Nonisothermal Kinetic Studies III: Rapid Nonisothermal–Isothermal Method for Stability Prediction

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Abstract □ A continuous nonisothermal–isothermal method for stability prediction was developed. The approach yields all necessary parameters for prediction, including reaction order. The experimental procedure involves changing the temperature of the samples being studied until degradation is rapid enough to proceed at a convenient isothermal rate for a sufficient number of half-lives with adequate analytical sensitivity so that the reaction order can be unambiguously determined. The analytical information obtained during the nonisothermal and isothermal portions of the experiment is utilized without curve matching in calculating the activation energy and determining the reaction order, reaction rate, and stability prediction at any desired temperature. Model experiments include the acid-catalyzed hydrolysis of acetylcholine bromide and the inversion of sucrose.

Keyphrases □ Nonisothermal kinetic studies—rapid nonisothermal–isothermal method for stability prediction □ Kinetics—rapid nonisothermal–isothermal method for stability prediction □ Stability prediction—rapid nonisothermal–isothermal kinetic method

Since the primary contribution to the field of nonisothermal kinetics by Borchardt and Daniels (1) in 1957, publications on this subject have appeared at a steady rate (2–8). When compared to isothermal methods, nonisothermal technology generally reduces the time and analytical effort needed to generate kinetic parameters. Although there are limitations (9) to the methods, these are being dealt with wherever possible (8).

Classical isothermal kinetics are often too burdensome to incorporate into predosage form development studies. A need exists for alternative short-term methodology so that the kinetic parameters needed for a knowledge of drug stability are known early in the preformulation phase. The nonisothermal methodology published to date has been subject to limitations (6, 7) of fixed time–temperature relationships, nonlinear curve fitting, and assumptions of reaction order. The method presented offers flexibility of time–temperature relationships, direct calculation of activation energy, and determination of reaction order.

In the proposed method, the investigator searches (through temperature adjustment and analytical determinations) for a temperature to run an isothermal experiment. Whereas this problem would normally be considered a screening study, it has been developed into a total experiment by mathematical treatment of data from the initial probing and subsequent isothermal rate determination.

THEORETICAL

A general method was developed which results in values for the reaction order, activation energy, and reaction rates from a single experiment without time–temperature restrictions or curve matching (5, 6). A time–temperature profile (Fig. 1) is obtained when using this technique, and it consists of two distinct parts: (*a*) a nonisothermal region from t_a to t_b, for which temperature can be any function of time, and (*b*) an isothermal region from t_b to t_c.

The concentration–time profile for the acid-catalyzed hydrolysis of acetylcholine bromide is shown in Fig. 2. The concentration function used is determined by the isothermal part. A function of concentration, $f(c)$, can be chosen that is linear under the isothermal conditions existing from t_b to t_c to describe a concentration, c, at time t when $c = c_b$ at $t = t_b$ (Eq. 1):

$$-[f(c) - f(c_b)] = k_f(t - t_b) \qquad \text{(Eq. 1)}$$

The $f(c) - f(c_b)$ is $c - c_b$ for a zero-order reaction [$f(c) = c$], is 2.303 log c/c_b for a first-order reaction [$f(c) = 2.303 \log c$], and is $1/c_b - 1/c$ for a second-order reaction [$f(c) = 1/c$]. The chosen function must hold for a reaction of a given order at all other temperatures. When the function of concentration, $f(c)$, which is linear against time under isothermal conditions, is plotted against time for the nonisothermal period from times t_a to t_b, it can be considered as a series of sequential isothermal reactions. Although it is relatively easy to describe the change in concentration from t_b to t_c, it is not easy to do so for t_a to t_b where the temperature is changing.

The approach used to resolve this problem is to divide this portion of the curve into a large number of equal segments. If these segments are "short enough," then the concentration function from t_a to t_b can be considered as a series of independent rates, each with its own rate constants (5, 6):

$$-\frac{f_b(c) - f_a(c)}{t_b - t_a} = \frac{k_1 + k_2 + k_3 \ldots k_i \ldots k_n}{n} = \bar{k}_n \qquad \text{(Eq. 2)}$$

According to this equation, the change in the concentration function with time equals the sum of the individual rate constants divided by the total number of constants. This result will equal the average rate constant. The relationship between the rate constant and activation energy, where a^* is the frequency factor, is:

$$k_i = a^* e^{-E_a/RT_i} \qquad \text{(Eq. 3)}$$

These equations can be combined to give Eq. 4, where Δt is the time interval of each short segment:

$$-\frac{f_b(c) - f_a(c)}{n\,\Delta t} = \frac{a^*}{n}\sum_{i=0}^{n-1} e^{-E_a/RT_i} = \bar{k}_n \qquad \text{(Eq. 4)}$$

