Integral Approach to Nonisothermal Estimation of Activation Energies

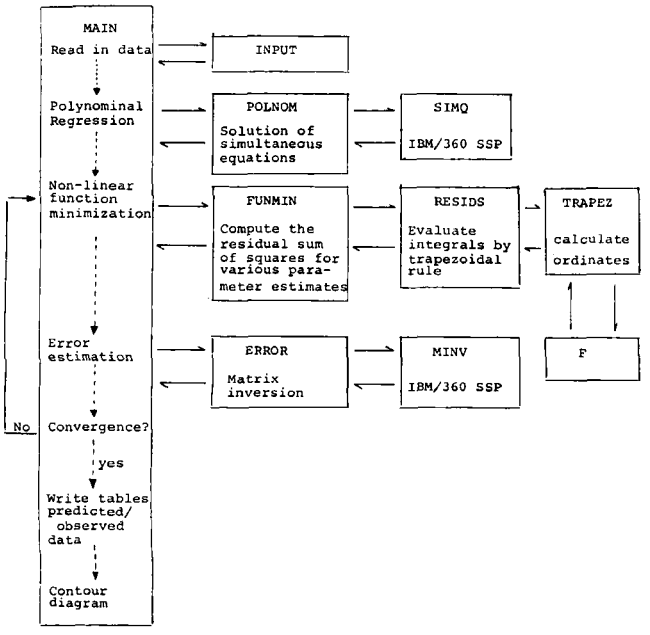
B. W. MADSEN*, R. A. ANDERSON*x, D. HERBISON-EVANS‡, and W. SNEDDON*

Abstract □ A simple experimental procedure is described, enabling estimates of the activation energy and preexponential factor for drug decomposition to be obtained from a single experiment. A computer program was written which bypasses the problem of slope determination and the consequent lack of final errors associated with graphical methods. It uses numerical integration and an iterative nonlinear least-squares treatment to ensure reliable and consistent parameter estimates and provides information on parameter uncertainties. The method is quite sensitive to deviations from the mathematical model and has been illustrated with the decomposition of riboflavin in alkaline solution.

Keyphrases □ Computer estimations, activation energies—integral approach to nonisothermal estimation, numerical integration and iterative nonlinear least-squares treatment □ Activation energies—integral approach to nonisothermal estimation, computer program, example from literature □ Decomposition—computer program for estimation of activation energy and preexponential factor of drug decomposition

The recent literature reflects a growing interest in nonisothermal methods for estimation of activation energies and the accelerated prediction of the stability of pharmaceuticals (1–4). Compared to the isothermal approach, these methods have been a compromise, reducing experimental effort but decreasing the accuracy of the final estimates.

The purpose of this paper is to show that the activation energy describing decomposition of a drug is a highly defined parameter. Provided computational facilities are available, the data obtained from a simple experimental procedure can be processed to give good activation energy estimates and, if required, rate constants at selected temperatures.



Scheme I—Computer flowchart of the program showing all subroutines

THEORETICAL

Decomposition of a drug is commonly represented by Eq. 1:

$$-\left(\frac{dc}{dt}\right)_i = kc_i^n \qquad \text{(Eq. 1)}$$

where c_i = concentration at time t_i, k = observed rate constant, and n = order of the reaction.

If the Arrhenius equation applies, then:

$$k_i = Ae^{-Ea/RT_i} \qquad \text{(Eq. 2)}$$

where A = preexponential factor, Ea = activation energy, R = universal gas constant, and T_i = absolute temperature at time t_i.

For the first-order case (n = 1), Eq. 1 can be rearranged; substituting for k gives Eq. 3:

$$-\frac{dc}{c_i} = Ae^{-Ea/RT_i}dt \qquad \text{(Eq. 3)}$$

To integrate Eq. 3, the series of temperature readings is first represented as a function of time. A polynomial is usually adequate for this, enabling Eqs. 4–6 to be formed:

$$-\int\frac{1}{c_i}dc = A\int e^{-Ea/RT(t)}dt \qquad \text{(Eq. 4)}$$

$$-\ln c_i = AI_i + \text{constant} \qquad \text{(Eq. 5)}$$

$$I_i = \int_{t_0}^{t_i} e^{-Ea/RT(t)}dt \qquad \text{(Eq. 6)}$$

Substitution for time t_0 in Eq. 5 shows that the constant term is $-\ln c_0$.

