2

Solution Kinetics

JENS T. CARSTENSEN

Madison, Wisconsin

Stability is not synonymous with chemical kinetics, yet most of the rate-limiting phenomena are either associated with chemical reactions or are describable by some equation system that bears a resemblance to those encountered in chemical kinetics. It is, therefore, of importance to lay the proper kinetic foundation before discussing the actual phenomena encountered in dosage forms.

These fundamental principles are most conveniently described by solution kinetics. The simpler a system is, the easier it is to make it reproducible, and it is therefore not surprising that the largest number of pharmaceutical publications on the subject of kinetics deal with solution systems. Furthermore, the more dilute a system is, the more it will adhere to ideal laws, and hence the largest number of publications to be found deal with dilute systems. There are obviously pharmaceutical dosage forms that are solutions, viz. oral, parenteral, nasal, ophthalmic, and otic solutions. Of these, it is only the parenteral and ophthalmic solutions that are chemically fairly simple, i.e. contain only a few number of components. These are systems that would behave similarly to the patterns described in, for instance, the chemical literature. In oral solutions, there are many ingredients (sweeteners, solubilizers, etc.), so that, here, one would expect definite vehicle effects and interaction possibilities.

The Stability Guidelines make certain requirements on basic stability that are best elucidated (or only elucidated) through solution kinetics: First of all it is necessary to develop a stability-indicating assay. This is defined in lines 111 of the 1987 Guidelines as "Quantitative analytical methods that are based on the characteristic structural, chemical, or biological properties of each active ingredient of a drug product and that will distinguish each active ingredient from its degradation products so that the active ingredient content can be accurately measured." The 1993 ICH Guidelines state,

> Analytical test procedures should be fully validated and the assays should be stability-indicating. The need or the extent of replication will depend on the results of validation studies (194–196).
>
> The focus may instead be on assuring the specificity of the assay ... of identified degradants as indicators of the extent of degradation via particular mechanisms (386–389).

This means that the assay must be capable of detecting quantitatively the amount of parent drug present, and identify, and to some degree quantitate, the decomposition products. Lines 265–277 of the 1987 Guidelines state, "When degradation products are detected, the following information about them should be submitted when available:

(a) Identity and chemical structure,
(b) cross-reference to any available information about biological effect and significance at the concentrations likely to be encountered,
(c) procedure for isolation and purification,
(d) mechanism of formation, including order of reaction...,
(e) physical and chemical properties,
(f) specifications and directions for testing for their presence at the levels or concentrations expected to be present."

Lines 141–144 further state that "the stability-indicating methodology should be

validated by the manufacturer (and the accuracy and precision established) and described in sufficient detail to permit validation by FDA laboratories."

In developing stability-indicating assay methodology, it is customary to deliberately decompose the drug in solution, so as to challenge the assay and insure its capability of separating the parent drug from decomposition products. It is obvious, also, that it is desired to establish the kinetic order of the decomposition.

1. THE ORDER OF A REACTION

The order of a reaction will be defined below, but in essence it determines how the degradation data are treated. That it is important to establish the order of a reaction is evident in that the 1993 ICH Guidelines specifically state,

> The nature of any degradation relationship will determine the need for transformation of the data for linear regression analysis. Usually the relationship can be represented by a linear, quadratic or cubic function on an arithmetic or logarithmic scale. Statistical methods should be employed to test the goodness of fit of the data on all batches and combined batches (where appropriate) to the assumed degradation line or curve (138–143).

They also state (in respect to mass balance),

> This concept is a useful scientific guide for evaluation data but it is not achievable in all circumstances. The focus may instead be on assuring the routes of degradation, and the use, if necessary, of identified degradants as indicators of the extent of degradation via particular mechanisms (385–389).

Although the presentation modes outlined in this quotation are not (at least not in the case of the quadratic or cubic functions) of scientific bent, it is obvious that efforts must be made, before the formal stability program is started, to establish the order of the reaction.

Establishing the order is, furthermore, of financial importance, because the establishing of expiration periods (which will be discussed later) depends, to some degree, on the investigator's capability of extrapolating the concentration of drug beyond the last time point of testing. The 1993 ICH Guidelines further state,

> Limited extrapolation of the real time data beyond the observed range to extend expiration dating at approval time, particularly where the accelerated data supports this, may be undertaken. However, this assumes that the same degradation relationship will continue to apply beyond the observed data and hence the use of extrapolation must be justified in each application in terms of what is known about the mechanism of degradation, the goodness of fit of any mathematical model, batch size, existence of supportive data, etc. (149–155).

The longest possible expiration period is, of course, economically desirable, and many of the efforts of the stability programs of pharmaceutical companies are geared towards lengthening this period. As for definition of the order or a reaction, if

$$A + B \rightarrow E \tag{2.1}$$

then the reaction rate is given by

$$\frac{dC}{dt} = -k_{(n+m)}[A]^n[B]^m \tag{2.2}$$

where C is the concentration of the species being studied, brackets denote concentrations of A and B, and k denotes a rate constant, then the reaction is said to be of the order $n+m$. The rate constant, in this writing, will most often carry the subscript denoting the order of the reaction whenever reaction orders are discussed and being distinguished. (A notable exception is the notation in the section dealing with pH profiles). The most important orders of interest in the pharmaceutical sciences are integral orders, i.e. those in which the sum of n and m is 0, 1, or 2. (Orders of higher than two are rare.)

As alluded to above, knowledge of the order of a reaction is of great importance in stability determination of drug substances, in particular in solution. The problem is frequently to judge whether the concentration-time profiles are linear (zero order) or curved (first or other order). When large amounts of data are at hand (e.g., at different temperature, where the order does not depend on temperature), then a data-consolidation technique described by Carstensen and Franchini (1994), Carstensen (1997), and Franchini and Carstensen (1994, 1999). The technique has later been used by Shalaev et al. (1997) in the study of solid-state methyl transfer reactions. For instance, the data in Fig. 1, when plotted linearly, give *fairly* good plots, but since there are only a few points it is difficult to say with reasonable certainty whether the data are, indeed, linear or curve-linear.

