RESEARCH ARTICLES

Estimation of All Parameters from Nonisothermal Kinetic Data

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Abstract □ A method is described which estimates (using least-squares analysis) all parameters (kinetic parameters and also zero and infinite time assays) from nonisothermal kinetic data. This method overcomes the time delays required for infinite time assays and biased estimates caused by the use of those assay results. Flexible temperature and data collection programs can be used. The mathematical models account for thermal volume expansion and the appropriate model is selected by statistical tests (provided the reaction is studied over ~90% decomposition). The models use a reparameterized Arrhenius equation in which the frequency factor is replaced by k_a, the rate constant at a specified temperature. This improves the numerical procedure and allows room temperature stability to be estimated directly.

Keyphrases □ Kinetics—estimation of all parameters from nonisothermal data □ Models, mathematical—estimation of all parameters from nonisothermal kinetic data □ Drug stability—estimation from nonisothermal kinetic data

Nonisothermal kinetic methods enable drug stability to be estimated at any temperature from a single test rather than multiple experiments as required by the traditional isothermal approach.

A logarithmic nonisothermal method has been proposed (1) as well as reciprocal (2), linear (3), polynomial (4), and stepped (5) temperature programs. Most methods have used manual and/or approximate procedures to estimate the kinetic parameters. One method (6) employed numerical integration (trapezoidal method) within a nonlinear, least-squares regression; however, this is only applicable where dependent (concentration) and independent (time) variables can be separated in the model.

All methods thus far have treated time zero and time infinity concentrations as known. When these parameters are not known to a precision greatly exceeding that of the data, then they should be estimated from the data (7). This also avoids the lengthy time delays necessary to determine values at infinity. Treating them as constants may lead to

biased kinetic parameter estimates as in isothermal experiments (8) and confidence limits might be falsely reduced (9).

In addition to these limitations of current methods, the effect of thermal volume expansion has not been considered.

The present report develops a nonrestrictive, numerical treatment which allows flexible temperature and data collection programs, estimates of all parameters, and accounts for thermal volume expansion in nonisothermal solution kinetics.

THEORY

The general form of the rate equation for a nonisochoric system (10) is:

$$-dM_1/dt = (1/V')^{v-1} k \prod_{i=1}^{i=j} M_i^{v_i} \qquad \text{(Eq. 1)}$$

where v_i is the exponent of the ith reactant, v is the reaction order (= $v_1 + v_2 + \ldots + v_j$), M_i is the number of moles of the ith reactant, V' is the solution volume which is a function of time, and k is the rate constant.

For a nonisothermal, nonisochoric system:

$$-dM_1/dt = (1/V')^{v-1} A e^{-E/(RT)} \prod_{i=1}^{=j} M_i^{v_i} \qquad \text{(Eq. 2)}$$

where A is the frequency factor, E is the apparent activation energy, R is the gas constant, and T is the absolute temperature.

From Eq. 2 the following apply:

$$-dM_1/dt = A e^{-E/(RT)} V' \qquad \text{zero-order} \qquad \text{(Eq. 3)}$$

$$-dM_1/dt = A e^{-E/(RT)} M_1 \qquad \text{first-order} \qquad \text{(Eq. 4)}$$

$$-dM_1/dt = A e^{-E/(RT)} M_1 M_2/V' \qquad \text{second-order} \qquad \text{(Eq. 5)}$$

It has been pointed out for isothermal kinetics (11) that if a reactant solution is prepared at room temperature and used at a different temperature, allowance should be made for thermal volume change. It is clear from Eq. 4 that first-order reactions are exempt from this requirement,

	Initial Parameter Estimates[b]		Iterations Required	
k_a, min^{-1}	E, kcal mole^{-1}	$10^{-10} A$, min^{-1}	k_a/E	A/E
0.20	14.0	0.37	5	>20
0.20	16.0	10.70	6	>20
0.10	14.9	0.84	2	5
0.33	14.0	0.60	8	9
0.06	16.0	3.20	5	>15
0.06	14.0	0.11	5	>20
0.12	14.9	1.01	4	9

[a] Parametric values are $E = 15.0$ kcal/mole, $A = 1.0 \times 10^{10}$ min^{-1}, $k_a = 0.10096$ min^{-1}, where k_a is the rate constant at 25° and the linear temperature program range is 25–45°. [b] For each test, the two models have the same initial sum of squares.

since a volume term does not appear in the equation; however, pseudo first-order reactions for which Eq. 5, with M_2 constant, is applicable, are not exempt.

For nonisothermal experiments, the change in molar concentration, C', $(C' = M/V')$ of a reactant is due to reaction and thermal volume change. For the case where all samples are cooled to the same temperature (T_s) before analysis, the measured concentration (C) is independent of thermal volume change, and $C = M/V$ where V is the volume at T_s.