Then Eq. 5 can be seen to give:

$$c_i = c_0e^{-AI_i} \qquad \text{(Eq. 7)}$$

The concentration data (c_i) are nonlinear with respect to the two unknown parameters A and Ea. With initial estimates, an iterative least-squares regression can be used to find the best estimates, minimizing Eq. 8 until convergence:

$$SS = \sum_{i=1}^{N}(c_{i_{obs}} - c_{i_{pred}})^2 \qquad \text{(Eq. 8)}$$

Equations similar to Eq. 7 can be derived for the zero- and second-order cases (Eqs. 9 and 10, respectively) and the data may be treated using the same nonlinear approach:

$$c_i = c_0 - AI_i \qquad \text{(Eq. 9)}$$

$$c_i = (AI_i + c_0^{-1})^{-1} \qquad \text{(Eq. 10)}$$

PROGRAMMING

A flow chart of the program is shown in Scheme I. The various subroutines perform the following functions.

INPUT: Data read in by INPUT include the number of data points (N); the order of the reaction (ORDER); initial estimates for the two unknown parameters (X_1 and X_2); various experimental information pertinent to a given run (HEAD); the dependent

Table I—Source Data for Nonisothermal Treatment of Riboflavin Decomposition in 0.1 *N* NaOH

Absorbance	Minutes	Temperature
0.640	0	21.0°
0.637	10	25.7°
0.633	20	29.4°
0.622	30	33.4°
0.618	40	37.7°
0.589	51.5	43.0°
0.568	60	46.0°
0.534	70	49.2°
0.488	80	53.5°
0.423	90	57.0°
0.351	100	61.0°
0.191	120	66.6°
0.124	130	70.5°

variable, in this case aqueous absorbance (AQABS); and time and temperature (TIME and TEMP, respectively). Time is changed from minutes to hours to minimize overflow in the subsequent polynomial regression, and the temperature is changed to absolute, as required by Eq. 2.

POLNOM: Initially, a fourth-order polynomial for the temperature on time regression was found adequate for simple monotonic temperature changes, with all of the absolute residual values less than 0.4% of the predicted values. To allow additional experimental flexibility in the time–temperature relationship, a seventh-order polynomial was used, giving residuals usually less than 0.3%. The number of data points in this regression was always greater than 12, making the occurrence of spurious stationary points impossible. An 8 × 8 matrix of simultaneous equation coefficients was fed into SIMQ[1], together with eight constant terms for an analytical solution. Then the observed and predicted data, residuals, and ratios were printed out for visual inspection.

FUNMIN: The Adaptive Simplex approach (5) to function minimization was programmed as a subroutine FUNMIN. This involves a direct search based on the sum of squares, making no assumptions about the function or its derivatives, so that initial estimates are not critical to successful execution. The only consequence of poor initial estimates is a longer processing time. FUNMIN calls on a function subroutine (RESIDS) which computes the residual sum of squares for any pair of parameter estimates (X_1 and X_2). It was sufficient to set the increments *(DX)* for the initial simplex at 50 and 10% for *A* and *Ea*, respectively. FUN-

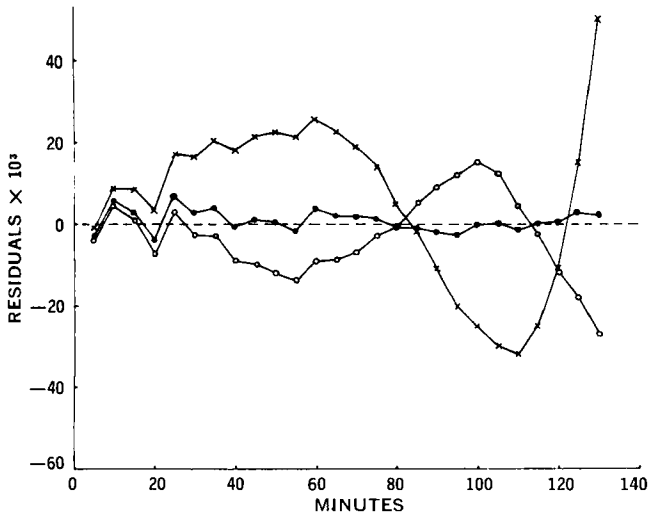


Figure 1—*Data processed according to zero-* (X), *first-* (●), *and second-* (○) *order kinetics. Along with other factors, an analysis of the residuals makes a decision on the best model simple.*

[1] IBM/360 Scientific Subroutine Package, 1967.