A *fractional life* is the length of time it takes for a product or drug substance to decrease to the level indicated by the fraction: the half-life, t_{50}, of a substance is the length of time it takes to decrease the content of active compound to 50% of its value. If, in Fig. 1, a given fractional life (e.g., t_{90} as shown in the figure) is read

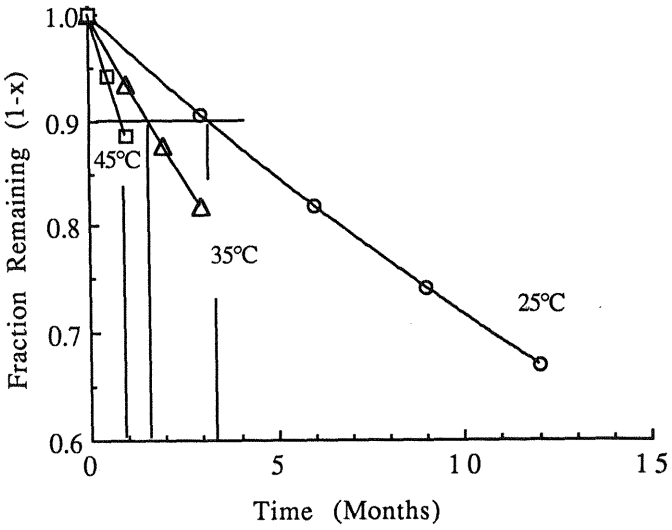

Fig. 1 Example of sparse data at three temperatures, such as is often encountered in early development of drug products.

Table 2.1 Example of Reduced Data Treatment in Kinetics

Time (Months)	Temperature (t_{90})	Fraction retained	Reduced time
0	25°C (3.5)	1.000	1.000
3		0.905	0.905
6		0.819	0.819
9		0.741	0.741
12		0.670	0.670
0	37°C (1.7)	1.000	1.000
1		0.935	0.935
2		0.875	0.875
3		0.800	0.800
0	45°C (0.95)	1.000	1.000
0.5		0.942	0.942
1		0.887	0.887

off the graphs, then the data may be consolidated. The t_{90} values shown are 45°C: 0.95; 35°C: 1.7; and 25°C: 3.5. The data in the first column in Table 1 are then reduced to the fourth column, and this is used as abscissa and the fraction retained (regardless of temperature) is used as ordinate in column 3, and the data are shown in Fig. 2.

The method allows better extrapolation tolerances, since the number of points is larger than for the individual temperatures. If, as in the unstable shown in Fig. 1, the t_{90} value is 3.5 months, then extrapolation could be made to $24/3.5 = 6.7$ half-lives, and the estimated potency after 24 months could be estimated with better precision than if only the 25°C data had been used.

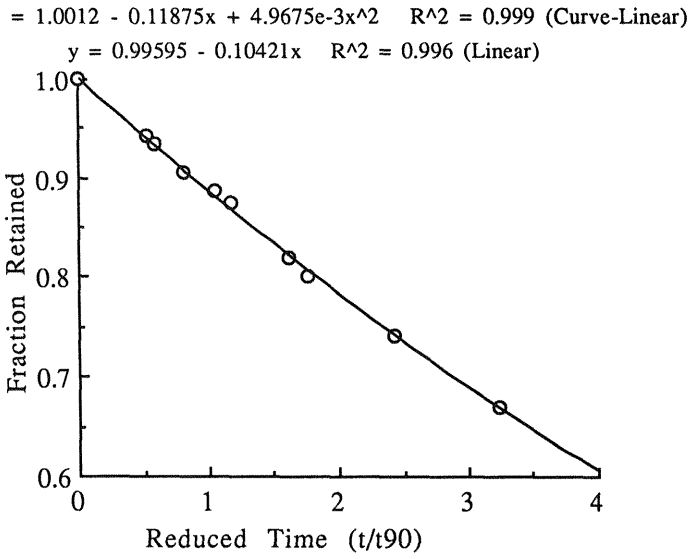

Fig. 2 Data from Table 1 plotted by reduced time treatment.

The fractional life should be *read off the graph* (Fig. 1) since at that point the reaction order is not known. Once the data are plotted, it is possible to execute curve fitting and estimate the best fit. In the case cited, the number of points is probably still too small to make a decision, but the indication is that the data are first order. Including data from even higher temperatures will help in this respect, but it is necessary that the order of reaction not change at the higher temperatures.

It has been mentioned that above 85% it is difficult to distinguish between different reaction orders. Li et al. (1998) report on apparent first-order plots for oxidation of a 4-[2-(2-amino-4-oxo-4,6,7,8-tetrahydro-3*H*-pyrimidino[5,4-6][1,4] thiazin-6-yl)-(S)-ethyl-2,5-thenoyl-L-glutamic acid. It is to be noted (from their Fig. 6) that there is definite downward curvature in the plots, and that they probably are S-shaped, as is discussed further in the chapter on oxidation. Here again, using a method of fractional times will help in deciding on which orders are plausible, or if a certain order of reaction can be ruled out.

It should, finally, be mentioned that Mälkki-Lame and Valkeile (1988) have described a method for transforming regression curves to the determination of reaction order of given situations in stability studies, using the Box–Cox technique and the Link function transformations.

2. THE ZERO-ORDER REACTION

There are not many truly zero-order reactions in the pharmaceutical field. It will be shown at a later point that there are several types of reactions that will appear to be zero order, i.e., are pseudo-zero order. The equation for zero-order reactions is

$$\frac{dC}{dt} = -k_0 \tag{2.3}$$

where C is concentration, t is time, and k_0 is the zero order rate constant.

It is seen that the unit of k is concentration units per time unit, e.g. molar per second. The integrated form of Eq. (2.3) is

$$C = C_0 - k_0 t \tag{2.4}$$

or

$$C_0[1 - a] = kt_a \tag{2.5}$$

where a is the fraction remaining at time t_a.

A quantity often utilized is the half-time, $t_{1/2}$, which is given by

$$t_{1/2} = \frac{C_0}{2k_0} \tag{2.6}$$

It is noted that this is dependent on the initial concentration.

Zero-order data may be graphed on plain Cartesian graph paper, using concentration as ordinate and time as abscissa. An example (Higuchi and Rheinstein, 1959) is shown in Fig. 1.

3. FIRST-ORDER REACTIONS

In this case Eq. (2.2) takes the form

$$\frac{dC}{dt} = -k_1 C \tag{2.7}$$

which integrates to

$$\ln\left[\frac{C}{C_0}\right] = -k_1 t \tag{2.8}$$

It is noted that the a-fractional life is given by

$$\ln[a] = -k_1 t_a \tag{2.9}$$

The most common of the a-lives is the half-life and the t_{90} (i.e., the point where 90% of the original concentration is left), which adhere to Eq. (2.9) by the equations

$$k_1 t_{/2} = -0.693 \tag{2.10}$$

$$k_1 t_{0.9} = -0.105 \tag{2.11}$$

Fig. 3 and Table 2 show an example of a straight first-order reaction.