Since temperature and volume are functionally related to time $[T = f_1(t); V' = V/f_2(t)]$, Eqs. 3–5 can be rewritten as:

$$-dC_1/dt = Ae^{-E/[Rf_1(t)]}/f_2(t) \quad \text{zero-order} \quad \text{(Eq. 6)}$$

$$-dC_1/dt = Ae^{-E/[Rf_1(t)]}C_1 \quad \text{first-order} \quad \text{(Eq. 7)}$$

$$-dC_1/dt = Ae^{-E/[Rf_1(t)]}C_1C_2f_2(t) \quad \text{second-order} \quad \text{(Eq. 8)}$$

where C_2 is the concentration of Reactant 2 at volume V or the constant catalyst concentration at V in the pseudo first-order case.

Model for Absorbance Measurement—If change in absorbance (D) is used to follow the reaction and the following assumptions are made: (a) the reaction goes to completion; (b) the absorbance is the sum of component absorbances due to reactant, product, and constant background; (c) all component absorbances obey the Beer-Lambert law; and (d) the sum of the moles of reactant and moles of product is constant, then:

$$C_1 = (D - D_\infty)/[(a_r - a_p)b] \quad \text{(Eq. 9)}$$

$$C_{10} = (D_0 - D_\infty)/[(a_r - a_p)b] \quad \text{(Eq. 10)}$$

where D_0 and D_∞ are the absorbances at zero and infinite times, respectively, and are constant, a_r and a_p are the absorptivities of reactant and product, respectively, b the cell path length, and C_{10} is the concentration of reactant 1 at time zero and T_s.

Recalling that for pseudo first-order reactions, Eq. 8 with C_2 constant is applicable, then substituting Eq. 9 into Eqs. 6–8 yields:

$$-dD/dt = Ae^{-E/[Rf_1(t)]} (a_r - a_p) b/f_2(t) \quad \text{zero-order} \quad \text{(Eq. 11)}$$

$$-dD/dt = Ae^{-E/[Rf_1(t)]} (D - D_\infty) \quad \text{first-order} \quad \text{(Eq. 12)}$$

$$-dD/dt = Ae^{-E/[Rf_1(t)]} (D - D_\infty) C_2 f_2(t) \quad \text{pseudo first-order} \quad \text{(Eq. 13)}$$

$$-dD/dt = Ae^{-E/[Rf_1(t)]} (D - D_\infty)^2 f_2(t)/[(a_r - a_p) b] \quad \text{second-order} \quad \text{(Eq. 14)}$$

Similar equations can be derived for other situations where the sum of moles of reactant and moles of product is not constant.

Equations 11–14 cannot be integrated analytically. However, they can be solved numerically (i.e., D values calculated at specified times) when initial values are supplied (i.e., $D = D_0$ at $t = 0$).

For isothermal kinetics where $f_1(t)$ is constant, these equations can be integrated to give the familiar forms. For example, Eq. 12 yields:

$$D = D_\infty + (D_0 - D_\infty)e^{-kt} \quad \text{(Eq. 15)}$$

The parameters (D_0, D_∞, A, and E for nonisothermal; D_0, D_∞, and k for isothermal) are chosen so as to minimize Eq. 16:

$$SS = \sum_{i=1}^{i=N} (D_i - \hat{D}_i)^2 \quad \text{(Eq. 16)}$$

Percent Reacted	Model			
	First-Order	Pseudo First-Order	Second-Order	Zero-Order
	k_a			
18	0.0537	0.0449	0.0063	0.1009
54	0.0992	0.0988	0.0469	0.1111
71	0.1002	0.0999	0.0605	0.1171
87	0.1004	0.1001	0.0735	0.1258
99	0.1002	0.1000	0.0866	0.1397
	E			
18	13.72	13.62	13.69	12.66
54	14.71	14.76	16.26	7.57
71	14.81	14.87	18.69	3.19
87	14.89	14.94	23.14	−2.91
99	14.97	15.02	41.32	−22.69
	D_0			
18	1.001	1.001	1.001	1.001
54	1.001	1.001	1.001	1.006
71	1.001	1.001	1.000	1.012
87	1.001	1.001	0.998	1.023
99	1.001	1.001	0.988	1.057
	D_∞			
18	−0.839	−1.198	−2.950	—[b]
54	0.031	0.030	−0.421	—
71	0.045	0.044	−0.240	—
87	0.049	0.049	−0.108	—
99	0.050	0.050	0.020	—

[a] Parameters and constants used to generate the first-order data were $k_a = 0.1$ hr^{-1}, $E = 15$ kcal/mole, $D_0 = 1.0$, $D_\infty = 0.05$ with a linear temperature program over the range 25–45° in all cases. Also $a_r = 1$, $a_p = 0$, $b = 1$, with the random error variance of 0.56×10^{-6}. [b] The zero-order model has no D_∞ term.

where D_i and $\hat{D}_i$ are observed and calculated absorbances, respectively, at the ith datum, and N is the number of data.