Table II—Polynomial Regression of Experimental Temperature Values on Time[a]

Hours	Observed Temperature	Calculated Temperature	Residuals	Ratios
0.	294.16°	294.29°	−0.131	0.9995
0.1667	298.86°	298.50°	0.361	1.0012
0.3334	302.56°	302.66°	−0.099	0.9997
0.5000	306.56°	306.87°	−0.306	0.9990
0.6667	310.86°	311.02°	−0.163	0.9995
0.8583	316.16°	315.64°	0.515	1.0016
1.0000	319.16°	318.96°	0.200	1.0006
1.1667	322.36°	322.81°	−0.446	0.9985
1.3334	326.66°	326.62°	0.037	1.0001
1.5000	330.16°	330.35°	−0.193	0.9994
1.6667	334.16°	333.84°	0.315	1.0009
2.0000	339.76°	339.89°	−0.134	0.9996
2.1667	343.66°	343.62°	0.044	1.0001

[a] Regression coefficients: 294.29, 26.33, −11.74, 41.28, −66.72, 52.69, −20.14, 29.71. Coefficients are listed in increasing order, *i.e.*, the constant term first, then the linear coefficient, *etc.*

MIN performs 24 iterations before returning to MAIN, and a maximum of 12 calls on FUNMIN is allowed.

RESIDS: This function subroutine is called by FUNMIN for each set of parameter estimates. To find the sum of squares, the right-hand side of Eq. 6 has to be evaluated. Using the trapezoidal rule, the integrals for each interval t_i to t_{i+1} are estimated separately since this involves less processing time. Then the total integral from zero time to any time t_i is obtained by summation. Then, for a chosen reaction order, a series of predicted concentrations can be calculated using Eq. 7, 9, or 10. Finally, a value for the sum of squares is computed using Eq. 8 and control returns to FUNMIN.

TRAPEZ and F: Subroutine TRAPEZ numerically estimates the integrals using the trapezoidal rule. That is, the interval is successively halved until the change in the integral is below some acceptable level (TEST). This criterion has a large effect on processing time, so it is important to find an acceptably accurate level. In the present work, concentrations were determined by measurement of absorbance values, and the error standard deviation of these readings was usually not greater than 0.003, so that in the worst case the relative error in primary absorbance data might be 0.003/0.1 = 3%. For a first-order reaction, this would correspond to an uncertainty of approximately 2% in the integral (I_i in Eq. 7). Since there is probably no point in performing subsequent calculations at better than a tenth of the error in the primary data, TEST was set at 0.2%. This value ensured fast processing, and reduction to a lower level did not alter the parameter estimates.

ERROR: The standard deviations of the derived parameters were estimated by matrix inversion using the sums of the cross-products of the partial derivatives (6). Normalization of this 2 × 2 matrix ensured that the off-diagonal elements of the unit matrix were never greater than 1×10^{-6}.

MAIN: After the errors have been computed, control returns to MAIN. If the difference between the final sum of squares from two successive calls on FUNMIN is less than 1%, then the program has converged at what most likely will be the true minimum. If not, then FUNMIN is called again, setting the new increments equal to the computed standard deviations.

When convergence has been achieved, it remains to print out a table of observed and predicted absorbance readings, residuals, and the residuals expressed as error standard deviations. If any data point is outside 3 *SD* (for 12–30 points), then a caution is printed out. The predicted rate constants for the various experimental temperatures also are listed in this table.

As a final aid in confirming convergence or in suggesting the direction of better initial estimates, a contour diagram is printed, showing the sum of squares for the two variables ±4 *SD*.

The program was written in FORTRAN IV[2], and all calculations were done on an automatic computer[3]. For average data

[2] A listing is available on request from the authors.
[3] 32K IBM 7040.

