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Influence of Nonequilibrium Sample Temperature on Stability Predictions Extrapolated from Elevated Temperature Studies

By MORTON W. SCOTT† and LEON LACHMAN‡

Assay results for a single sample stored in an elevated temperature oven for a known period of time may be in error if instantaneous heating and cooling of the sample is not achieved. For usual kinetic studies however, the storage behavior of a group of samples which have been placed in the oven at the same time is followed. Theoretical and experimental considerations are presented to show that in the case of apparent first-order reactions the assay values for all samples in the group are subjected to the same degree of error. Rates of degradation calculated from the assay data are identical to those measured under isothermal conditions. Consequently, decomposition rates obtained under the usual oven storage conditions, where time lags in heating and cooling samples exist, give useful information for estimating product stability.

CONSIDERABLE PROGRESS has been made in recent years in applying chemical kinetic principles for the prediction of the shelf life of pharmaceutical products. The techniques employed are gaining general acceptance in the pharmaceutical industry and have been the subject of an increasing number of literature reports.

In one early investigation, Garrett and Carper (1) predicted the color stability of a multisulfonamide preparation using colorimetric measurements of samples subjected to thermally accelerated degradation. Later, Swintosky, *et al.* (2), used chemical kinetics in predicting the shelf life of oral penicillin G procaine suspensions. In another report, Garrett (3) examined the thermal degradation of ascorbic acid, vitamin B_{12}, folic acid, and other components of a multivitamin preparation. The author was able to arrive at satisfactory estimates of room temperature stability from data obtained under accelerated test conditions. McLeod (4) used a similar approach to predict expiration dates for other multivitamin preparations.

These reports and others on the subject (5–7) have suggested a simple experimental procedure for the evaluation of product stability. The procedure generally followed involves storing the product, preferably packaged in its final market container, in three or four individual ovens maintained at constant elevated temperature. Periodically samples are removed from these ovens and assayed. The loss of active ingredient is calculated, and rates of decomposition for the

Received April 28, 1961, from the Warner-Lambert Research Institute, Morris Plains, N. J. and the Research Department, Ciba Pharmaceutical Products Inc., Summit, N. J.
Accepted for publication June 20, 1961.
Presented to the Scientific Section, A.PH.A., Chicago meeting, April 1961.
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several temperatures are obtained. These rates are plotted according to the Arrhenius relationship. Extrapolation of the Arrhenius plot gives the estimate of room temperature stability.

Errors which may arise in this procedure were recently discussed (8). In particular, it was observed that considerable time may elapse before samples placed in elevated temperature ovens reach thermal equilibrium. A time lag also may occur while the sample returns to room temperature after it is removed from the oven. These time lags lead to incorrect estimates of the actual storage interval at elevated temperature to which the samples are exposed. In order to correct for these errors, the concept of "Equilibrium Temperature Time Equivalent" or ETTE has been introduced. The ETTE is defined as the effective time of storage at oven temperature. It is calculated from the assay data by computer techniques, provided that an estimate of the heat transfer coefficients can be obtained.

Because of the increasing importance of accelerated testing procedures in industry, it seemed appropriate to explore in detail the errors introduced in stability predictions by heating and cooling lag times. This was done by using a theoretical and experimental approach to the problem.

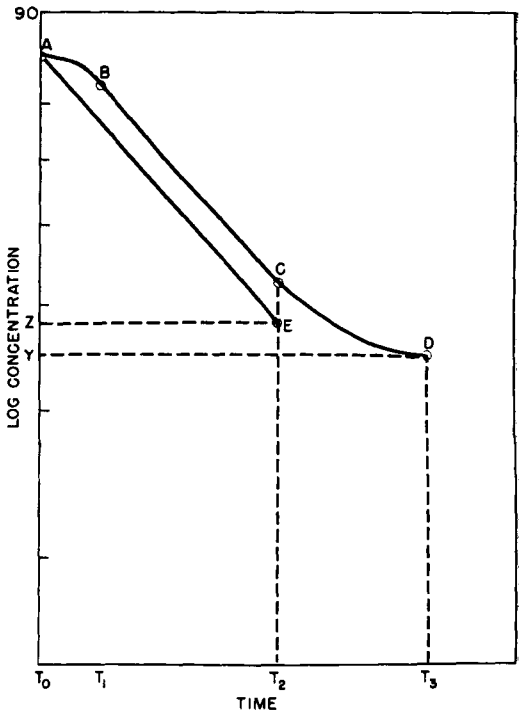

Fig. 1.—Time-concentration history for apparent first-order decomposition with heating and cooling lags, line *A-D;* and without lag, line *A-E.*

