acids, the latter being present either in food or generated in the small intestine by the action of digestive enzymes on dietary triglycerides. Such a competition, depending on the nature and relative concentration of the fatty acid, could cause a reduction in the therapeutic efficiency of cholestyramine to sequester certain bile salt anions. Studies to determine the nature and extent of any competition which may exist between bile salt anions and other physiologic substances for the binding positions on cholestyramine are in progress and will be the subject of subsequent communications.

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Flexible Nonisothermal Stability Studies

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Abstract □ A method is described which allows *ad libitum* temperature adjustment during the course of a nonisothermal kinetic study. The data obtained are compared to theoretical degradation patterns to obtain from a single experiment activation energy, reaction rates, and stability predictions at any desired temperature. The inversion of sucrose and the hydrolysis of ethyl acetate are studied to demonstrate the validity of the theory and the advantages of the method.

Keyphrases □ Stability studies—flexible, nonisothermal □ Kinetic equations—flexible nonisothermal stability studies □ Sucrose inversion—nonisothermal stability methodology □ Ethyl acetate hydrolysis—nonisothermal stability methodology □ Polarimetry—analysis

The field of nonisothermal kinetics has grown considerably in popularity since the classic treatment by Rogers (1) was published in 1963. Since then numerous publications have appeared in the literature utilizing nonisothermal techniques (2, 3). Others have introduced new ideas and techniques to the field (4, 5). The objective of this study is to eliminate the need for a fixed time-temperature profile during the course of a nonisothermal study. The advantages of such an approach lie in the freedom to change temperature at a rate consistent with analytical findings and also in minimizing experimental requirements. The method involves the subjection of a solution of the substance for study to changing temperature to provide sufficient breakdown for calculation of activation energy, reaction rates, and stability predictions. The degradation is controlled by adjusting the rate of change of temperature according to analytical findings during the experiment. The time–temperature data are fitted to a polynomial expression of sufficient degree to describe the changes. This relationship and the experimental data are then combined and utilized to synthesize a series of degradation pathways corresponding to different levels of activation energy. The curves are compared to the experimental analytical data to obtain the correct energy of activation for the reaction. Utilizing this activation energy and the analytical data, reaction rate and stability calculations can be made.

THEORETICAL

Consider a drug in solution degrading according to some unchanging reaction order as in Fig. 1. Drug concentration can then

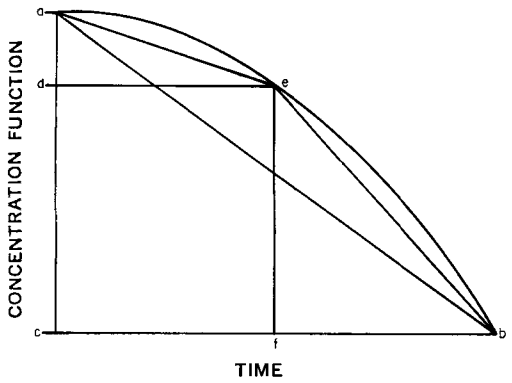

Figure 1—*Hypothetical drug degradation in solution during a flexible nonisothermal experiment.*

be considered to be a time-dependent function, where the instantaneous rate of change of drug concentration is related to the total loss in drug concentration by the following expression:

$$f(b) - f(c) = \int_c^b f'(t)dt \qquad \text{(Eq. 1)}$$

where $f'(t)$ represents drug concentration as a function of time. Dividing both sides of Eq. 1 by $(b - c)$ yields

$$\frac{f(b) - f(c)}{b - c} = \frac{\int_c^b f'(t)dt}{b - c} \qquad \text{(Eq. 2)}$$

If tc to tb is divided into two equal increments,

$$\frac{f(b) - f(c)}{b - c} = \frac{[f(b) - f(f)/(b - c)/2] + [f(f) - f(c)/(b - c)/2]}{2} \qquad \text{(Eq. 3)}$$

which is equivalent to saying that the tangent of triangle *abc* is equal to the arithmetic average of the tangents of triangles *aed* and *ebf*. If this concept is extended to include more triangles of equal base, the tangents of these triangles will approach slopes on the curve *aeb* as the number of triangles is increased. This is a somewhat intuitive way of looking at the following expression:

$$\frac{f(b) - f(c)}{b - c} = \frac{k_1 + k_2 + k_3 + \cdots k_i + \cdots k_n}{n} \qquad \text{(Eq. 4)}$$

where k_i represents the slope of the ith triangle and n equals the number of triangles being considered. Since each triangle describes some equal and individual interval Δt, the question is raised as to how large a value of n is needed for the tangent of each triangle to approach the slope on the curve *aeb* for each interval Δt.