Table III—Summary of the Convergence History

	Iteration Number	Preexponential Factor, $\times 10^{13}$ hr^{-1}	Activation Energy, kcal mole^{-1}	Sum of Squares
Initial estimates		2.0	21.0	
Current value	1	2.903	20.370	5.655×10^{-5}
SD		0.273	0.063	
Final value	2	2.876	20.364	5.653×10^{-5}
SD		0.270	0.063	

and fair initial estimates, the usual execution time was 2–4 min.

EXPERIMENTAL

The degradation of riboflavin under aqueous alkaline conditions was chosen to illustrate the technique. Generally, the method followed the procedures used to study the reaction between octatomic sulfur and triphenylphosphine in benzene (7) and to follow the acid-catalyzed hydrolysis of ethyl acetate (8).

A solution of riboflavin (10^{-4} *M*) and sodium hydroxide (0.05 or 0.1 *N*) was heated so that the temperature rose conveniently over the temperature range of interest. At selected times, samples were taken, quenched in acetic acid, and assayed spectrophotometrically as previously described (9). The temperature of the solution was noted each time a sample was taken for analysis, giving three corresponding sets of primary data: concentration, time, and temperature.

To obtain the best combined parameter estimates from different experiments, the weighted means and standard deviations were calculated according to Eqs. 11–13:

$$w_i = \frac{1}{s_i^2} \qquad \text{(Eq. 11)}$$

$$\bar{x} = \frac{\sum w_i x_i}{\sum w_i} \qquad \text{(Eq. 12)}$$

$$\bar{s} = \frac{1}{\sqrt{\sum w_i}} \qquad \text{(Eq. 13)}$$

where s_i = standard deviation of an individual point x_i, w_i = weight for the point x_i, $\bar{x}$ = weighted mean, and $\bar{s}$ = weighted standard deviation.

The uncertainty in the predicted rate constant at a given temperature was computed using Eq. 14:

$$S_k = \sqrt{\left(S_A \frac{\partial k}{\partial A}\right)^2 + \left(S_{Ea} \frac{\partial k}{\partial Ea}\right)^2} \qquad \text{(Eq. 14)}$$

where S_k, S_A, and S_{Ea} are the uncertainties in the predicted rate constant and estimated preexponential factor and activation energy, respectively.

The nonisothermal estimates were confirmed by designing an isothermal experiment using the same assay procedure and 50 data points at temperatures of 20, 35, and 50°. Final parameter estimates were computed by weighted least squares (10).

RESULTS AND DISCUSSION

Table I lists typical experimental results for input to the program, and Tables II–V summarize the results of computations on this data set. Provided the reaction is followed through at least two half-lives and that the data are spread uniformly over the whole range, only 12 points are needed to define the two unknown parameters sufficiently. If additional experimental effort is indicated, it is probably better spent in repeating the whole run than in gathering more data points for the given experiment. This approach represents a distinct advance in experimental effort over the more standard isothermal procedure. Adjustment of the heating rate is completely flexible, and temperature may be increased at a rate chosen to achieve the desired degree of decomposition in a convenient experimental time. This ensures that estimates can be gained from the first experiment.

One assumption made in this method is that c_0 (the first sample taken) is known without error. Since the temperature is changing least rapidly at the commencement of a run, it should be the most certain of the assay results. By taking a duplicate and averaging the results, the error incurred should not be great. At the cost of increased processing time, the alternative is to have three variables in the regression; the program is sufficiently general to be easily modified when this is needed.

Table IV—Comparison of Experimental and Predicted Data

Observed Absorbance	Predicted Absorbance	Residuals, $\times 10^{-3}$	Scatter as *SD*	Predicted Rate Constants, hr^{-1}
0.637	0.6370	−0.02	−0.01	0.0367
0.633	0.6322	0.75	0.33	0.0558
0.622	0.6247	−2.71	−1.19	0.0869
0.618	0.6130	5.00	2.21	0.138
0.589	0.5921	−3.08	−1.36	0.240
0.568	0.5697	−1.74	−0.77	0.325
0.534	0.5339	0.10	0.05	0.447
0.488	0.4857	2.33	1.03	0.680
0.423	0.4239	−0.89	−0.39	0.948
0.351	0.3502	0.80	0.35	1.37
0.191	0.1927	−1.72	−0.76	2.28
0.124	0.1229	1.10	0.49	3.21