THEORETICAL TREATMENT

Figure 1 illustrates the decomposition history of a hypothetical pharmaceutical product stored at elevated temperature when degradation occurs by apparent first-order reaction. At zero time (T_0) the sample is placed in the storage oven and proceeds to warm to the equilibrium storage temperature at time T_1. During this heating interval the product temperature rises continuously, and breakdown occurs at an increasing rate. This is indicated by the changing slope of the line *A-B*. At time T_1, the sample temperature has reached its equilibrium value, and decomposition has reached its limiting rate. This maximum rate of breakdown continues at a constant level over the remaining time interval of actual storage in the oven. This period is indicated by the line *B-C*. At time T_2, the sample is removed from the oven and cooled to room temperature. While cooling, the decomposition rate gradually decreases as indicated by the changing slope of line *C-D*. The assay value obtained at the conclusion of the study, time T_3, is found to be Y units.

A hypothetical situation where the product is instantly heated to oven temperature, held at this temperature over the entire storage interval, and then instantly cooled back to room temperature is shown as a dashed line in Fig. 1. In this case, the rate of decomposition is constant and results in an assay value of Z units at time T_2. This value is higher than that obtained in the first example (Y units). The difference between Y and Z represents the error arising because of heating and cooling lag times. The presence of this $Y-Z$ error was illustrated in a similar fashion by Erickson, *et al.* (8), and served as the starting point for the development of the ETTE concept.

It is important to recognize, however, that in the usual kinetic study we are not primarily interested in the absolute assay value of any one particular sample. What is desired for the stability prediction are the rates of decomposition at the various elevated temperatures. These rates are obtained generally by following the decomposition history of a series of samples which have been placed in oven storage.

Figure 2 illustrates a theoretical decomposition history for a series of samples stored at oven temperature for various time intervals. First-order kinetic principles have been assumed to apply. It was also assumed that all samples were placed in the oven at the same time and heated at the same rate to the equilibrium oven temperature. Further, it was assumed that all samples cool to room temperature at the same rate after they are removed from the oven. These assumptions are completely in accord with industry practice and are justified by the results of Erickson and co-workers (9). It is important to note that in the present study no assumptions were made (or required) that the rates of heating (to oven temperature) or cooling (to room temperature) are equal to each other.

For the analysis shown in Fig. 2, all samples are placed in the oven at time T_0 and heated to equilibrium oven temperature at time T_1. The first sample is held in the oven until time T_2 and then cooled to room temperature by time T_3 giving an assay

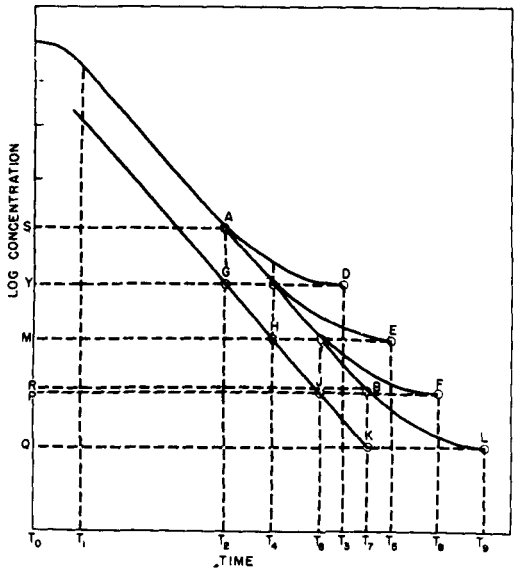

Fig. 2.—Time-concentration history for a series of samples decomposing under apparent first-order kinetics.

value of Y units. This plot is identical with that shown in Fig. 1.