Replacing the temperature in Eq. 4 by its corresponding time function gives Eq. 5, in which $G(t_i)$ is the time function:

$$\frac{a^*}{n}\sum_{i=0}^{n-1} e^{-E_a/RG(t_i)} = \bar{k}_n = -\frac{f_b(c) - f_a(c)}{n\,\Delta t} \qquad \text{(Eq. 5)}$$

It remains to be determined how large n must be to define the nonisothermal curve. This is accomplished by utilizing the relationship given in Eq. 6:

$$\lim_{n\to\infty}\left[1 + \sum_{i=2}^{n} e^{(E_a/R)(T_i - T_1)/(T_i T_1)}\right]/n = L \qquad \text{(Eq. 6)}$$

The origin of this relationship was described previously (6). It can be shown that the value of this expression approaches a constant value for a specific activation energy as n becomes larger. Therefore, by evaluating the expression for increasing values of n, a

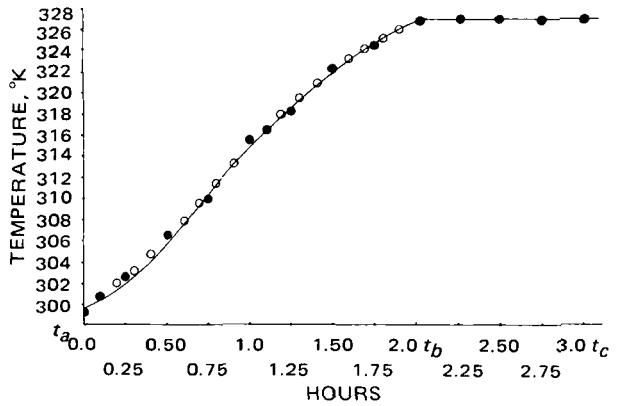

Figure 1—*Time-temperature relationship for the acid-catalyzed hydrolysis of acetylcholine bromide. Key:* O, *mathematical fit; and* ●, *experimental points.*

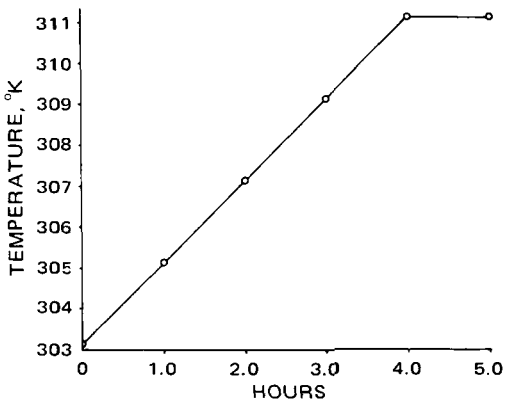

Figure 3—*Time-temperature relationship for the acid-catalyzed inversion of sucrose.*

value of n can be chosen that results in a constant not significantly different from the preceding one.

Finally, an expression can be derived that relates the activation energy to the average rate constant over the nonisothermal region, the rate constant for the isothermal region (k_j), and the length of time of each short segment:

$$-\frac{n[f_b(c) - f_a(c)]}{(t_b - t_a)k_j} = \frac{n\bar{k}_n}{\Delta t k_j} = \sum_{i=0}^{n-1} \exp\left[\frac{E_a}{R}\left(\frac{T_i - T_j}{T_i T_j}\right)\right] = C \quad \text{(Eq. 7)}$$

Making a series expansion of the exponentials results in Eq. 8, in which a, b, c, *etc.*, are the temperature terms and X is E_a/R. This equation can be evaluated by Newton's method to obtain the energy of activation:

$$n + (a + b + c \ldots)X + \frac{(a^2 + b^2 + c^2 \ldots)}{2!}X^2 + \frac{(a^3 + b^3 + c^3 \ldots)}{3!}X^3 + \ldots = C \quad \text{(Eq. 8)}$$

EXPERIMENTAL

Acetylcholine Bromide—A solution of 25 g of acetylcholine bromide (recrystallized from absolute ethanol) in 500 ml of 0.1 *N* HCl was prepared, and 20-ml aliquots were pipetted into 50-ml ampuls which were heat sealed. These ampuls were immersed in a water bath fitted with a thermoregulator[1] and thermometer (0.1° graduations). The thermometer was immersed in an unsealed ampul containing the acetylcholine bromide solution, and the temperature of this solution was allowed to equilibrate before the nonisothermal run was begun. An initial sample was taken, and the nonisothermal run was begun by hand manipulation of the thermoregulator at an appropriate rate of temperature increase. Samples were taken for analysis at convenient times, and the temperature was continuously recorded.