Computational Procedures—*Temperature–Time Relationship* $f_1(t)$—For each data set, an orthogonal polynomial regression program, ORTHO (12), was used to establish the polynomial of lowest order which adequately described the temperature–time data. This enables flexible temperature programs to be employed. ORTHO uses the Gram–Schmidt orthonormalization procedure with an iterative straightening refinement (13).

Volume–Time Relationship $f_2(t)$—Data for the specific volume (V'_{sp}) of water at various temperatures are available (14). ORTHO was used to establish a polynomial relationship for this data over the experimental temperature range (30–92°),

$$V'_{sp} = 0.99992806 - 0.35791641 \times 10^{-4} T \quad \text{(Eq. 17)}$$
$$+ 0.72094751 \times 10^{-5} T^2 - 0.42243321 \times 10^{-7} T^3$$
$$+ 0.21949816 \times 10^{-9} T^4 - 0.47002556 \times 10^{-12} T^5$$

where T is degrees centigrade for this equation only. All residuals about this regression were less than 7×10^{-7} ml/g and were randomly distributed.

Since the relationship $f_1(t)$ was established for each data set, and the general relationship $V'_{sp} = f(T)$ (Eq. 17) is available, the volume–time relationship $f_2(t)$ was established for each set.

Relationships $T = f_1(t)$ and $V'_{sp} = f(T)$ were used in the nonlinear regression program, MARQDT.

Optimization Routine—The function to be minimized (Eq. 16) contains nonlinear parameters so an iterative least-squares regression is required. A nonlinear regression program (MARQDT) was developed for this purpose. The program uses the Marquardt algorithm (15) with minor modifications (12). The advantages of this method have been described previously (16).

Initial estimates are required for the parameters. When these are not available from prior knowledge, satisfactory values can be obtained by one of the differential nonisothermal methods (17), provided thermal volume effects are neglected. These estimates are improved iteratively until the convergence test (the relative change in parametic estimates in consecutive iterations is less than 1 in 10^4) is satisfied.

Numerical Differentiation—The Marquardt algorithm requires partial derivatives with respect to each parameter at each datum. Since integrated functional equations are not available for nonisothermal kinetics, the partial derivatives were estimated numerically. These were computed using the two-point central difference method. Choice of the step length has been discussed previously (18).

Table III—Goodness of Fit Statistics for Various Models Fitted to Nonisothermal First-Order Simulated Data [a]

Percent Reacted	Model			
	First-Order	Pseudo First-Order	Second-Order	Zero-Order
		$10^6 V_r$ [b]		
18	0.56	0.56	0.56	0.55
54	0.56	0.56	0.59	5.5
71	0.56	0.56	0.72	21
87	0.56	0.56	1.6	73
99	0.56	0.56	13	279
		Probability [c]		
18	NS	NS	NS	NS
54	NS	NS	NS	S
71	NS	NS	NS	S
87	NS	NS	S	S
99	NS	NS	S	S

[a] See Table II. [b] V_r is the residual variance (i.e., residual SS/df) and should be compared with the error variance of 0.56×10^{-6}. [c] Probabilities (NS = not significant at $p = 0.01$ level) for runs, mean square successive difference, and F tests were similar.

Numerical Integration—A fourth-order Runge–Kutta method with error estimation and step size control was used in MARQDT for the solution of Eqs. 11–14 (19). The method has been tested extensively on a number of derivative functions and found to perform excellently provided relative rather than absolute truncation errors are used to control step size.

In this work, a relative truncation error of 10^{-5} was used for sum of squares (SS) calculations, and 10^{-4} for partial derivative evaluations. This error in $\hat{D}_i$ for SS calculations was about one-fiftieth of the random error in the data and so did not contribute significantly to the calculated SS value. Further decrease in the truncation error did not lead to significant changes in the parameter estimates but only increased computational time.

Initial values are required for a particular solution of a differential equation. In the present work these were $D = D_0$ at $t = 0$ with D_0 being treated as a parameter.

Statistical Aspects—*Goodness of Fit*—The adequacy of the kinetic model was judged in two ways. First, the residuals were examined for nonrandomness or trends. If the model is adequate or the fit is good, the residuals should be randomly distributed in time. They were examined visually and by the runs (20) and mean square successive difference (21) statistical tests. Second, the residual variance about the regression was compared with the error variance of the experimental system $(F\text{-test})$ a knowledge of which was available from prior testing. However, in nonlinear regressions the residual variance is not an unbiased estimate of the pure error even when the model is correct, so this F-test is only approximate (16).

Confidence Limits—In nonlinear regressions, exact confidence limits for the parameters are not available; however, the linear estimates calculated from the variance–covariance matrix and residual variance are often adequate approximations (16). A Monte Carlo method has been described (22) to test the accuracy of these linear approximations. This test involves the following steps:

1. Estimate best-fit parameters and their approximate confidence limits;
2. Generate data using the best-fit estimates and add normally distributed random error with a variance equal to that of the original data set;
3. Estimate best-fit parameters for the data set;
4. Repeat steps 2 and 3 a number of times (15 has been used) and determine the confidence limits for the distribution of best-fit estimates.