Consider the zero-order case where

$$-k_i = \frac{C_{(i+1)} - C_i}{t_{(i+1)} - t_i} \qquad \text{(Eq. 5)}$$

C here representing concentration. If Δt is very small the slope over this interval could approximate the rate over an isothermal interval. The rate constant corresponding to k_1 of Eq. 4 may be represented by the following expression,

$$k_1 = a^* e^{-E/RT_1} \qquad \text{(Eq. 6)}$$

where E is activation energy, R is the universal gas constant, and T is temperature.

The concentration at the end of the first interval would be

$$C_1 = C_0 - a^* \Delta t e^{-E/RT_1} \qquad \text{(Eq. 7)}$$

If the temperature is changed to T_2 for the same increment of time and then to T_3 and so on, then after n increments the concentration would be

$$C_n = C_0 + \sum_{i=1}^{i=n} - \Delta t a^* e^{-E/RT_i} \qquad \text{(Eq. 8)}$$

Therefore,

$$\frac{C_n - C_0}{n\Delta t} = \frac{a^*}{n} \sum_{i=1}^{i=n} -e^{-E/RT_i} = \bar{k}_n \qquad \text{(Eq. 9)}$$

where k_n represents the average rate constant for n discrete values. For the time interval t_c to t_b of Fig. 1, temperature will most likely be related to time in a manner which can be described mathematically. The assumption is thus made that

$$T_i = G(t_i) \qquad \text{(Eq. 10)}$$

where the relationship can be obtained perhaps by some polynomial-fitting technique. Consider the function

$$-\left(\frac{a^*}{t_b - t_c}\right) e^{-E/RG(t_i)} \qquad \text{(Eq. 11)}$$

An upper Rieman sum for the interval t_c to t_b would be

$$\sum_{i=1}^{n} - \frac{(t_b - t_c)}{n} \left(\frac{a^*}{t_b - t_c}\right) e^{-E/RG(t_i)} = \frac{a^*}{n} \sum_{i=1}^{n} -e^{-E/RG(t_i)} \qquad \text{(Eq. 12)}$$

As the value of n approaches infinity, this sum will approach

$$-\frac{1}{t_b - t_c} \int_b^c a^* e^{-E/RG(t)}\, dt = \bar{k} \qquad \text{(Eq. 13)}$$

which implies through Eqs. 2 and 4 that for very large n a discrete curve over the interval t_c to t_b should approach the smooth curve, and the average value of k should not differ significantly from the quantity

$$\frac{f(b) - f(c)}{b - c}$$

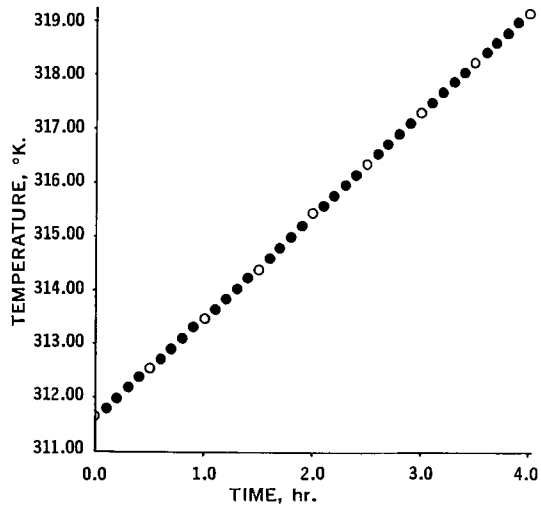

Figure 2—*Time–temperature relationship for nonisothermal acid-catalyzed inversion of sucrose (a). Key: ●, mathematical fit; and ○, experimental points.*