In stability situations it is required to monitor both the disappearance of the drug and the appearance of decomposition product(s). In most cases there is more than one decomposition product (and simple cases of this will be treated below). In the simplest case there is only one decomposition product. There are cases of this, e.g., aspirin in simple systems (Carstensen et al., 1985; Carstensen and Attarchi, 1988a, 1988b) decomposes, by a pseudo-first-order reaction, in a simple fashion, i.e. to salicylic acid and acetic acid. In such cases, if the assay of the decomposition product is fairly good, the decomposition can be monitored best by monitoring

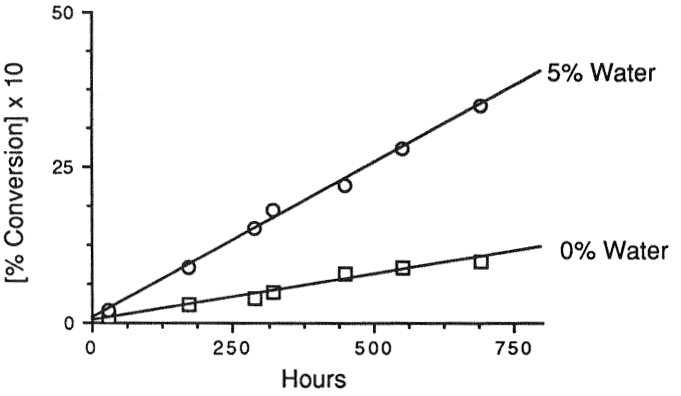

Fig. 3 Decomposition of vitamin A acetate (to anhydrovitamin A). The least squares fits are (with 5% water) $y = 0.48 + 0.015x$; (without water) $y = 0.75 + 0.05x$. (Figure constructed from data published by Higuchi and Rheinstein, 1959.)

Table 2.2 Decomposition of Decarboxymoxalactam

Time (Min)	% Retained	ln[% Retained]	% Decomposed
0	100	4.61	0
10	78	4.36	32
20	50	3.91	50
30	38	3.64	62
40	27	3.30	73
50	17	2.83	83

Source: Reconstructed from data published by Hashimoto et al. (1984).

the appearance rate of the decomposition product, which should follow the reaction

$$[B] = A_0[1 - \exp(-k_1 t)] \tag{2.12}$$

An example of this is shown in Table 2.

It should be noted that whenever this approach is taken, it is mandatory still to monitor the content of parent drug, because mass balance should persist throughout the reaction period. (If the molar quantities do not sum up to A_0 (within experimental error), then either the reaction is not simple A → B, or the analytical procedure fails in aged samples). The easiest way of plotting the data in Table 2 is obviously to subtract each [B] figure from A_0 and plot is as [A]. One might then argue that one might simply plot the experimental value of [A]. But for fairly stable systems, the values of [A] may not differ (decrease) much and may be masked by experimental error. The percentage change in [B], however, is substantial, as seen in the table, and plotting becomes more meaningful (see Fig. 4).

There are many reported instances of first-order reactions in solution. For instance, Jordan (1998) has shown that timolol and propanolol decompose by straight first-order kinetics at pH 7.4. Aso et al. (1997) have shown that aqueous solutions of cephalothin decompose by first-order kinetics and that they follow

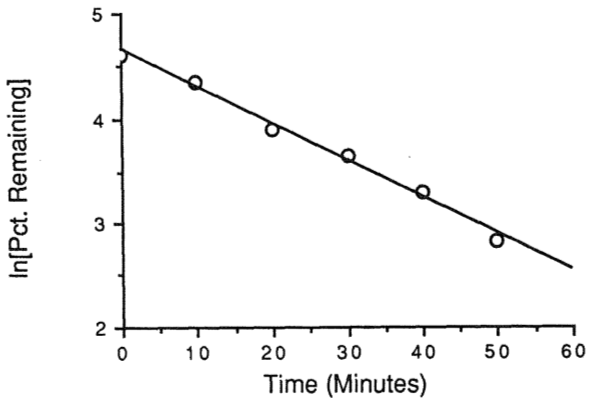

Fig. 4 Plot of first-order data from Table 1. (Graph constructed from data by Hashimoto et al., 1984.)

an Arrhenius equation. Hammad and Müller (1998) have found clonazepam to degrade first-order in phosphate buffers at pH 7.4 and to adhere to an Arrhenius equation.

Heat conduction *microcalorimetry* has been used as a method to evaluate stability and excipient stability by a series of researchers Angerg et al., (1988, 1990, 1993). Hansen et al. (1989), and Wilson et al. (1995) have described the general method and results interpretation. Oliyai and Lindenbaum (1991) have studied the decomposition of ampicillin in solution by means of microcalorimetry.

4. FIRST-ORDER REACTIONS WITH MORE THAN ONE END PRODUCT

The considerations above have assumed that the scheme is simply a reaction of type $A \rightarrow B$, but often there is more than one decomposition product.

4.1. Consecutive Reactions of The First Order

The 1993 ICH Guidelines state that mass balance (or material balance) is

> The process of adding together the assay value and levels of degradation products to see how closely these add up to 100 per cent of the initial value, with due consideration of the margin of analytical precision (382–384).

It is possible that the primary decomposition product itself is not stable, and in such cases the reaction scheme is

$$A \underset{k_1}{\rightarrow} B \underset{k_2}{\rightarrow} C \tag{2.13}$$

In other words, there will be more than one decomposition product. If all the products can be identified and quantitated, then it follows that *the number of moles of A, B, and C should always add up to the initial number of moles of A.* It is noted that it is the number of moles that must add up. Addition on a weight basis would be futile if there is a substantial difference between the molecular weights of the drug and the products. The guidelines recognize that it can be difficult, at times, to ascertain mass balance, partly due to analytical precision.

More often it is "unknowns" that cause the problem. If C were not identified, for instance, and was detected as a peak in a HPLC chromatogram, then its "content" is often stated as the area under the peak, using the drug as the unit of measure. But if, for instance, a UV detector is used, and C is lacking the amount of chromophores that A possesses, then the area under the C peak may grossly underestimate the amount of C.

An example of this is chlorbenzodiazepine, which hydrolyzes to the lactam form, and then further to the benzophenone (Carstensen et al., 1971). In fact in this reaction, for some of the benzodiazepines, C can progress further with the formation of the carbostyril and the acridone derivative, and some of the steps are associated with equilibrium conditions.

The rate equations governing scheme (2.13) are

$$\frac{d[A]}{dt} = -k_1[A] \tag{2.14}$$

$$\frac{d[B]}{dt} = -k_2[B] + k_1[A] \tag{2.15}$$

and

$$\frac{d[C]}{dt} = k_2[B] \tag{2.16}$$

These simultaneous differential equations may be solved by conventional means and yield the following results:

$$[A] = A_0 e^{-k_1 t} \tag{2.17}$$

$$[B] = A_0 \frac{k_1}{k_2 - k_1}(e^{-k_1 t} - e^{-k_2 t}) \tag{2.18}$$

and

$$[C] = A_0 - [A] - [B] \tag{2.19}$$

It is noted that the above expressions refer to molar quantities. An example of consecutive reactions is shown in Table 3 and Fig. 5. The table shows $C = 100 - [A] - [B]$. It often happens that one of the decomposition products is difficult to assay for, and in such a case, it may be obtained by difference, provided that mass balance is checked occasionally, e.g., in the early stages and at the end. Of course, there are reactions that have a multitude of end products, and in such cases it is conventional to assume that if e.g. a HPLC peak is less than 0.5% then it is considered negligible. This may be dangerous, because (especially if it is a constant wavelength peak), the actual molar content of the product(s) in the peak may be more than 0.5% (in which case mass balance would be lost).