This analysis need not be carried out every time a particular model is run, but should be used to check the linear approximations at least once for each model.

EXPERIMENTAL

Materials—p-Nitrophenyl acetate (I) was prepared by acetylation of p-nitrophenol (23) and recrystallized from fractionated benzene and petroleum ether (40–60° fraction) mixed solvent to a constant melting point (77.6°). p-Nitrophenol was recrystallized to constant melting point (115°) from fractionated benzene, and both materials were stored in

Table IV—Parameter Estimates and Goodness of Fit Statistics for Various Models Fitted to Nonisothermal Second-Order Data [a]

Model	k_a	E	D_0	D_∞	Probability [b]
Second	0.1004	15.09	1.001	0.0512	NS
First	0.0739	−15.71	0.989	−0.1289	S
Pseudo first	0.0737	−15.64	0.989	−0.1287	S
Zero	0.0711	−30.57	0.968	—	S

[a] See Table II for parameter and constant values used to generate data over 90% reacted. [b] $p = 0.001$, S = significant, NS = not significant.

vacuum desiccators in the dark until required for use. Thermal analysis indicated these samples to be greater than 99.9% pure.

Concentrated hydrochloric acid[1] was diluted with glass distilled water to produce sufficient dilute acid for all experiments. This acid was standardized against freshly dried sodium carbonate (analytical reagent grade) using methyl orange indigo carmine indicator (24). Replicate analyses $(n = 4)$ indicated the acid to be $0.2396 M$ (SE mean $= \pm 0.0003 M$).

Equipment—A 1-liter, closed, stirred, glass reaction vessel was immersed in a 60 liter polyethylene glycol 600 bath. In isothermal experiments the temperature was controlled by a circulating heater[2], while a variable speed programmer[3] was used for nonisothermal tests.

Temperatures inside the reaction vessel were monitored with a four-lead platinum resistance thermometer[4].

Samples were withdrawn automatically *via* polytef tubing, cooled to 32° in a jacketed coil, then passed into a 2-mm quartz flow-cell for spectrophotometric[5] analysis.

Analysis—The rate of hydrolysis was followed spectrophotometrically at 318 nm (2). Spectra recorded during the reaction showed a single iso-
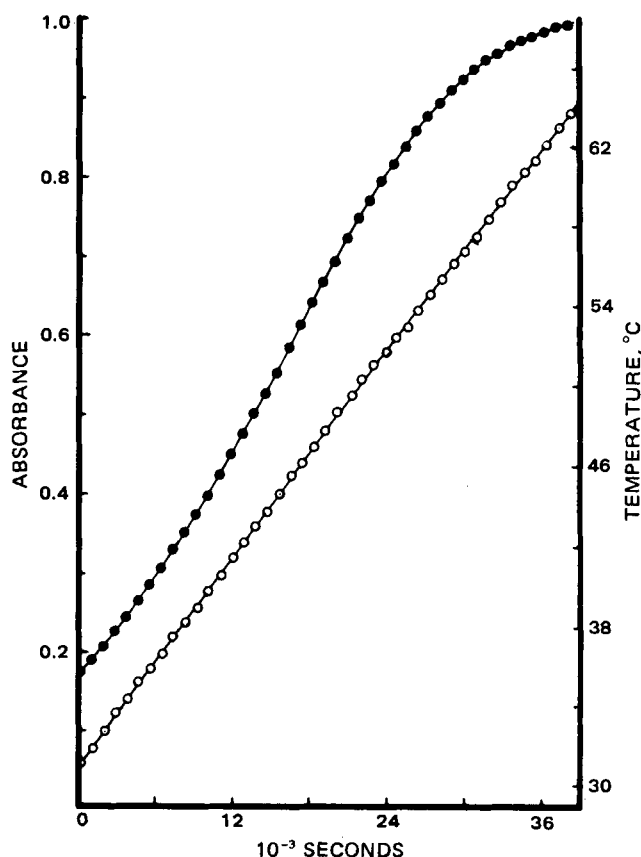

Figure 1—*Typical temperature program* (O) *and reaction data* (●) *with fitted curves for the nonisothermal acid catalysed* (0.2396 M HCl) *hydrolysis of I. The temperature time relationship determined by ORTHO was* T(K) 303.911 + 0.904339 × 10^{-3} t − 0.105973 × 10^{-8} t^2.

[1] Baker Analyzed Reagent, Baker Chemical Co.
[2] Model ED Unitherm, Gebruder Haake.
[3] Gebruder Haake Temperature Programmer, model PG12.
[4] Degussa element, type P4.
[5] Perkin-Elmer model 124 double-beam spectrophotometer.