Table I—Convergence for the Acid-Catalyzed Inversion of Sucrose (Experiment a) for the Expression

$$\left\{1 + \sum_{i=2}^{n} \exp E/R[(T_i - T_1)/(T_iT_1)]\right\}\Big/n$$

Value of n	Value of Expression for $E = 10$ kcal.	$E = 15$ kcal.	$E = 20$ kcal.	$E = 25$ kcal.	$E = 30$ kcal.
100	1.228	1.360	1.512	1.684	1.883
200	1.222	1.355	1.504	1.676	1.874

In order to utilize Eq. 4, the magnitude of n needed for the equation to be of use must be determined. If all rates in Eq. 4 are expressed in terms of one rate (k_1), then

$$\frac{f(b) - f(c)}{b - c} = \{k_1 + k_1 \exp E/R\,[(T_2 - T_1)/(T_1T_2)] + \cdots \times k_1 \exp E/R[(T_i - T_1)/(T_iT_1)] + \cdots k_1 \exp E/R[(T_n - T_1)/(T_nT_1)]\}/n \quad \text{(Eq. 14)}$$

which may be expressed as

$$\frac{f(b) - f(c)}{b - c} = k_1\frac{\left\{1 + \sum_{i=2}^{n} \exp E/R[(T_i - T_1)/(T_iT_1)]\right\}}{n} \quad \text{(Eq. 15)}$$

The convergence of the expression

$$\left\{1 + \sum_{i=2}^{n} \exp E/R[(T_i - T_1)/(T_iT_1)]\right\}\Big/n$$

for various values of E at increasing levels of n can be used to find a value for n. Upon obtaining n, Eq. 15 can be used to calculate k_1 for a particular activation energy. The remaining rates necessary to synthesize a model degradation pathway for this energy of activation are calculated through the Arrhenius equation. The calculations are repeated for a series of activation energy values to obtain a family of degradation pathways. The experimental degradation pathway is then compared to the theoretical pathways to obtain the energy of activation for the reaction. Rates can be calculated using Eq. 15 and the Arrhenius equation. Stability predictions can then be made by integration of the proper rate equations. The arguments presented in the theory are applicable to reaction orders other than zero and can be realized through the same type of reasoning.

EXPERIMENTAL

Sucrose—A 40% w/v sucrose solution in distilled water was prepared and 400 ml. of this solution thoroughly mixed with 200 ml. 0.05 *N* HCl. Aliquots (30 ml.) of this stock solution were pipeted into 50-ml. ampuls which were flame sealed and immersed in a water bath. The water bath was fitted with a thermoregulator[1] and thermometer (0.1° graduations). The thermometer was immersed in an unsealed ampul containing the reaction mixture for determination of the temperature inside the reaction solution. 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 water bath temperature was increased by manual manipulation of the thermoregulator at an appropriate rate, with samples being removed for analysis at convenient intervals and the temperature continuously recorded.

For analysis the ampuls were cooled and opened, and the solution was diluted with 50 ml. 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 reading in the usual manner.

[1] 115-v., 60-cycle thermoregulator, Brownell Sci. Div., Will Corp., Rochester, N. Y.