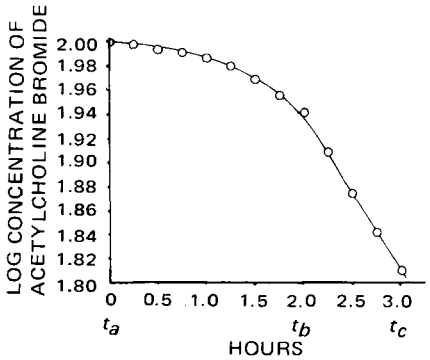

Figure 2—*Log concentration* versus *time relationship for the nonisothermal–isothermal hydrolysis of acetylcholine bromide.*

For analysis, the ampuls were quickly cooled under ice water. Five milliliters of solution was pipetted into a 250-ml erlenmeyer flask containing 50 ml of ice water, and the flask was immersed in ice water. A titration with 0.05 *N* NaOH (three titrations per sample) was performed, using phenolphthalein as the indicator. Correction was made for the initial hydrochloric acid content of the solution, with the remainder of sodium hydroxide consumed being proportional to the amount of acetic acid present. The moles (average of the three titrations) of acetic acid present were subtracted from the acetylcholine bromide initially in solution and used in the first-order calculations.

Sucrose—A 40% (w/v) sucrose solution in distilled water (400 ml) was thoroughly mixed with 0.05 *N* HCl (200 ml) in a 1-liter erlenmeyer flask. The flask was then immersed in a water bath fitted with a thermoregulator[1] and thermometer (0.1° graduations). This mixture was constantly stirred with a magnetic stirrer to prevent a temperature gradient. Sufficient time was allowed for equilibration of the temperature in the reaction solution before the nonisothermal run was begun. Following temperature equilibration, an initial sample was taken and the thermoregulator was fitted with a 2-rpm motor, which increased the temperature of the water bath by 2°/hr. The samples were removed for analysis every 15 min while the temperature was continuously recorded.

For analysis, aliquots (30 ml) were pipetted into 100-ml beakers and immediately diluted with 50 ml of 0.1 *N* NaOH. This solution was read on a polarimeter[2], using the sodium D line. Sucrose concentration is directly proportional to $(\alpha_t - \alpha_\infty)$, where α is the optical rotation. The rotation at time infinity, α_∞, is determined by heating a sample at 90° for 2–3 hr, diluting with 0.1 *N* NaOH, and then reading in the usual manner.

RESULTS AND DISCUSSION

The time-temperature relationship for the nonisothermal segment of Fig. 1 for the acid-catalyzed hydrolysis of acetylcholine bromide may be described by Eq. 9:

$$T = 298.5 + 14.743t + 33.742t^2 - 1.8099t^3 \quad \text{(Eq. 9)}$$

This equation was utilized in testing the convergence of Eq. 6 in order to determine the value of n to be used in Eq. 8. Table I shows that a value for n of 200 is adequate to describe the nonisothermal portion of the curve in Fig. 2. The criterion used to estimate convergence is a variation of less than 1% in Eq. 6 per change in n of 100. The value of n and Eq. 9 allow calculation of the coefficient terms of Eq. 8. The constant C is evaluated for the total experiment by use of the total nonisothermal concentration loss (2.90×10^{-2} hr^{-1}), the isothermal rate (1.38×10^{-1} hr^{-1}), and the value of n.

[1] A 115-v, 60-cycle thermoregulator, Bronwell Scientific Division, Will Corp., Rochester, N.Y.

[2] Model 70, O. C. Rudolph and Sons, Inc., Caldwell, N.J.