Table V—Parameter Estimates and Goodness of Fit Statistics for Various Models Fitted to Nonisothermal Zero-Order Data [a]

Model	k_a	E	D_0	D_∞	Probability[b]
Zero	0.1004	14.95	1.000	—	NS
First	0.0019	15.19	1.000	−50.32	NS
Pseudo first	0.0018	15.25	1.000	−53.40	NS
Second	1×10^{-5}	15.16	1.000	−252.0	NS

[a] See Table II for parameter and constant values. [b] $p = 0.001$, NS = not significant.

bestic point (292 nm) indicating a one-to-one reaction and stability of the absorbing product, p-nitrophenol. The final spectrum was identical with that of p-nitrophenol.

A Beer–Lambert calibration, using replicate samples, confirmed linear concentration–absorbance relationships for I and p-nitrophenol, and that the component absorbances were additive. Since it was shown that spectra in acid and water were identical, this calibration was performed in distilled water, thereby minimizing decomposition problems.

Procedure—One liter of acid was allowed to equilibrate thermally in the closed reaction vessel. About 80 mg of I dissolved in 2 ml of fractionated ethanol was added to produce an ~0.0004 M solution which was bubbled with high purity nitrogen. The temperature programmer was started when I was added and the first sample withdrawn 2 min later. Samples were withdrawn automatically, cooled to 32°, and assayed directly, the concentrations used being such that the infinite time absorbance was ~1. At each sampling, reaction vessel temperature, sample absorbance, and time data were automatically recorded.

Design—About 30 data were collected during each experimental run. Triplicate isothermal tests were performed at 39.26, 49.03, 58.68, and 68.24°. Triplicate nonisothermal tests at each of four different approximately linear heating rates (~3, 6, 12, and 18°/hr) were used. These all started at about 30° and continued until decomposition exceeded 90% giving a maximum temperature of about 76° (for 18°/hr).

RESULTS AND DISCUSSION

Reparameterization of the Model—For first-order nonisothermal kinetics with a linear temperature program, exact simulated data were generated using parameter values of $A = 1 \times 10^{10}$ min^{-1}, $E = 15$ kcal/mole, $D_0 = 1$, $D_\infty = 0$, and $T = 298.15 + t$. D_0 and D_∞ were then held constant at their parametric values, while A and E were estimated. Convergence of the program was very slow, even for initial estimates close to the true values.

Sum of squares contours for the data in question were generated and found to be very elongated in the direction of the A parameter. It has been pointed out (16) that in such cases, slow convergence of any iterative estimation procedure is likely. Clearly, A is an ill-determined parameter; that is, large changes in A cause small changes in SS. In retrospect, it is apparent why A is ill-determined. It is the rate constant at infinite temperature. Since data are usually collected in the range 25–90°, estimation of A involves an extrapolation outside the data, and such estimates are poorly determined.

Conditioning can often be improved by reparameterizing the model. In the present study, this was done by replacing A with $k_a \, e^{E/(RT_a)}$ in Eqs. 11–14, where the new parameter k_a is the rate constant at T_a, a temperature within the experimental range. Sum of squares contours for various T_a values were found to be well-rounded ellipses, i.e., the parameters were well-conditioned. Since little change occurs in the conditioning with changes in T_a, provided it is within or near the experimental range, it may be advantageous to choose $T_a = 25°$. This would allow the room tem-

Table VI—Kinetic Parameter Estimates for the Acid Catalysed Hydrolysis of I [a]

Source	$E \pm SE$, kcal/mole	$10^5 k_a \pm SE$, sec^{-1}
Nonisothermal[b]	16.98 ± 0.03	1.904 ± 0.010
Isothermal[c]	17.00 ± 0.04	1.912 ± 0.009
Reference 2[d]	21 ± 2	1.9 ± 0.4
Reference 2[e]	18	
Reference 25[f]		2.054
Reference 26[g]	17.2	