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

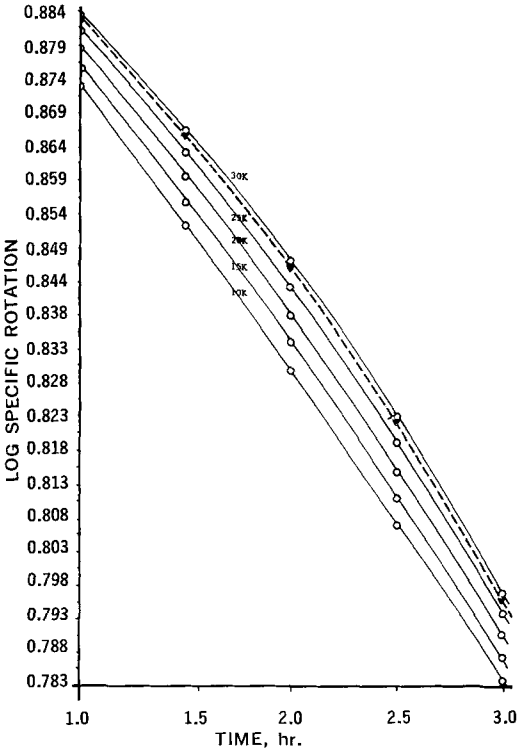

Figure 3—*Model degradation curves for nonisothermal acid-catalyzed inversion of sucrose (a). Key: - - -, experimental data.*

Ethyl Acetate—A solution of 25 ml. (22.45 g., 0.255 mole) of ethyl acetate in 500 ml. 0.1 *N* HCl was prepared (0.5096 mole/l.) and 25-ml. aliquots pipeted into 50-ml. ampuls which were heat sealed. These ampuls were immersed in a water bath fitted with a thermoregulator and thermometer (0.1° graduations). The thermometer was immersed in one of the unsealed ampuls containing the ethyl acetate solution, and the temperature of this solution was allowed to become constant. 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 temperatures continuously recorded.

The ampuls were quickly cooled under ice water. Five milliliters of solution was pipeted into a 250-ml. conical flask containing 50 ml. ice water and the flask immersed in ice water. A titration with 0.05 *N* NaOH was then performed using phenolphthalein as the indicator. Correction was made for the initial HCl content of the solution, with the remainder of NaOH consumed being proportional to the amount

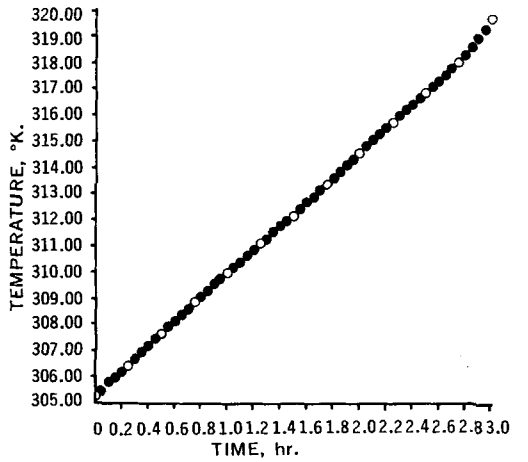

Figure 4—*Time–temperature relationship for nonisothermal acid-catalyzed hydrolysis of ethyl acetate. Key: ●, mathematical fit; and O, experimental points.*

Table II—Convergence for the Acid-Catalyzed Hydrolysis of Ethyl Acetate for the Expression

$$\left\{1 + \sum_{i=2}^{n} \exp E/R[(T_i - T_1)/(T_iT_1)]\right\}\Big/n$$

Value of n	Value of Expression for $E = 10$ kcal.	$E = 15$ kcal.	$E = 20$ kcal.	$E = 25$ kcal.	$E = 30$ kcal.
100	1.488	1.836	2.288	2.881	3.661
200	1.481	1.826	2.275	2.863	3.635

Table III—Convergence for the Acid-Catalyzed Inversion of Sucrose (Experiment b) for the Expression

$$\left\{1 + \sum_{i=2}^{n} \exp E/R[(T_i - T_1)/(T_iT_1)]\right\}\Big/n$$

Value of n	Value of the Expression for $E = 10$ kcal.	$E = 15$ kcal.	$E = 20$ kcal.	$E = 25$ kcal.	$E = 30$ kcal.
100	1.454	1.755	2.126	2.587	3.158
200	1.448	1.749	2.119	2.579	3.150

of acetic acid present. The average value of three titrations per sample was taken and the moles of acetic acid present calculated and subtracted from the ethyl acetate initially in solution for use in the first-order calculations.