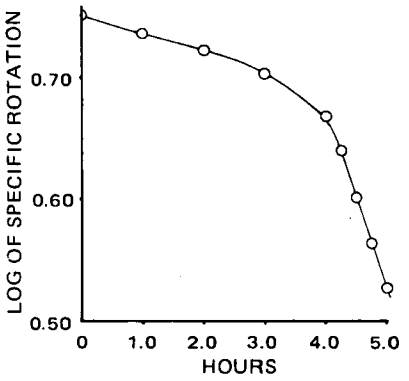

Figure 4—*Log specific rotation* versus *time relationship during a nonisothermal–isothermal acid-catalyzed inversion of sucrose.*

The coefficient terms for Eq. 8 were evaluated for hours 0–2 of the nonisothermal segment, and an equation was formed as follows:

$$157.15 - 0.03668 \times 10^{-1}X + 0.8288 \times 10^{-5}X^2 - 0.2125 \times 10^{-8}X^3 + 0.5867 \times 10^{-12}X^4 - 0.1695 \times 10^{-15}X^5 + 0.5049 \times 10^{-19}X^6 - 0.1537 \times 10^{-22}X^7 = 0 \quad \text{(Eq. 10)}$$

When each set of analytical points was considered and Eq. 8 was revised to include the time segment between these values, the mean activation energy calculated was 17.4 ± 0.9 kcal. The reported value was 17.5 kcal (10). Since the number of analytical points was nine, the number of equations and activation energies considered was $[m!/2(m-2)!]$ or 36 where $m = 9$. The plus or minus value represents 1 *SD* from the mean. For the sucrose experiment (Figs. 3 and 4), the nonisothermal time–temperature relationship is linear and may be expressed by:

$$T = 303.16 + 2t \quad \text{(Eq. 11)}$$

The convergence testing, as shown in Table II, yields a value of $n = 200$. The total loss in drug concentration (Fig. 4) during the nonisothermal portion of the experiment (1.843×10^{-2} hr^{-1}), the isothermal rate (1.539×10^{-1} hr^{-1}), and the value of n are used to calculate a value of 78.24 for C of Eq. 8 for the total experiment. When the coefficient terms of Eq. 8 are calculated, the expression for the total experiment is:

$$78.24 - 0.1278 \times 10^{-1}X + 0.1872 \times 10^{-5}X^2 - 0.2816 \times 10^{-9}X^3 + 0.4343 \times 10^{-13}X^4 - 0.684 \times 10^{-17}X^5 + 0.1097 \times 10^{-20}X^6 - 0.1788 \times 10^{-24}X^7 = 0 \quad \text{(Eq. 12)}$$

Newton's method of solution for this equation yielded a value of 29.2 kcal, which is higher than the literature values of 25.5–27.0 kcal (8). When the five analytical points were used in sets, the 10 resulting equations yielded a mean activation energy of 29.2 kcal (*SD* ± 0.9 kcal). This high value may be due to nonapplicability of the Arrhenius equation over the entire temperature span of the experiment. For each experiment, the linearity of the isothermal segment of Figs. 2 and 4 was assumed to be indicative of reaction order.

The parameters necessary for prediction of stability, that is, activation energy, an isothermal rate, and reaction order were thus obtained from a single continuous experiment. Predictive calculations can easily be made with these parameters by use of the Arrhenius equation and integration of the proper rate equation. It is not necessary to consider all of the mathematically possible sets of nonisothermal analytical values in setting up equations of the type depicted by Eqs. 10 and 12, as was done in this study. Experience in this technique does, however, suggest the use of at least five nonisothermal analytical points and 10 equations to yield 10 activation energies, which then may be considered in determining a mean activation energy.

Table I—Convergence of the Acid-Catalyzed Hydrolysis of Acetylcholine Bromide According to Eq. 6

n	E_a = 10 kcal	E_a = 20 kcal	E_a = 30 kcal
100	2.519	7.361	23.97
200	2.506	7.312	23.76

Table II—Convergence of the Acid-Catalyzed Inversion of Sucrose According to Eq. 6

n	E_a = 10 kcal	E_a = 20 kcal	E_a = 30 kcal
100	1.677	2.981	5.627
200	1.668	2.962	5.584

SUMMARY

This general method for determining activation energy, order of reaction, and rate constants might be very useful for studying reactions that occur slowly at room temperature. The method is subject to limitations imposed by assay precision, the constancy of activation energy, and the applicability of the Arrhenius equation. Equilibrium situations and kinetic salt effects may also present problems in this and other reported nonisothermal methods.

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ACKNOWLEDGMENTS AND ADDRESSES

Received January 6, 1970, from *Merrell-National Laboratories, Division of Richardson-Merrell Inc., Cincinnati, OH 45215*

Accepted for publication January 14, 1975.

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