[a] $T_a = 30°$. [b] Mean of 12 experiments. [c] Least-square estimates based on 12 experiments (three replicates at four temperatures). [d] Their nonisothermal estimates, k_a being calculated from their second-order rate constant at 25° using [H$^+$] = 0.2396 M and $E = 17.0$ kcal/mole. [e] Their isothermal estimate. [f] Determined from their second-order rate constant as above. [g] For phenyl acetate.
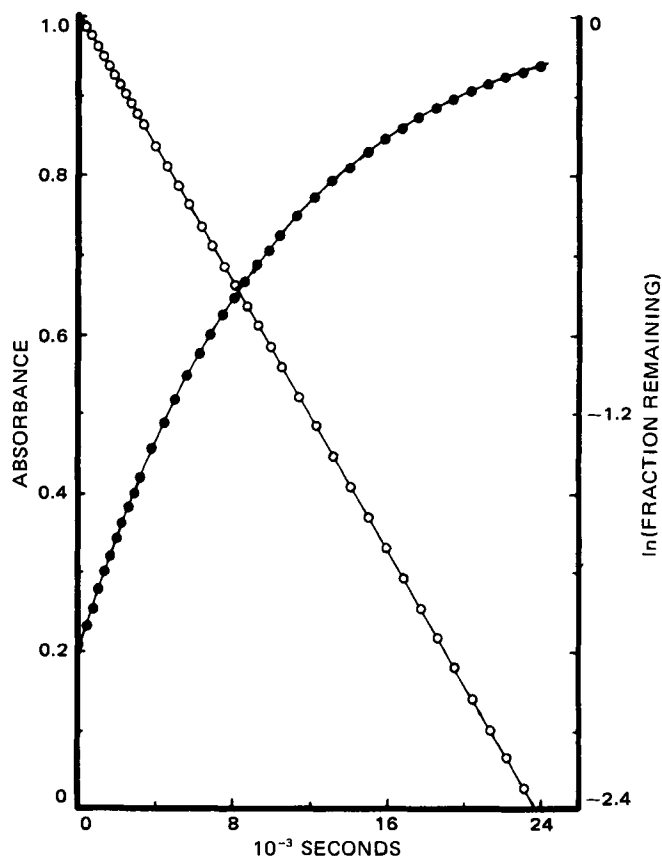

Figure 2—*Typical data for the isothermal acid catalysed (0.2396 M HCl) hydrolysis of I showing the first-order, nonlinear, least-squares curve (●) and the more usual linearized logarithmic plot (○). D_∞ from the nonlinear regression was used to calculate the fraction remaining.*

perature rate constant to be estimated directly, and approximate confidence limits would also be immediately available.

The new model was tried on the same first-order simulated data and convergence was rapid. A comparison of the new and old models for this typical data set, for differing initial estimates, is shown in Table I. In all cases the program converged to the parametric values and the new model was superior. Convergence was acceptable, since the initial estimates were far worse than normally would be expected.

It is clear that A may be of theoretical interest, but k_a is of greater interest in practical stability studies and its use facilitates the numerical procedure. In all subsequent computations, the reparameterized models were used.

Goodness of Fit—To test the ability to select the appropriate kinetic model, simulated data (40 pairs) were generated and normally distributed random error was added. The variance of the random error was that of the experimental system. The parameter estimates for zero-, first-, pseudo first-, and second-order fits to first-order data are given in Table II while the statistics for judging the goodness of fit are in Table III.

In all cases, ~95% confidence limits of the first-order estimates bracketed the parametric values. This, along with the excellent agreement between the residual variances (about the regression) and error variance (Table III), indicated the program was functioning correctly.

With the reaction 18% complete, the 95% confidence limits (−0.087–0.194) for the first-order estimate of k_a bracketed the parametric value, but the point estimate is about half the parametric value. This shows the danger of estimating rate constants from data covering a low percentage reacted, and of the need to compute confidence limits.

At high percentages reacted, the estimated E values for the zero-order model were negative. These values were expected, since the decrease in concentration causes a decrease in reaction rate for first-order kinetics; this is not accounted for in the zero-order model. Consequently, the reaction rate apparently decreased with temperature rise.

From Table III, it is clear that at the added random error level the various kinetic models could not be distinguished below 18% decomposition. At 54% reacted the zero-order model was inadequate, at 87% the second-order was inadequate, but the first- and pseudo first-order models

Table VII—Effect of Treating D_0 and/or D_∞ as Constants on the Remaining Parameter Estimates

Row	D_∞, Absorbance Units	D_0, Absorbance Units	$10^4 k_a$, sec^{-1}	±95% Confidence Unit, %	E, kcal/mole	±95% Confidence Unit, %	10^6 Residual Variation, (absorbance units)^2
1	0.9825	0.1721	0.1897	0.54	17.06	0.48	0.16
2	I − CL[a]	0.1722	0.1896	0.47	17.08	0.22	0.16
3	I + CL	0.1720	0.1899	0.47	17.04	0.22	0.16
4	0.9845	II − CL[a]	0.1912	0.38	16.96	0.43	0.24
5	0.9805	II + CL	0.1882	0.39	17.16	0.43	0.24
6	I − CL	II + CL	0.1883	0.38	17.13	0.20	0.24
7	I + CL	II + CL	0.1883	0.40	17.10	0.22	0.27
8	I − 0.01	0.1729	0.1877	0.94	17.36	0.45	0.61
9	I + 0.01	0.1713	0.1916	0.87	16.78	0.43	0.55
10	I − 0.01	II	0.1891	0.65	17.30	0.35	0.70
11	I + 0.01	II	0.1902	0.60	16.83	0.33	0.63

[a] I and II are Row 1 estimates of D_∞ and D_0. I − CL means D_∞ was treated as a constant with value I minus the 95% confidence limit of a reading (0.0008 absorbance unit).

remained indistinguishable, even at 99% decomposition. When the error variance was reduced by a factor of 10, the second-order model was then inadequate at 71% reacted, but the first- and pseudo first-order models were adequate. Attempts to separate these two models by increasing the temperature range to 60° while maintaining 90% reacted were unsuccessful.