RESULTS AND DISCUSSION

The time–temperature relationship for the acid-catalyzed inversion of sucrose is illustrated in Fig. 2. This relationship is described by the following polynomial expression,

$$\text{temp.} = 311.66 + 1.7482t + 0.086257t^2 - 0.013662t^3$$

When the expression was utilized to calculate the number of rates necessary to describe a degradation curve adequately for the experiment, 200 rates were found necessary. The criterion used to determine convergence was a change of less than 1% with an increase in n of 100 in the value of that portion of Eq. 15 used to test convergence. The convergence for this experiment is shown in Table I.

The model degradation pathways for the five activation energies considered in Table I were calculated and are represented in Fig. 3. Hours 1–3 of the experiment are represented. The model curves tend to lie very close to each other for the early and final stages of the experiment and do not contribute to an accurate activation energy determination. The experimental points are averages of results from six separate samples and yield an activation energy of about 28 kcal. This is slightly higher than the reported values of 25–27 kcal. (6).

The time–temperature relationship for the acid-catalyzed hydrolysis of ethyl acetate is shown in Fig. 4. The relationship can be described by the following polynomial expression,

$$\text{temp.} = 305.26 + 4.4450t + 1.6114t^2 - 2.2298t^3 + 0.98356t^4 - 0.070209t^5 + 0.011573t^6 - 0.034019t^7 + 0.0076531t^8$$

The convergence testing for this experiment is shown in Table II and resulted in a value of 200 rates to describe adequately the model degradation curves.

The model curves for this experiment are shown in Fig. 5 for hours 0.45–2.55 of the 3-hr. experiment. Segments of the model curves were eliminated for the reasons given for the sucrose experiment. Since the analytical points in this experiment were scattered, they were averaged and fitted to a second-degree polynomial to yield the smooth experimental curve shown in Fig. 5. The activation energy is approximately 17 kcal., which is in good agreement with the values of 16.5–17.3 kcal. reported in the literature (7).

The time–temperature profile of a second sucrose inversion experiment is given in Fig. 6. This experiment was essentially a test of the limits of flexibility of this method. A fair mathematical fit to this time–temperature relationship is given by the following equation,

$$\text{temp.} = 311.23 + 54.445t - 20.70t^2 - 350.95t^3 + 406.57t^4 + 187.83t^5 + 50.62t^6 - 899.25t^7 + 776.93t^8 - 193.08t^9$$

The convergence testing for this experiment showed a requirement of 200 rates to describe degradation. The results are given in Table III.

The rapid convergence is probably due to the brevity of the experiment. The model curves for the experiment are shown in Fig. 7. The experimental curve is not as smooth as in the first sucrose experiment, but the activation energy of about 27 kcal. is in good agreement with the sucrose experiment The model curves for this experiment are drawn for hours 0.45–0.9 of the experiment. The tendency of the curves to group very closely together at the beginning and second half of the experiment was the reason for their elimination. In the three experiments the assumption of first order was made. Reaction orders other than first are handled by choosing the proper concen-

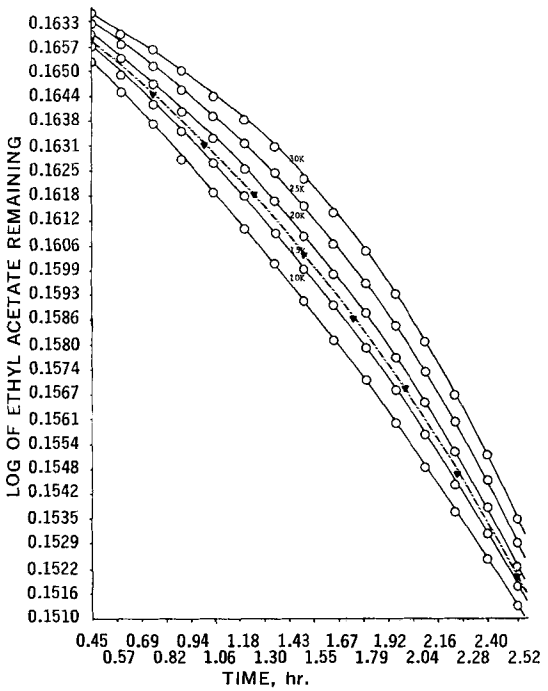

Figure 5—*Model degradation curves for nonisothermal acid-catalyzed hydrolysis of ethyl acetate. Key:* ▼, *experimental curve; and* ○, *model curves.*

