting the first-order computation using the data plotted in Fig. 3. Additional scales on the nomograph, based on the relationships defined in Table IV, can be provided for this purpose. Estimates of shelf-life also were computed from the data in Table III in which zero- and second-order ($r = 1$) degradation were assumed. The results shown in Fig. 4 suggest that the degradation is best represented by a second-order process, since this shows minimum scatter. This conclusion is verified by plotting the data. However, still the conclusion is made that the most probable shelf-life is 18–21 months in both instances.

With the single exception of first-order reactions, adjusted specific rates are functions of initial

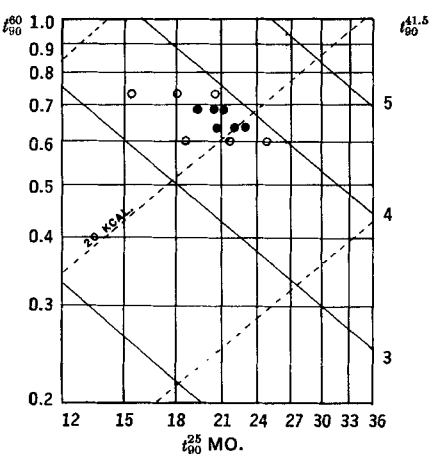

Fig. 4.—Section of a stability chart showing the results of analysis of data in Table III, assuming, O, zero-order degradation and, ●, second-order degradation.

[4] A 1° temperature difference between sample and that presumed to be set in the oven maintained over the storage period can result in significant error. Consider the following example. The estimated shelf-life of a product (obtained using the chart in Fig. 1), stored in a low-temperature oven set at 40.5° instead of 41.5° (actual α = 0.888), was 25 months. Assuming no other source of error, its actual shelf-life would be about 19 months if ΔH_a = 28 Kcal./mole and about 21 months if ΔH_a = 18 Kcal./mole.

potency (Table IV). The stability of such systems will consequently be functions of potency. One must allow for this when comparing the stability of systems with different initial potencies. If there is no other source of error and degradation is carried beyond 10% loss in potency, one would expect that a zero-order process, treated as a first-order degradation, would tend to predict a shorter than actual shelf-life, its magnitude depending on the extent of potency loss. Second-order processes treated similarly would predict a greater than actual shelf-life. At less than 10% potency loss, the converse would be true.

Pseudo first-order degradation is probably most frequently observed in practice. Errors that may arise when the second component in the reaction is not in significant excess have been computed (10). One example is shown in Fig. 3 for the case where a drug degrades by reaction with some other component in the system initially present in a concentration two times that of the drug. Degradations of this type may be analyzed as first-order kinetics with little error. On the other hand, where the concentration of drug is greater than that of the second component of the degradation ($r < 1$), predicted shelf-lives may appear to be significantly greater or less than that predicted by the use of the proper kinetics, depending on the extent of potency loss. In this case, the reaction is limited by the second component. If the drug is in great excess, even though the apparent initial rate of loss is high, the system still may meet requirements for stability. If this type of reaction is suspected, the degradation should be carried to the point where a significant reduction in the rate of potency loss is observed.

SUMMARY AND CONCLUSIONS

A method to evaluate stability data relating to the storage of pharmaceuticals at constant elevated temperature has been described. The method is based on the design of a nomographic chart to facilitate the analysis of data. This stability chart shows the relation between times required to observe 10% potency loss in a product stored at two elevated temperatures and room temperature over a range of heats of activation. Its use requires acceptance of the validity of the Arrhenius equation as an index of the influence of temperature on the degradation rate. This assumption appears justified on the basis of published reports of stability data.

The advantages inherent in using such a chart are manifold. It is a convenient time-saving device to eliminate the need for complicated computations and curve fitting. Consequently, its use minimizes bias in the analysis of data introduced by the investigator. The first assay value obtained in a study can be subjected to immediate analysis. The chart allows the ready application of prior information, *e.g.*, heats of activation concerning the degradation to this analysis. Data can be analyzed directly and easily as they become available. It is not necessary to wait until the entire or most of a stability study has been completed to arrive at some conclusion. Scatter in shelf-life estimates is shown graphically, thus giving an immediate picture of the uncertainty inherent in the data. This graphic presentation leads to the selection of the mode or 3-month interval delineated on the chart, in which the greatest number of estimates fall as the best estimate of shelf-life. The chart will prove most useful where limited data must be analyzed and where the only concern may be the acceptance or rejection of a formulation, depending upon specific criteria which are established, *e.g.*, a 12- or 24-month shelf-life.

The application of the chart has been extended to an analysis of the effect of errors on predictions made using it, especially those arising from faulty assays, timing, and temperature control. The extent to which these errors influence the validity of estimated shelf-lives must be weighed, whatever the technique employed, to evaluate the raw stability data. If sufficient information has been obtained in a study to make statistical analysis feasible, the total effect of all errors on the estimated shelf-life is reflected in the computed confidence limits. In cases exemplified by the data discussed in this report, the stability chart can be used to establish confidence limits based on the estimated effects of errors in the data.