Second-order and zero-order data were generated using parameter and constant values given previously (Table II) and covering 90% reacted with a temperature range of 20°. The parameter estimates and probabilities for second-order data are given in Table IV and for zero-order data in Table V.

At 90% decomposition and at the random error level used, the second-order equation was the only adequate model (Table IV). The other

Figure 3—*Arrhenius plot for the acid catalysed (0.2396 M HCl) hydrolysis of I, $T_a = 30°$. Points cover the range of the experimental rate constants.*

models were shown to be inadequate by the statistical tests and the meaningless E estimates.

The probabilities in Table V indicate that zero-order data can be described equally well by all models. Additionally, the kinetic parameter estimates (k_a, E) were reasonable for all models (although if prior stability information were available, the k_a estimates from the incorrect models would be suspect). However, the D_∞ estimates for the incorrect models were unacceptable, since absorbances of −50 and −252 are meaningless. Therefore, the first-, pseudo first-, and second-order models were rejected because of their D_∞ estimates and the zero-order model was accepted by default and because it was not rejected by the statistical tests.

For zero-order reactions, the rate increases continuously with time due to temperature rise and comes to an abrupt halt at zero concentration. For first-order, the rate increases initially but then decreases when the effect of lowered concentration exceeds that due to the increased k value. In second-order reactions, the decrease in rate occurs at a lower percentage reacted since rate is proportional to concentration squared. Therefore, all models appear similar before the inflection points and, consequently, zero-order reactions covering a high percentage decomposition appear like first- and second-order reactions covering a small percentage reacted. From Table V, the 90% reacted zero-order model was adequately fitted by first- and pseudo first-order models covering ~2% ($D_\infty \sim -50$) or a second-order model with ~0.5% decomposition ($D_\infty = -252$).

Data from the nonisothermal hydrolysis of I, over ~90% decomposition, were treated similarly. Graphs for a typical temperature program and reaction together with the ORTHO and MARQDT (pseudo first-order) fits are shown in Fig. 1. In all cases, zero- and second-order models were inadequate but the first- and pseudo first-order nonisothermal models were adequate. Pseudo first-order rate constants were estimated from isothermal data using nonlinear least-squares regression (Eq. 15). Plots of a typical data set and the fitted curve are shown in Fig. 2 along with the usual linearized graph. The rate constants were corrected for thermal volume expansion and then k_a and E were estimated by linear least-squares regression using the logarithmic form of the reparameterized Arrhenius equation (Fig. 3). Nonisothermal parameter estimates were in good agreement with isothermally determined values. These estimates are shown in Table VI with literature values.

Confidence Limits—In all cases tested, the approximate confidence limits did not greatly exceed the Monte Carlo limits (usually only by a factor of <1.5). This indicates that the approximate error estimates cause less confident parameter estimates than is justified. However, in a
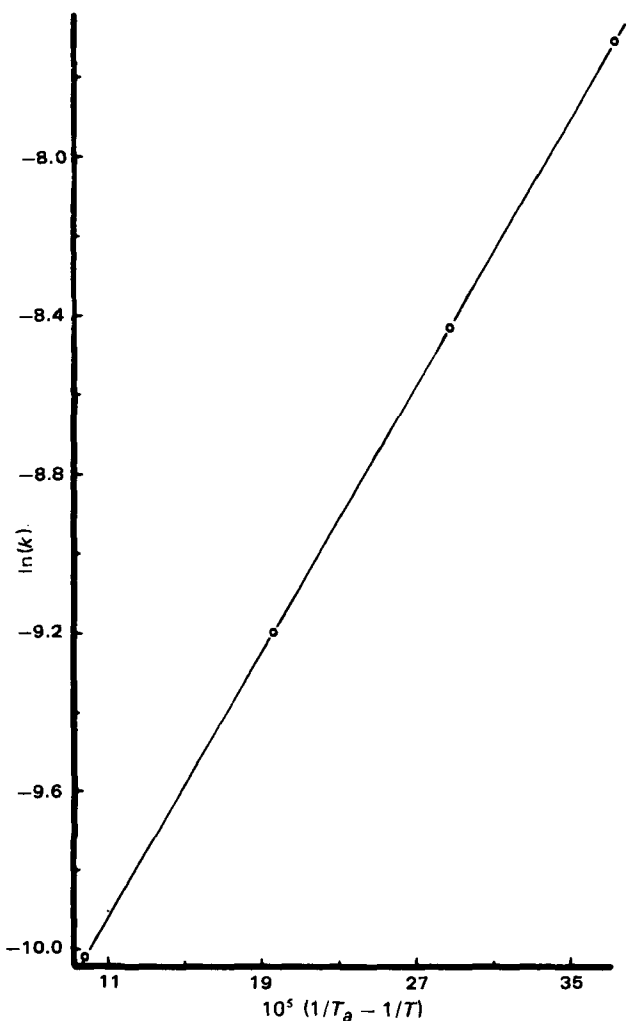
Table VIII—Variations in Parameter Estimates when Thermal Volume Expansion is Neglected [a]