The second sample is held at oven temperature until time T_4 and then removed from the oven and cooled to room temperature at time T_5, giving a final assay value of M units. Similarly, samples 3 and 4 are held at oven conditions for times T_6 and T_7, respectively, and then allowed to cool to room temperature to yield final assay values of P and Q units, respectively.

The individual assay values of Y, M, P, and Q units, together with the respective storage time intervals for each sample, constitute the entire data collection obtained for this particular temperature condition. It must be noted, however, that although assays are normally performed on samples which have cooled to room temperature, the storage interval is usually measured to the time the sample is actually removed from the oven. The data points used to plot the breakdown rates will therefore have the coordinates of (Y, T_2), (M, T_4), (P, T_6), and (Q, T_7) for the first, second, third, and fourth samples, respectively. These points have been designated in Fig. 2 by the letters G, H, J, and K.

A straight line has been drawn through the points G, H, J, and K in Fig. 2. Further, the slope of this constructed line has been set equal to the slope of the line A-B, which in turn equals the slope of line B-C in Fig. 1. The analysis which follows shows that points G, H, J, and K do fall on a straight line with a slope equal to that of line A-B. It should be emphasized that the slope of line A-B is equal to the rate constant for decomposition at constant temperature and is the parameter required for the Arrhenius plot. The slope of line A-B can be set equal to

$$\frac{R - S}{T_7 - T_2} = k \qquad \text{(Eq. 1)}$$

In coordinate notation, point G has been written as (Y, T_2) while point K has the coordinates of (Q, T_7). These points can be denoted also as ($S-X$, T_2) and ($R-Z$, T_7), respectively, where X and Z are concentration units such that

$$\ln S - \ln X = \ln Y \qquad \text{(Eq. 2)}$$

$$\ln R - \ln Z = \ln Q \qquad \text{(Eq. 3)}$$

For first-order and pseudo first-order rate mechanisms, the change in the logarithm of concentration is independent of initial concentrations and dependent only on the time interval during which the reaction occurs. This is true regardless of the temperature at which the reaction takes place. Consequently, if the rate of cooling from oven to room temperature is assumed to be equal for all samples, all samples will show an equal and constant change in the logarithm of concentration during the cooling step. Thus, the fall of X units in the logarithm of concentration from S to Y during the cooling of sample 1 will equal the fall of Z units in the logarithm of concentration from R to Q during the cooling of sample 4. Therefore, X and Z are equal to each other. Points G and K can now be expressed as ($S-X$, T_2) and ($R-X$, T_7), respectively. The slope of line G-K therefore becomes

$$\frac{(R - X) - (S - X)}{T_7 - T_2} = \frac{R - S}{T_7 - T_2} \qquad \text{(Eq. 4)}$$

This slope is identical to that for line A-B as shown in Eq. 1.

By repeating this procedure, it can be established that lines G-H, H-J, and J-K also have slopes equal to that for line A-B. Since G-H, H-J, J-K, and G-K can be shown to have the same slope and have points in common, points G, H, J, and K must be on the same line.

Individual assay points collected after sample decomposition at elevated temperatures do not recognize the errors introduced by heating and cooling lags. The foregoing theoretical analysis demonstrates however that the slope of the line drawn through these points represents, without error, the rate constant of decomposition desired for the Arrhenius plot.

EXPERIMENTAL

In order to illustrate the validity of the preceding theoretical treatment, two experiments were performed on a simple dye system degrading according to apparent first-order kinetics. The color stability at 87 ± 0.5° of aqueous solutions of FD&C Blue No. 2 (0.002%) in phosphate buffer, pH 6.72, was investigated. Residual color was measured spectrophotometrically at 610 mμ with a Cary model 11 recording spectrophotometer. Beer's law held for the dye in buffer solutions over the concentration range studies. Two different procedures were used to bring the color solutions to thermal equilibrium.