The effect of known precision in assays can be readily ascertained. This analysis has indicated that replication, particularly of the low-temperature samples, can result in less scatter and, therefore, greater confidence in shelf-life estimates. While the assay error may be most important at low temperatures, when the observed extent of degradation may be small, timing errors have significant effects only if the sampling time is short. Therefore, they will prove most critical in the case of the first sample withdrawn from the high-temperature oven. Errors arising from lack of knowledge concerning the true temperature of the sample will be most severe if the actual α value is less than the value used to design the chart, leading to predictions of greater than actual shelf-life. It would seem that special attention should be paid to temperature control and recording of the true sample temperature during the course of a study.

Some assumption concerning reaction type must be made if limited data are to be analyzed. Computations based upon first-order kinetics are most useful. If sufficient data have been accumulated, this assumption can be tested by computing t_{90} values, assuming different orders, and noting their effect on reducing scatter of shelf-life estimates. However, the same conclusion will be drawn with respect to the most probable shelf-life, irrespective of the actual order, if computations are based on observed degradation not greatly different from 10% potency loss.

An optimized stability testing program has been outlined. This was based on the selection of elevated temperatures at equal intervals on a $1/T$ scale, a factor which simplifies the design of the stability chart, and on a predetermined assay schedule derived from consideration of reference degradations. The use of such a schedule with the chart facilitates interpretation of degradation data.

The methods described in this report are not intended as substitutes for more elaborate stability studies which yield sufficient data to enable the meaningful application of statistical analysis. They can be used, however, as an adjunct to such studies, especially in their early stages. They are intended as convenient alternates to the usual graphical approach and the rules of thumb com-

monly employed and provide a means of maximizing the information derived from minimum data.

Existing stability testing programs are based on the use of prescribed temperatures which are not altered from product to product. While the stability chart described in this report has been designed for a recommended stability testing program, it may be viewed generally as a prototype for other possible designs. Charts can be prepared for any combination of temperatures desired, whatever the value of α. The methods described can be applied to fit existing stability testing facilities. The use of a nomographic technique with conventional stability calculations has made the chart practical for routine application.

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Some Interfacial Properties of a Nonaqueous Emulsion

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Emulsions of glycerin and olive oil were formed using anionic surface-active agents. The two surfactants used were condensation products of ammonia and of 2-amino-2-methyl-1,3-propanediol (AMP) with the fatty acids available in olive oil. The minimum concentration of ammonia needed for emulsification was found to be 0.6 mg./100 ml. and 0.84 mg./100 ml. at phase volumes of 0.40 and 0.58, respectively. The minimum concentration of AMP was 2 mg./100 ml. and 2.7 mg./100 ml. for phase volumes of 0.40 and 0.58, respectively. Total interfacial area was calculated from photomicrographs of the emulsions and correlated with interfacial tensions. The effective mean molecular areas of the surfactants, in the low concentrations employed, were sufficient to form 89 to 98 per cent interfacial coverage.

PREVIOUS PUBLICATIONS (1, 2) have reported on the preparation of nonaqueous emulsions of olive oil and glycerin with the use of anionic, cationic, and nonionic surface-active agents. McMahon and co-workers (1) have demonstrated that oil can be emulsified with as little as 0.01% of amine employed. These workers also suggested that still lower concentrations might be effective in emulsifying this system. Further work with much lower concentrations of two anionic surfactants [condensation products of ammonia and of 2-amino-2-methyl-1,3-propanediol[1] (AMP) with the fatty acids available in olive oil] confirmed this hypothesis. Attention was also focused on the interfacial film available at these lower concentrations. Since oleic acid is the predominant fatty acid found in olive oil (3), it was assumed that the surfactant at the interface could be represented simply as an ammonium or an AMP oleate. Therefore, if the dimensions of the oleate molecule and the total interfacial area in the emulsion were known, it would be a relatively simple matter to calculate the total interfacial coverage.

The dimensions reported for various oleate salts vary greatly. Kremnev and Kagan (4) reported values for the molecular areas of different oleate salts ranging from 50 Å.^{2} for sodium oleate to 258 Å.^{2} for cesium oleate. Calculation of the total interfacial coverage, based on a model with a dimension of 50 Å.^{2} showed a greatly insufficient monomolecular layer in the present system; whereas if the calculation is based on a 258 Å.^{2} model, an almost continuous monomolecular layer is shown. Thus, a knowledge of molecular dimensions in the glycerin–oil system is essential to the accurate calculation of interfacial coverage.

This study was designed to determine the minimum concentration of surfactant needed for emulsification and to obtain the interfacial tension data needed to confirm the effective area of the surfactant molecule.

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