The fact that the predicted temperature readings from the polynomial regression do not fit the observed temperatures exactly (absolute residual values not greater than 0.2% in Table II) is not a problem, since positive and negative fluctuations are averaged out during integration. If the regression was so bad that all of the residuals were positive and +0.1%, an average error of +0.1% would be introduced in the computed integrals, which is well within the experimental level of ±2% discussed previously. Pragmatically, as reported previously (11), the parameter estimates are fairly insensitive to temperature errors with such an integral nonisothermal approach. For example, if all the temperature readings from the data in Table I are lowered by 1°, then the computed activation energy changes from 20.36 ± 0.06 to 20.24 ± 0.06[4] kcal mole^{-1}, and the preexponential factor changes from $(2.9 \pm 0.3) \times 10^{13}$ to $(2.6 \pm 0.2) \times 10^{13}$ hr^{-1}.

The final results for the two systems studied (0.05 and 0.1 *N* sodium hydroxide) are shown in Table VI, together with the isothermal results. Parameter variation between runs is probably better than the normally tolerated levels, and agreement with the isothermal method is good. However, previous workers (1, 9) quoted slightly lower values for the activation energy (Table VII). Hughes (11) commented that higher results may be obtained with nonisothermal techniques, and part of this discrepancy may be due to faulty data treatment in the classical isothermal approach. When the same isothermal data in Table VI were processed by taking log absorbance, linearly regressing on time, and then making an unweighted linear regression of log *k* on reciprocal temperature, the parameter estimates were slightly depressed (Table VII).

The approach presented here is sensitive to deviations from the expected order decomposition, as could be due to change of mechanism or approach to equilibrium. Attainable experimental preci-

[4] All calculated uncertainties in this report are given as standard deviations.

Table V—Total Sum of Squares Dependence on the Two Nonlinear Variables about the Best Estimates

Pre-exponential Factor, $\times 10^{13}$ hr^{-1}	Activation Energy, kcal mole^{-1}								
	20.12	20.18	20.24	20.30	20.36	20.42	20.48	20.54	20.60
3.96	0.108E-0	0.799E-1	0.562E-1	0.368E-1	0.215E-1	0.104E-1	0.330E-2	0.229E-3	0.101E-2
3.69	0.872E-1	0.624E-1	0.417E-1	0.253E-1	0.130E-1	0.482E-2	0.671E-3	0.423E-3	0.391E-2
3.42	0.675E-1	0.459E-1	0.285E-1	0.153E-1	0.624E-2	0.123E-2	0.153E-3	0.286E-2	0.914E-2
3.15	0.490E-1	0.309E-1	0.171E-1	0.736E-2	0.173E-2	0.674E-4	0.223E-2	0.800E-2	0.172E-1
2.88	0.322E-1	0.180E-1	0.795E-2	0.201E-2	0.565E-4	0.195E-2	0.748E-2	0.164E-1	0.285E-1
2.61	0.178E-1	0.783E-2	0.194E-2	0.580E-4	0.202E-2	0.764E-2	0.167E-1	0.288E-1	0.437E-1
2.34	0.687E-2	0.148E-2	0.103E-3	0.256E-2	0.865E-2	0.181E-1	0.307E-1	0.460E-1	0.637E-1
2.06	0.732E-3	0.401E-3	0.387E-2	0.109E-1	0.213E-1	0.346E-1	0.507E-1	0.690E-1	0.893E-1
1.79	0.151E-2	0.663E-2	0.152E-1	0.270E-1	0.416E-1	0.588E-1	0.781E-1	0.991E-1	0.122E-0