To ascertain that the unidentified products are not toxic (and since they are unidentified, specific toxicity cannot be checked), it is conventional, as well, in such cases, to degrade a sample considerably and check its toxicity. It is worthwhile,

Table 2.3 Photolysis of Cefotaxime

Time (hours)	Cefotaxime A (% moles)	Anti-isomer, B (% moles)	*C* by difference[a]
0	100	0	0
0.25	82	10	8
0.5	70	15	15
0.75	55	18	27
1	43	19	38
1.5	28	18	54
2	20	15	65
3	10	10	80
4	5	5	90

[a] Column 3 not reported by Lerner et al. (1988). C may be more than one product.
Source: Constructed from data published by Lerner et al. (1988).

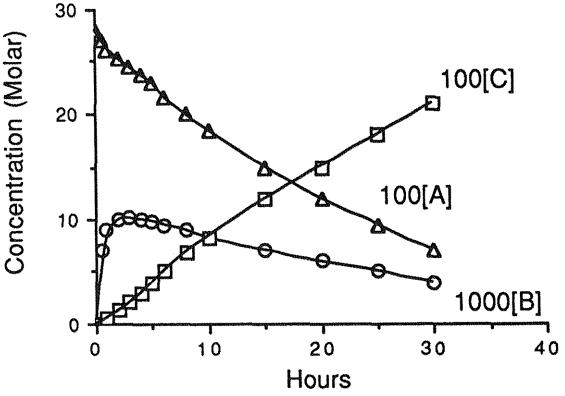

Fig. 5 The A → B part of cefatoxime photolysis. C_{total} has been obtained as 100-[A]-[B], but this does not account for the possibility of other reactions of C. (Graph constructed from data by Lerner et al., 1988.)

however, also to check the toxicity at intermediate points, because C might be toxic, but degrade into nontoxic products, and the toxicity of a partly degraded sample might be worse than that of a fully degraded sample).

The A → B → C reaction is rather common; for instance, it has been reported by Misra et al. (1993).

There continues to be, in present literature, reports of this type of reaction; for instance, Archontaki et al. (1998) reported on the decomposition of nordazepam and showed typical A–B–C plots with the A degradation being first order, the B profile having a maximum, and the C profile having the typical upswing. Burke et al. (1997) reported on the decomposition of theo-m-GLA and found it to be biexponential.

Buur and Bundgaard (1984) and Beal et al. (1993, 1997) reported that the hydrolyses of 3-acetyl- and 3-propionyl-5-FU were biexponential and found that an initial equilibrium of 3-acyl-5FU with O^2-acyl-5FU, which then hydrolyzed to 5-FU, explained this.

4.2. Parallel Reactions

If A can decompose into two species, B and C, then the reactions may be represented by:

$$A \rightarrow B \qquad \text{(rate constant } k_1\text{)} \tag{2.20}$$

and

$$A \rightarrow C \qquad \text{(rate constant } k_2\text{)} \tag{2.21}$$

The rate equation is

$$\frac{dA}{dt} = -k_1[A] - k_2[A] = -(k_1 + k_2)[A] \tag{2.22}$$

Table 2.4 Parallel Reactions (5-azacytosine decomposition)

Time (hours)	5-azacycytosine ($\times 10^4$ Molar)	5-azouracil ($\times 10^4$ Molar)	Nonchromophoric compounds ($\times 10^4$ M)
0	1.65	0	0
0.5	1.4	0.13	0.3
1	1.18	0.20	0.5
1.5	1.00	0.25	0.6
2	0.85	0.27	0.67
2.5	0.72	0.29	0.72

Source: Table constructed from data by Notari and de Young (1975).

which integrates to

$$\ln\left[\frac{A}{A_0}\right] = -[k_1 + k_2]t \tag{2.23}$$

At any given time the (molar) ratio of formation of B and C is given by

$$\frac{[B]}{[C]} = \frac{k_1}{k_2} \tag{2.24}$$

An example of this is shown in Table 4 and Fig. 6.

Other examples are those of Visconti et al. (1984), who have studied the degradation profile of cadralazine in aqueous solution. The reaction consists of four parallel reactions. Fabre et al. (1984) have shown that 3-acetoxymethylcephalosporin, cefotaxime sodium salt, in aqueous solution, decomposes by the scheme

$$\text{Cefotaxime} \xrightarrow{(k_2)} \text{lactone} \xrightarrow{(k_3)} \text{products}$$

$$\text{Cefotaxime} \xrightarrow{(k_2)} \text{products}$$

i.e., a combination of a parallel and a consecutive reaction.

5. EQUILIBRIA

Frequently a reaction will proceed and level off. In such cases there is often an equilibrium:

$$A \longleftrightarrow B \tag{2.25}$$

with an equilibrium constant, K, given by

$$\frac{[B]}{[A]} = K \tag{2.26}$$

Denoting the forward rate constant $k_>$ and the backwards rate constant $k_<$ it follows that when equilibrium has been achieved (at $t = \infty$), the amount going to the right in the reaction must equal the amount going to the left, i.e.

$$k_>[A] = k_<[B] \tag{2.27}$$

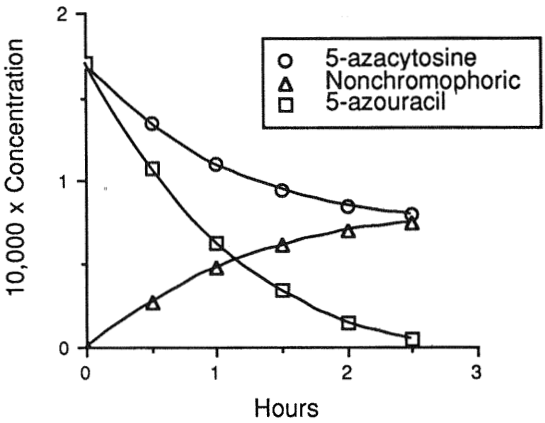

Fig. 6 Example of parallel reactions: decomposition of azacytosine. (Graph constructed from data published by Notari and deYoung, 1975.)