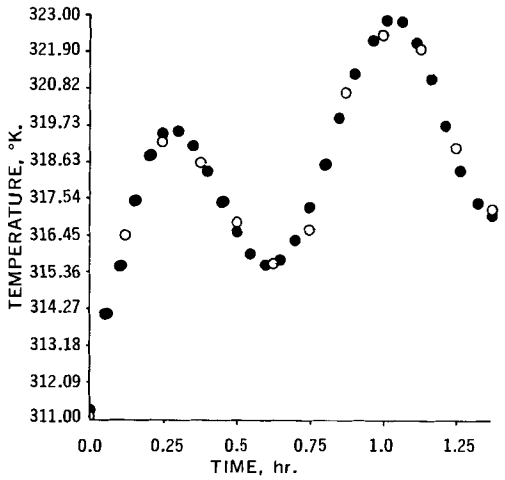

Figure 6—*Time–temperature relationship for nonisothermal acid-catalyzed inversion of sucrose (b). Key:* ●, *mathematical fit; and* ○, *experimental points.*

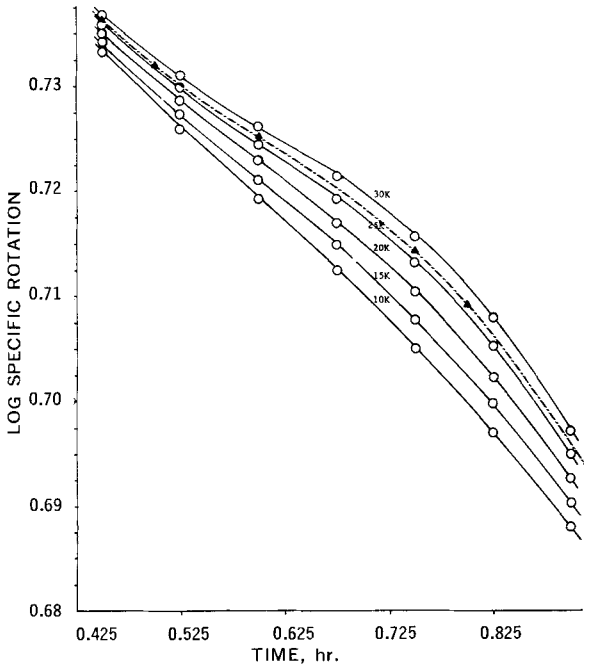

Figure 7—*Model degradation curves for nonisothermal acid-catalyzed inversion of sucrose (b). Key: ▼, experimental curve; and O—, model curves.*

tration function for calculation of model degradation pathways. The calculations in the study were performed utilizing a quiktran terminal.[3]

SUMMARY

A method which eliminates the disadvantages of fixed time–temperature relationships in nonisothermal kinetic studies has been developed. The investigator utilizing this method may change temperature during the experiment to fit analytical findings. The data obtained from the single experiment allow calculation of activation energy, reaction rate, and shelf-life prediction. To obtain this information a series of theoretical degradation pathways are synthesized by utilizing the experimental time-temperature relationship and initial and final analytical points. The concentration–time plot for degradation is then compared to the model curves to obtain the energy of activation for the reaction. Calculations using analytical data and the activation energy are then used to determine reaction rates and stability predictions at desired temperatures. In addition to the flexibility of temperature adjustment introduced, the advantages of the method lie in the use of a single experimental unit, the analysis of one set of samples, the shorter time required for completion of the experiment, and the use of readily available laboratory equipment. Disadvantages (8) lie in the need for a separate experiment to determine the order of the reaction, the nonapplicability of the technique in situations where equations cannot be made linear (as in equilibria), the need to compensate for ionic strength effect with change in temperature, and the sometimes difficult task of fitting an equation to the experimental time–temperature data. The approach is also limited by assay precision, constancy of activation energy, and applicability of the Arrhenius equation.

[3] The quiktran program used for the entire treatment will be provided to interested parties upon request.

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