Table VI—Best Parameter Estimates for Riboflavin Decomposition

	Experiment Number	Number of Data Points	A, $\times 10^{13}$ hr^{-1}	Ea, kcal mole^{-1}
0.05 N NaOH	1	12	1.2282 ± 1.241	20.017 ± 0.717
	2	13	2.1104 ± 0.383	20.366 ± 0.124
	3	26	1.341 ± 0.0980	20.0872 ± 0.0490
Weighted mean and SD			1.39 ± 0.09	20.12 ± 0.04
Weighted least-squares isothermal estimates			1.21 ± 0.98	20.0 ± 0.5
0.1 N NaOH	4	12	2.8793 ± 0.270	20.3637 ± 0.0626
	5	12	1.9194 ± 0.451	20.0851 ± 0.161
	6	14	4.0999 ± 0.594	20.565 ± 0.0963
	7	20	1.4734 ± 1.70	19.8780 ± 0.8005
Weighted mean and SD			2.8 ± 0.2	20.39 ± 0.05
Weighted least-squares isothermal estimates			3.3 ± 4.2	20.3 ± 0.8

Table VII—Comparison of Results with Previous Literature Reports on the Decomposition of Riboflavin in 0.05 N NaOH

	Nonisothermal	Rogers (1)	Guttman (9)	Isothermal Unweighted Least Squares
Preexponential factor $\times 10^{13}$, hr^{-1}	1.6 ± 0.4	—	—	0.8 ± 0.75
Activation energy, kcal mole^{-1}	20.09 ± 0.05	17.85	19.2	19.7 ± 0.4
Rate constant at 20°, hr^{-1}	0.014 ± 0.002	0.018	0.016	—

Table VIII—Simulation of an Equilibrium Situation[a]

Observed Absorbance	Predicted Absorbance	Residuals, $\times 10^{-3}$	Scatter as Error SD
0.637	0.6365	0.44	0.09
0.633	0.6312	1.80	0.37
0.622	0.6320	−0.97	−0.20
0.618	0.6105	7.48	1.53
0.589	0.5890	0.03	0.00
0.568	0.5665	1.48	0.30
0.534	0.5312	2.75	0.56
0.488	0.4848	3.24	0.66
0.423	0.4262	−3.19	−0.65
0.351	0.3570	−5.99	−1.22
0.202	0.2082	−6.17	−1.26
0.150	0.1400	10.00	2.04

Sum of squares = 2.65×10^{-4}
Absorbance error = ±0.0049
A ($\times 10^{13}$ hr^{-1}) = 0.4 ± 0.10
Ea (kcal mole^{-1}) = 19.1 ± 0.2

[a] This table should be compared to Table IV.

Table IX—Effect of Using Absolute Absorbance or Log Absorbance Values as Dependent Variable in Nonlinear Regression

Dependent Variable (c_i)	0.05 N NaOH	0.1 N NaOH
Absolute absorbance:		
A, $\times 10^{13}$ hr^{-1}	1.3 ± 0.1	2.9 ± 0.3
Ea, kcal mole^{-1}	20.09 ± 0.05	20.36 ± 0.06
Log absorbance:		
A, $\times 10^{13}$ hr^{-1}	0.8 ± 0.1	2.6 ± 0.6
Ea, kcal mole^{-1}	19.7 ± 0.1	20.3 ± 0.2
Number of data points	26	12

sion is readily established (in this work, not greater than ±0.003 absorbance unit) and if the breakdown does not follow such a simple integer order, the program will make the best fit possible and return a large error standard deviation. Together with non-normality of residuals, this should serve as a warning that all is not well.

If an equilibrium situation applies, then the greatest deviation will occur in the later time readings. Sensitivity to such a system was tested by simulation and the results are shown in Table VIII. The last two absorbance readings in Table I were increased from 0.191 to 0.202 and from 0.124 to 0.150, and then this new data set was fed into the program. It will be seen that the uncertainty in the absorbance readings increases from ±0.0023 to ±0.0049, the residuals are no longer randomly distributed, and the last point is accounting for approximately 40% of the total sum of squares.