or

$$\frac{k_>}{k_<} = \frac{[\mathrm{B}]}{[\mathrm{A}]} = K \tag{2.28}$$

Denoting by A_∞ the infinity concentration of A (and hence by $A_0 - A_\infty$ the infinity concentration of B), Eq. (2.27) may be written

$$k_>[A_\infty] = k_<[A_0 - A_\infty] \tag{2.29}$$

which may be rewritten

$$A_\infty = \frac{A_0 k_<}{k_< + k_>} \tag{2.30}$$

The rate equation for Eq. (2.25) is

$$\begin{aligned} -\frac{dA}{dt} &= k_>[\mathrm{A}] + k_<[\mathrm{B}] = k_>[\mathrm{A}] + k_<[A_0 - A] \\ &= k_>[\mathrm{A}] + k_<[A_0] - k_<[\mathrm{A}] \\ &= [k_> + k_<]\{A - A_\infty\} \end{aligned} \tag{2.31}$$

which integrates to

$$\ln[A - A_\infty] = [k_> + k_<]t + \ln[A_0 - A_\infty] \tag{2.32}$$

or

$$\ln\left[\frac{A - A_\infty}{A_0 - A_\infty}\right] = -[k_> + k_>]t \tag{2.33}$$

The work regarding the hydrolysis of hydrocortisone butyrate by Yip et al. (1983) is an example of this type of decomposition combined with an $\mathrm{A} \rightarrow \mathrm{B} \rightarrow \mathrm{C}$

Table 2.5 Decomposition of Progabide in pH 1.75 Buffer

Time (min)	Concentration C (Molar)	ln[C-0.0055]
0	0.00896	−5.666
7	0.0076	−6.166
13	0.00700	−6.502
19	0.00660	−6.812
25	0.00612	−7.386
31	0.00600	−7.601

Source: Table constructed from data published by Farraj et al. (1988)

reaction, with the equilibrium occurring between A and B. Ghebre-Sellassie et al. (1984) have described the epimerization of benzylpenicilloic acid in alkaline media and shown it to be an equilibrium between 5R,6R-benzylpenicilloic acid with penamaldic acid (enamine).

Table 5 and Fig. 7 show data by Farraj et al. (1988) showing a leveling effect. If the data in Table 4 represent a simple equilibrium, then the equilibrium level could be obtained by iteration, by assuming different values for the equilibrium level and choosing the one giving best linearity in the form

$$\ln[C_\infty - C] = -kt + \ln[C_\infty - C_0] \tag{2.34}$$

In this case $C = 0.0055$ and the data are plotted in this fashion in Fig. 8, but it should be underscored that they have simply been used as an example. The alkaline hydrolysis of chlorambucil (Owen and Stewart, 1979) is another example of an equilibrium situation.

Beal et al. (1993) tested the hydrolysis of 3-acetyl-5-fluorouracil and showed equilibrium kinetics, as did Prankerd et al. (1992) in the case of rifampicin.

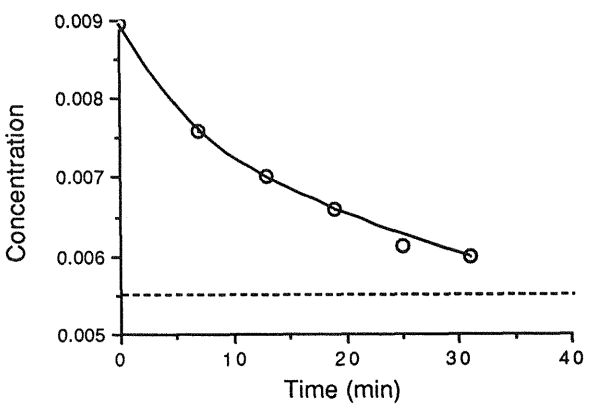

Fig. 7 Decomposition of progabide in pH 1.75 buffer. (Graph constructed from data reported by Farraj et al., 1988.)

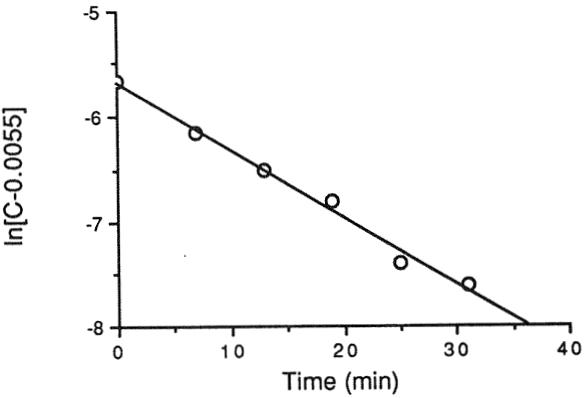

Fig. 8 Data from Fig. 6 treated using a niveau level of $C = 0.0055$. Least squares fit is $y = -0.764 = 0.0127x$ ($R = 0.97$). (Graph constructed from data reported by Farraj et al., 1988.)

5.1. Steady-State Situations

If a situation occurs where $A \rightarrow B \rightarrow C$ and the latter is fast, the kinetics can be simplified by assuming that [B] is "at steady state" throughout the time course. This, obviously cannot be true at the onset. The equations governing this situation are

$$\frac{d[A]}{dt} = -k_1[A] \tag{2.35}$$

$$\frac{d[B]}{dt} = k_1[A] - k_2[B] = 0 \tag{2.36}$$

$$\frac{d[C]}{dt} = k_2[B] \tag{2.37}$$

where the steady state has been imposed by setting the expression in Eq. (2.36) equal to zero. Hence

$$[A] = A_0 e^{-k_1 t} \tag{2.38}$$

and since it follows from Eq. (2.36) that

$$[B] = \frac{k_1[A]}{k_2} \tag{2.39}$$

then Eqs. (2.38) and (2.39) inserted into Eq. (2.37) give

$$\frac{d[C]}{dt} = k_1 e^{-k_1 t} \tag{2.40}$$

which integrates to

$$C = C_\infty[1 - e^{-k_1 t}] \tag{2.41}$$

i.e., the reaction occurs as if B were not in the picture at all.

In general, if there is such a fast step in the first step of a complex reaction, it is not incorrect to consider it an A–B–C reaction.

The same arguments can be made in the case of A → B → C → D reaction where the B → C was much more rapid than the others, and in such a case it would be justified to think of this as an A → C → D reaction. To be more exacting, a steady-state approach would probably be better. It should be pointed out however, that the steady-state approach is a fundamental approximation, and if it is used, then the reasonableness of the approximation should always be checked.