Experiment A—Instantaneous Heating and Cooling.—A 2.0-ml. aliquot of a concentrated stock solution of the dye at 25° was pipetted into 300 ml. of buffer solution preheated to 87 ± 0.5° and maintained at this temperature in a constant temperature bath. The solutions were mixed for 20 seconds to obtain uniform color distribution. The temperature of the buffer solution was not lowered by the

addition of the dye solution, thereby indicating instantaneous attainment of thermal equilibrium. At selected time intervals, 25-ml. aliquots of the solution were removed from the reaction mixture and quickly transferred to a glass-stoppered 50-ml. Erlenmeyer flask. These flasks were placed immediately into a dry ice-acetone bath at −67°. Cooling of the sample to 25° was obtained within 20 seconds by this procedure. Absorbance measurements were performed at 610 mμ for residual color. The change in the observed absorbance with time for this system is plotted in Fig. 3 as line *A*.

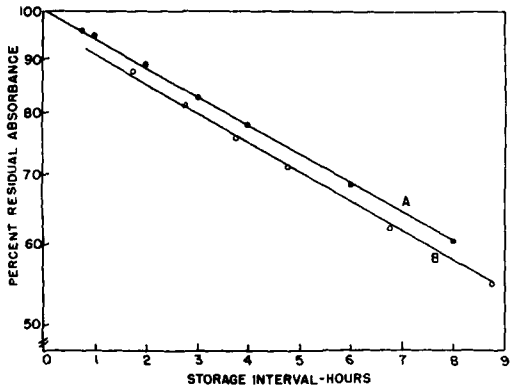

Fig. 3.—Apparent first-order plots for loss in absorbance of FD&C Blue No. 2 aged at 87°. Curve A, instantaneous heating and cooling; curve B, heating lag, 45 minutes, cooling lag, 105 minutes.

Experiment B—Slow Heating and Cooling.—This experiment was designed to introduce significant lags in both the heating and cooling of the dye solution. For this work a 2-ml. aliquot of concentrated stock solution of dye at 25° was pipetted into 300 ml. of buffer solution at 25°. The solutions were mixed to give uniform color distribution. The mixed solution was then placed in a constant temperature bath and heated to 87°. The solution required 45 minutes to reach an equilibrium temperature of 87 ± 0.5°. Periodically, 25-ml. aliquots of the solution were removed from the reaction flask and placed into a 50-ml. glass-stoppered Erlenmeyer flask. These samples were stored at room temperature to cool. A period of 105 minutes was required for the samples to reach 25°. Absorbance measurements were then taken. The data collected in this study are presented in Fig. 3 as line *B*. Storage intervals plotted in Fig. 3 were measured from the time samples were placed in the elevated temperature bath to the time they were removed.

DISCUSSION AND CONCLUSIONS

From the data shown in Fig. 3 it is evident that the rate of color loss follows first-order kinetics for experiment A (instantaneous heating and cooling). The rate constant at 87° for the isothermal reaction is expressed by the slope value of line *A* and is equal to 4.21×10^{-2} hr.$^{-1}$. It is also possible to read a $t_{80\%}$ value (3.6 hours) and other $t_{\%}$ values directly from the curve. These parameters often are used as convenient indexes of decomposition rate in the Arrhenius plot.

The degradation observed in experiment B (slow heating and cooling) also follows first-order kinetics once isothermal storage conditions are achieved (after 45 minutes). The data obtained in this experiment lie on a straight line (line *B*) which is parallel to that for the isothermal reaction. The rate constants as measured by the slopes of the lines therefore are equal. The experimental study thereby confirms the theoretically derived conclusion that valid rates of degradation can be measured directly from assay data which may be subject to (absolute) error as a result of heating and cooling lag times. Results obtained from customary stability testing of pharmaceutical dosage forms stored in constant temperature ovens therefore can be used with confidence to give accurate estimates of product stability.

Although data collected in such studies will give linear relationships with correct slope, the line constructed through the individual data points may be displaced from that obtained under isothermal conditions. Extrapolation of the decomposition data generally will not give a concentration intercept value equal to the experimentally determined concentration at zero time (100%). The rate of decomposition therefore cannot be correctly expressed in terms of $t_{\%}$ values which assume a linear relationship whose intercept on the concentration axis is equal to 100% (zero % decomposition).

When heating and cooling lags occur during the course of a stability study at elevated temperature, rate constants must be obtained from the slope of decomposition line. This slope should be calculated directly from the coordinates of two points which actually fall on the line. Although a high level of confidence is generally placed on the initial (zero time) assay value of the product, this value should not be employed in establishing the rate of decomposition.

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