Weighting of data points should also be considered. In this

Table X—Effect of Treating Decomposition Data According to Different Mathematical Models, Namely Zero, First, and Second Order[a]

	Order		
	Zero	First	Second
A, hr^{-1}	$(7.0 \pm 7.0) \times 10^6$	$(1.3 \pm 0.1) \times 10^{13}$	$(1.6 \pm 0.5) \times 10^{19}$
Ea, kcal $mole^{-1}$	11.2 ± 0.7	20.09 ± 0.05	28.7 ± 0.2
Absorbance error	± 0.021	± 0.0029	± 0.010

[a] In this case, the data confirm literature reports stating the decomposition is first order.

case, the dependent variable (c_i) is measured using a UV spectrophotometer. The reported uncertainty in such an instrument is ±0.005 absorbance unit[5], which represents a constant absolute error. Therefore, it is more correct to use the absolute absorbance values than the log absorbance. In a least-squares procedure, the former approach would minimize as far as possible to a constant absolute error, whereas the latter minimizes toward a constant relative error. Log weighting of the absorbance readings invariably returned a slightly lower value for the activation energy than when the absolute values were used and often produced nonrandom residuals. The biggest factor against using log absorbance in this nonisothermal technique is the comparatively larger relative standard deviations for the two parameters (Table IX).

The nonisothermal approach is very sensitive to the order of the reaction, so prior isothermal experiments need not be made to establish order. Data can be submitted for analysis, and the consequences of treating a reaction by the wrong mathematical model are clearly pointed out (see Fig. 1 and Table X). In this instance, an examination of the residuals clearly shows the reaction is first order, and this is substantiated by the smaller parameter and absorbance error standard deviations.

[5] Manual, Perkin-Elmer model 124 double-beam spectrophotometer.

REFERENCES

(1) A. R. Rogers, *J. Pharm. Pharmacol.*, **15**, 101T(1963).

(2) S. P. Eriksen and H. Stelmach, *J. Pharm. Sci.*, **54**, 1029(1965).

(3) H. V. Maulding and M. A. Zoglio, *ibid.*, **59**, 333(1970).

(4) A. I. Kay and T. H. Simon, *ibid.*, **60**, 205(1971).

(5) J. A. Nelder and R. Mead, *Computer J.*, **7**, 308(1965).

(6) M. G. Kendall and A. Stuart, "The Advanced Theory of Statistics," vol. II, Charles Griffin and Coy, London, England, chap. 19.

(7) R. E. Davis, *J. Phys. Chem.*, **63**, 307(1959).

(8) R. A. Anderson and M. Campbell, *Aust. J. Pharm. Sci. Suppl.*, **105**, S81(1971).

(9) D. E. Guttman, *J. Pharm. Sci.*, **51**, 811(1962).

(10) D. L. Bentley, *ibid.*, **59**, 464(1970).

(11) M. A. Hughes, *Proc. Soc. Anal. Chem.*, **9**, 162(1972).

ACKNOWLEDGMENTS AND ADDRESSES

Received June 1, 1973, from the *Department of Pharmacy and the ‡Basser Department of Computer Science, University of Sydney, Sydney, N.S.W. 2006, Australia.

Accepted for publication December 10, 1973.

[x] To whom inquiries should be directed.

NOTES

Quantitative GLC Analysis of Plasma Cholesterol

P. A. HARRIS[x] and K. L. HARRIS

Abstract □ A GLC method is described for the quantitative analysis of cholesterol in plasma. The method was applied to the analysis of total cholesterol in 152 human plasma samples and compared with the results obtained by a standard automated colorimetric procedure on the same samples with values ranging from 100 to 472 mg %. The results of the two methods when subjected to a linear regression analysis yielded a sample correlation coefficient of 0.969 and a standard error of the estimate of ±15.2. The precision of the GLC method was determined by repeated total cholesterol analysis of the plasma of three human subjects. Similar results were obtained with all three. One of these had a mean total cholesterol of 172 mg % in 19 determinations with a standard error of the mean of ±1.0. Results with a single sample may be obtained in less than 2 hr, and one technician may obtain the results on 40 samples in 1 day using manual techniques. The GLC procedure clearly separates cholesterol from desmosterol and lanosterol. Adjustments in the volume and type of solvents allow quantitative determination of as little as 1.0 μg/sample. Evidence indicates that the method may be used for determining nonesterified cholesterol in the plasma.

Keyphrases □ Cholesterol—quantitative GLC analysis in plasma, compared to colorimetric method □ GLC—analysis, cholesterol in plasma, compared to colorimetric method

The determination of cholesterol in plasma is most often accomplished by procedures that require strong acids and/or ferric chloride to yield colored products. These methods measure the total cholesterol con-