The steady-state approach is often used, particularly in Michaelis–Menten type kinetics. Here, as an example, let us consider the situation, often occurring, that *many* low level decomposition products are encountered. There are different regulatory views on this, one being that no more than 1% of a product may be formed for it to be considered a minor decomposition product. The situation is hazy, at best, at all times, because often the compounds are unknown. In such cases the "amount" of the decomposition product in the small peak is estimated by the ratio of its area to that of the main peak. But if the decomposition product has a different λ_{max} then this estimate is incorrect, and this is likely to occur if, for instance, in an HPLC setup a single-wavelength UV detector is used.

A well documented and elucidated example is the case reported by Vilanova et al. (1994), who showed in alkaline hydrolysis of cefotaxime the presence of deacetylcefotaxime, the 7-epimer of cefotaxime, the 7-epimer of deacetylcefotaxime, the exocyclic methylene compound, and examine compounds. With such an array of decomposition products, it is important to establish the major products, and treat, in approximation, the decomposition in this light. In the simplest case, cefotaxime shows an A–B–C and A–D–E reaction, with two B curves and three C curves.

Another case that serves as such an example is the case of relaxin oxidation by hydrogen peroxide reported by Nguyen et al. (1993) shown in Fig. 9 where there are two intermediates (B and C) showing maxima and a final product, D, showing the monotonically increasing pattern.

6. PSEUDO-ZERO-ORDER REACTIONS

When only small amounts of decomposition occur, it is difficult to distinguish between zero- and first-order reactions. This is because for small values of x (< 0.15)

$$\ln[1 - x] \approx -x \tag{2.42}$$

where x is the fraction decomposed. If the initial amount of drug substance is A_0, then the fraction decomposed is

$$\frac{A_0 - A}{A_0} = 1 - \frac{A}{A_0} = x \tag{2.43}$$

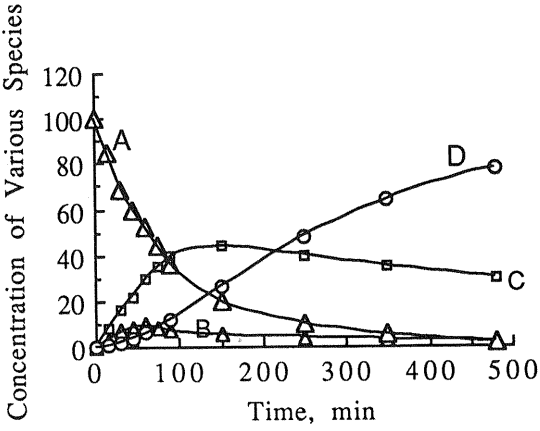

Fig. 9 Decomposition of an A → B; A → C; B → C → D reaction. (Graph constructed from data published by Nguyen et al., 1993.)

or

$$\frac{A}{A_0} = 1 - x \tag{2.44}$$

A first order reaction would require that

$$\ln \frac{A}{A_0} = -kt \tag{2.45}$$

but this may, via Eq. (2.37), be written

$$\ln[1 - x] \approx -x = -kt \tag{2.46}$$

or

$$x = 1 - \frac{A}{A_0} = kt \tag{2.47}$$

which may be written

$$A = A_0 - A_0 kt \tag{2.48}$$

i.e. a zero-order reaction. Since it actually was a first-order reaction [Eq. (2.45)] such a situation is referred to as a pseudo-zero-order reaction.

A set of data is shown in Table 6, treated in zero-order fashion in Fig. 10A and in first-order fashion in Fig. 10B; and it is seen that the fits are comparable. The least squares fit data are shown in Table 6.

It is noted that different time intervals are used for the different temperatures, and it is one of the tasks, before starting studies at higher temperatures, to establish what the time intervals should be. There is no sense in e.g. testing at 3, 6, and 9 months at 55°C, if all the drug is lost after 3 months' storage.

Table 2.6 Assays for an Arrhenius Study

	Potency (°C)				
Months	15	25	37	45	55
0	100	100	100	100	100
3		99.5	98.5	96.5	91
6		99	97	93	83
9		98.5	95.5		
12	99.5	98.5			
18		97.5			
24	99.0	96.5			
36	98.5				
48	98.0				
k_0 %/mo	0.042	0.0017	0.05	1.17	3
$\ln[k_0]$	−3.17	−1.77	−0.69	0.16	1.10

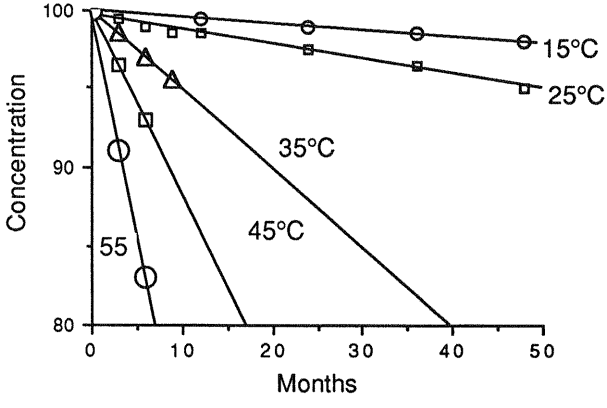

Fig. 10A Data from Table 5 treated by zero-order kinetics.

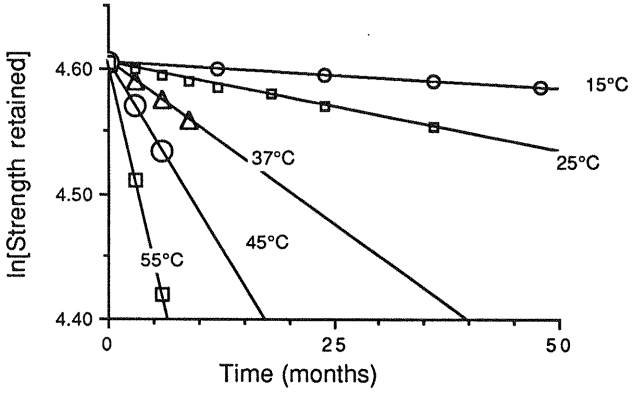

Fig. 10B Data from Table 5 treated by first-order kinetics.

7. THE ARRHENIUS EQUATION

Rate constants are, of course, a function of temperature, and the data shown in Table 5 are graphed in Figs. 10A and 10B.

If rapid results are desired for a given product, it is at times a practice to store it at elevated temperatures. The purpose of this is to force sufficiently large degrees of decomposition in a short time, so that they may be assessed with accuracy. The data in Table 6 are artificially precise, and with a bit of assay error, the 25°C data would not show a discernible loss after 6 months. Is it possible to get some idea of what the loss would actually be, and what it would be after 24 months, without having to wait too long? To get an answer to this (an estimate, not a precise answer) is one of the reasons that Arrhenius plotting is carried out for drug products. The method is actually quite precise in solution systems.