Temperature Range	Model		
	Zero-Order	Pseudo First-Order	Second-Order
		k_0	
20	0.09973	0.1002	0.1002
40	0.09949	0.1004	0.1003
60	0.09900	0.1007	0.1004
		E	
20	15.07	14.94	14.96
40	15.09	14.92	14.94
60	15.13	14.90	14.93

[a] Exact nonisothermal simulated data were generated using: $k_a = 0.1$, $E = 15.0$, $D_0 = 1.0$, $D_\infty = 0.05$, $a_r = 1$, $a_p = 0$, $b = 1$, $T = 298.15 + t$, and $T_a = 25°$ for various models incorporating the volume correction term.

practical sense, the differences were not large and errors caused by accepting the approximate values would be safe.

D_0 and D_∞ as Constants—The errors resulting from treating D_0 and D_∞ as constants were investigated using a typical set of I data. Initially, all parameters (k_a, E, D_0, and D_∞) were estimated (row 1, Table VII) and the residual variance from this fit was used to obtain the 95% confidence limits of a reading. Variables D_0 and/or D_∞ were then treated as constants and the remaining parameters were re-estimated from the data. Constant values for D_0 and D_∞ were best estimates from the four parameter fit ±95% confidence limits of a reading (0.0008 absorbance unit). It is acceptable that D_0 be determined to this precision, since for nonisothermal kinetics, the reaction initially proceeds slowly. However, determination of D_∞ may involve time delays with instrument drift, mechanism changes, *etc.*, so an uncertainty of 0.01 absorbance unit would not seem unreasonable. Table VII shows the results of these refits.

It is apparent that fixing D_0 and D_∞ reduces the 95% confidence limits of E as expected, while those of k_a are reduced except where the increase in residual variance outweighs the decrease caused by fixing D_0 and D_∞ (Table VII). In a practical sense it is unlikely that the decreases in 95% confidence limits would cause serious errors, but the inflated residual variances make the model suspect, making the choice of the appropriate reaction order difficult.

Errors in k_a and E estimates were quite small (0.16 and 0.23% range, respectively) when D_∞ was treated as a constant in error by the 95% confidence limit of a reading (Rows 2 and 3 in Table VII), but similar errors in D_0 caused considerable biases in the estimates (1.6 and 1.2% ranges for k_a and E, respectively). When the error in D_∞ was increased to 0.01 absorbance unit, k_a and E ranges were 2.1 and 3.4% in the worst case. These errors must be seen in light of the experimental precision and, hence, the approximate confidence limits. The 95% confidence limits for k_a and 99% limits for E for Rows 4 and 5 and 8 and 9 in Table VII do not bracket the best (Row 1) estimates. That is, biased estimates resulted from using constant (incorrect) values for D_0 and D_∞.

Thermal Volume Expansion—Comparison of the first- and pseudo first-order estimates in Table II indicates that errors caused by neglecting thermal volume expansion are small for this model. However, to ascertain the magnitude of expected biases for all models, exact simulated data (no added random error) were generated. Data (40 pairs) covering 90% decomposition and temperature ranges of 20, 40, and 60° were produced using reparameterized Eqs. 11, 13, and 14. Parameters were re-estimated using these equations and again without the volume correction term [$f_2(t)$]. In all cases where the complete equations were used, the estimates agreed (to 6 correct digits) with the parametric values. Errors caused by neglecting the volume correction are shown in Table VIII.

The biases observed are quite small (*e.g.*, pseudo first-order, 40° range, biases are +0.4% in k_a and −0.53% in E). These percentages varied little with parameter values (*e.g.*, with $k_a = 0.01$ and $E = 30$, the errors were +0.4% in k_a and −0.3% in E). Assuming an error variance of 0.56×10^{-6}, the 95% confidence limits for the 40° pseudo first-order range are ±0.61% for k_a and ±0.64% for E. These limits bracket the parametric values.

The bias in k_a depended on the T_a chosen. For $T_a = 90°$, the error was −1.9% but this increased error must be compared with the wider 95% confidence limits of ±2.5%. Thus, the bias errors are relatively small. As seen previously, fits with and without volume correction terms (first- and pseudo first-order, Table III) are equally good. Therefore, the need for a volume correction term was not evident from the experimental system, and justification for the term is theoretical (10).

From a practical viewpoint it appears that failure to correct for volume expansion has a negligible effect on kinetic parameter estimates from a single data set; however, in the long term, slightly biased estimates will result and the inclusion of the term is warranted.

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