The temperature dependence of a chemical reaction (as long as it is the rate-determining rate constant that is being treated) follows the so-called Arrhenius equation given by

$$\ln[k] = -\frac{E_a}{RT} + \ln[Z] \tag{2.49}$$

or its antilogarithmic form,

$$k = Z \exp\left[-\frac{E_a}{RT}\right] \tag{2.50}$$

where E_a is the activation energy, R is the gas constant, and T is the absolute temperature (°K) obtained by adding 273.15° to the degrees Celcius (Centigrade).

Often the variable $1000/T$ is used (because the numbers then are between 2 and 4 rather than between 0.002 and 0.004 and hence are easier to handle). The slope of a plot according to Eq. (2.42) is still E_a/R, but E_a will now be in kCal (rather than in cal) per degree per mole.

An example of this type of treatment of the first-order data in Fig. 10A is shown in Table 7.

A similar table may be constructed for zero-order treatment, and the graphical presentation is shown in Fig. 10A. When the rate constants are plotted according to Eq. (2.49), then Fig. 11 emerges.

Table 2.7 Least Squares Fit Parameters from Fig. 10B

Temperature °C	Temperature °K	k (mo^{-1})	$1000/T$	$\ln[k]$
15	288.15	0.00042	3.473	−7.783
25	298.15	0.0014	3.356	−6.571
37	400.15	0.0052	3.224	−5.259
45	408.15	0.0118	3.145	−4.440
55	418.15	0.031	3.049	−3.471

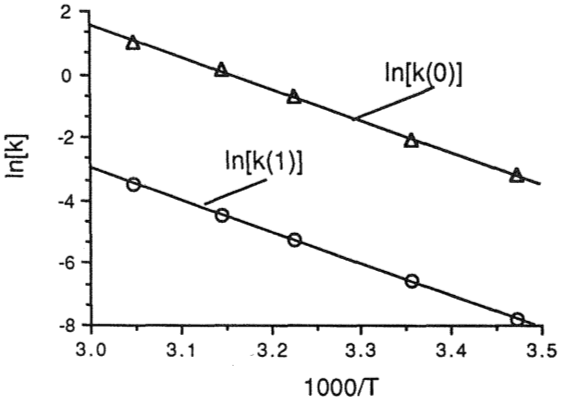

Fig. 11 Data from Figs. 10 A and B treated by the Arrhenius equation.

The Arrhenius equations for the two types of plotting are

$$\ln[k_0] = 31.63 - 10.03(1000/T) \tag{2.51}$$

$$\ln[k_1] = 27.631 - 10.2(1000/T) \tag{2.52}$$

It is noted that both plotting modes give good results and about the same activation energy. Using a value of $R = 1.99$ cal/degree-mole gives an activation energy of about 20 kCal/mole. *This is quite a common activation energy for many reactions.*

If only the data at the three high temperatures had been present, they could have been plotted as in Fig. 11 and extrapolated to 25°C (where $1000/T = 3.356$). Inserting this into Eq. (2.49) gives

$$[k_{25}] = 27.631 - 10.2 \times 3.356 = -6.601 \text{ or } [k_{25}] = 0.00136$$

which is close to the value in Table 7.

One can now construct a curve, as shown in Fig. 12, which is an extrapolated curve. The data points from Table 6 are shown for comparison. In general, extrapolations are not that good, but in solution systems they frequently approximate the curve that, after time has elapsed, is the actual curve. A case in point is flurogestone decomposition at pH 7.3 reported by Kabadi et al. (1984).

Arrhenius plotting can also be carried out by using t_{90} data. Since $k_1 t_{90} = -0.105$ it follows that

$$\ln[k_1 t_{90}] = \ln[t_{90}] + \ln[k_1] = \ln[t_{90}] - \frac{E_a}{RT} + \ln[Z] = -0.105$$

or

$$\ln[t_{90}] = \frac{E_a}{RT} - \ln[Z] - 0.105 \tag{2.53}$$

i.e. a plot of $\ln[t_{90}]$ versus $1000/T$ will be linear and the slope will be E_a/R, where E_a will be in kCal (rather than in cal) per degree per mole.

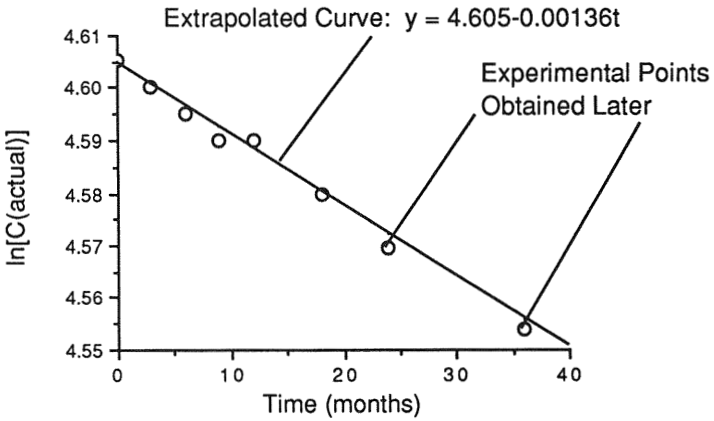

Fig. 12 Extrapolated decomposition curve of a solution obtained (e.g., in January 1993 after three months accelerated data) compared with the actual data accumulated in time and plotted 33 months later (i.e., in October 1995).

Table 2.8 Data from Table 6 Plotted by the t_{90} Method

Temperature	$1000/T$	k_1 (mo^{-1})	t_{90} (months)[a]	$\ln[t_{90}]$
55°C	3.049	0.031	3.40	1.233
45°C	3.145	0.0118	8.93	2.189
37°C	3.224	0.0052	20.26	3.009

[a] Calculated from k_1 values from the equation $kt_{90} = -0.1054$.

Table 8 shows the data from Tables 6 and 7 plotted by the t_{90} method. The data are shown in Fig. 13. The least squares fit equation in Fig. 13 is $\ln[t_{90}] = -29.695 + (10.142/T)$, and it is noted that the slope (activation energy) is the same as in Fig. 11 (where the slope is 10.153). The small difference lies in rounding off errors in calculating t_{90} from the k_1 values.

The t_{90} value at room temperature is determined to be 4.072 [Eq. (2.53)]. If plotted on semilogarithmic paper, the value will emerge directly, and this type of plotting is more easily understood by those not familiar with kinetics than plots of the type in Fig. 11.

7.1. Cyclic Testing

One advantage of testing at higher temperatures is that it is possible to construct decomposition profiles at nonconstant temperature. It should be pointed out that extended room temperature is defined in the USP, 1990, as between 15 and 30°C but is now 15–25°C. Stability studies are usually carried out isothermally, e.g., as mentioned, at 25°C. The rationale for testing temperatures will be discussed at a later point in the book; suffice it to say at this point that a product in the marketplace will never experience an isothermal shelf history.

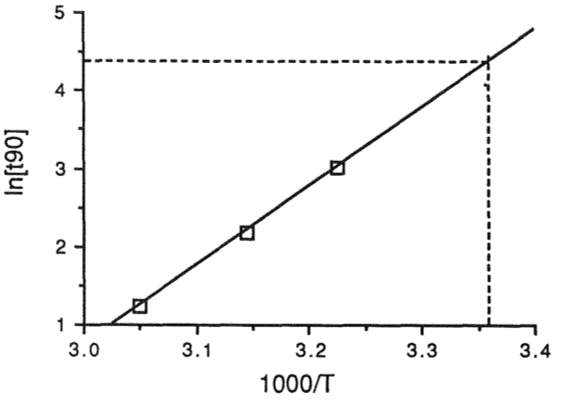

Fig. 13 Arrhenius plotting using t_{90}.

At best there will be daily fluctuations. This concept was investigated by Carstensen and Rhodes (1986), in the following fashion. Suppose a product is stored at 25°C with daily fluctuations of ±5°C. This means that the dependence of T on time, t (in days, if the cycle is one day), is given by

$$T = T_1 + T_2 \sin(2\pi t) \tag{2.54}$$

This is shown in Fig. 12.

Assume, as well, that the reaction is zero order, i.e.

$$C = C_0 - kt \tag{2.55}$$

Introducing Eqs. (2.50) and (2.54) into this and applying the situation to a differential time element dt, gives

$$dC = \left[Z \exp\left[-\frac{E}{R\{T_1 + T_2 \sin(2\pi t)\}}\right]\right] dt \tag{2.56}$$

so that to obtain the concentration after a time period t of cyclic storage, where $0 < t < 1$ day, is given by

$$C = C_0 - \int_0^t Z \exp\left(-\frac{E}{(R\{T_1 + T_2 \sin(2\Pi t)\}}\right) \tag{2.57}$$

These types of integrals can be solved by computer programs. The loss in e.g. one year will be 360 times that after one day, and so on.

The authors calculated the loss after 3 years of storage, using different activation energies, and using k_{25} (isothermal) = 0.01%, and using a daily cycle with a fluctuation of ±5°C. They arrived at the results in Table 9.

It is seen (Table 9, Fig. 14) that as long as the activation energy is less than 22 kCal per mole, the percent increase in the amount lost after a given storage period is less than 10%. For example, if a dosage form lost 5% after 3 years storage at static room temperature, it would lose 5.5% after 3 years of cyclic room temperature. This point will be of importance in the following section.

Table 2.9 Cyclic Versus Constant 25°C Data. $k_{25} = 0.01\%$ Per Day

E, kCal per mole	Loss after 3 years	Percent increase in loss
10	11.16	1.9
15	11.38	3.9
20	11.77	7.5
25	12.27	12.1
30	12.89	17.7

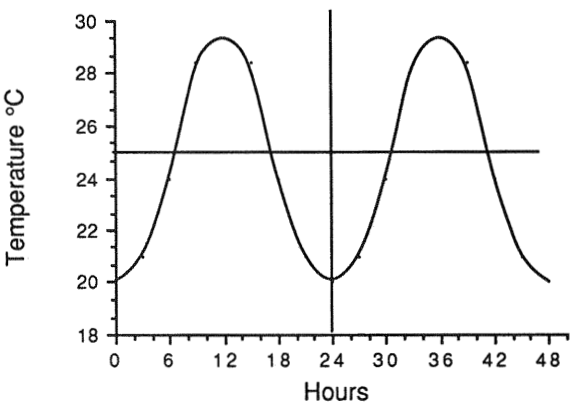

Fig. 14 Daily temperature fluctuations according to Eq. (2.54).

It is noted that any type of cycle can be used, e.g. seasonal cycles could be used as well. The problem of cyclic testing (for chemical stability) is raised from time to time. But to decide on a cycle is difficult, and it is much more rational to use the data from accelerated studies to produce the desired profile.

7.2. Nonisothermal Kinetics

It is possible, rather than studying a reaction at a fixed temperature (isothermally), to vary the temperature in a given fashion, and fit the data to Eq. (2.50).

For a zero-order reaction we can write

$$\frac{C_0}{C} = kt = \left[Z \exp\left(-\frac{E_a}{RT} \right) \right] \cdot t \tag{2.58}$$

We may allow T to vary in a given manner, e.g., in the simplest case as

$$\frac{1}{T} = a - bt \tag{2.59}$$

where a and b are the constants that we input into a programmable temperature

Table 2.10 Alkaline Decomposition of Riboflavin by Isothermal and by Several Nonisothermal Programs

Temperature program	Rate constant at 25°C	Activation energy (kcal/mole)	Reference
Isothermal	0.016	19.2	Cole and Leadbeater (1966)
Linear up	0.014	20.1	Guttman (1962)
Linear up	0.016	20.3	Rosenberg et al. (1984)
Log up	0.018	17.9	Madsen et al. (1974)
Log up[a]	0.015	20.9	Rosenberg et al. (1984)
Log down	0.015	18.9	Rosenberg et al. (1984)

[a] Triplicate experiments.
Source: Table constructed from data published by Rosenberg et al. (1984).

bath. By inserting Eq. (2.59) into Eq. (2.58), the concentration profile in time becomes

$$\frac{C_0}{C} = Z\exp\left[-\frac{E_a}{R}(a - bt)\right] \tag{2.60}$$

or, logarithmically,

$$\begin{aligned}\ln\left[\frac{C_0}{C}\right] &= \ln[Z] - \frac{E_a}{R}(a - bt) \\ &= \left[\ln\{Z\} - \frac{aE_a}{R}\right] + \left(\frac{bE_a}{R}\right)t\end{aligned} \tag{2.61}$$

This gives rise to a linear plot when $\ln[C_0/C]$ is plotted versus t, and (since a and b are the constants for the program we have chosen for our temperature bath), the slope (divided by b/R) will give us the value of E_a; and now $\ln[Z]$ can be obtained from the intercept.

The same procedure can be used for first-order reactions, although they are somewhat more complicated. In such a case a computer program is best, and curves can be generated to match the curve obtained experimentally.

Table 10 shows some of the investigations that have been carried out in the last 20 years, using this procedure.

7.3. Kinetic Mean Temperature

Since stability studies carried out in an industrial setting are isothermal, there has been a fair amount of discussion over the last 2 decades as to what the actual temperature of the study ought to be. Up to 1993, the FDA required stability studies to be carried out at 30°C for the approval of expiration periods. In contrast, the European community would consider the United States as an area where 25°C would be the appropriate temperature to require for isothermal testing.

The resolution of the problem in Europe came from by Futscher and Schumacher (1972), who established the climate zones, and Wolfgang Grimm (1985,