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Statistical evaluation of non-isothermal prediction of drug stability. II. Experimental design for practical drug products

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Summary

Reliability of the non-isothermal method for stability prediction of practical drug products was evaluated by the Monte Carlo method in comparison with the isothermal method. In addition to the error in measurement of drug contents, the error in temperature control was taken into account as an element of experimental error. From the simulated experimental data (simulated up to 20% degradation), expiration dating periods at 25 and 40 °C ($t_{90(25)}$ and $t_{90(40)}$) were estimated as kinetic parameters. Non-linear temperature programs expressed by power functions of time were employed, as well as linear ones. Examination of the sampling distributions for the estimates obtained indicated that if appropriate temperature programs were employed, the non-isothermal method could provide more reliable estimates of $t_{90(25)}$ and $t_{90(40)}$ than the isothermal method in some cases; especially when the dependence of the degradation rate on temperature showed non-linear Arrhenius plots. Typical experimental designs were proposed for the non-isothermal method which were applicable to stability prediction of drug products whose shelf-lives have a wide range. The usefulness and limitations of the non-isothermal method in prediction of drug stability are herein discussed.

Introduction

Non-isothermal kinetic methods have been applied to accelerated stability testing of pharmaceuticals, and the shelf-lives predicted have been reported to coincide with those predicted by isothermal methods in many cases (Tucker and Owen, 1982; Hempenstall et al., 1983; Inaba and Mizutani, 1986). The statistical reliability of their methods, however, has not been examined.

In a previous paper (Yoshioka et al., 1987) we developed an equation to allow direct estimation of shelf-life and activation energy from the data obtained under non-isothermal conditions, and evaluated the reliability of the non-isothermal method by the Monte Carlo method of computer simulations. Examination of the estimates obtained under linear temperature programs indicated that the non-isothermal method was superior to the isothermal method in some aspects such as reduction of the experimental period. The accuracy of the estimates obtained by the non-isother-

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mal method, however, depended largely on the experimental conditions, and was governed mainly by the extent of drug degradation and the temperature range achieved during the experiment. It was suggested there that non-isothermal experiments designed without careful consideration might provide meaningless estimates.

Regardless of the several merits of the non-isothermal method that were indicated, some problems remain to be considered before the non-isothermal method is applied to stability prediction of drug products, whose self-lives have a wide range and which may degrade in various reaction orders under various mechanisms.

First, change in the reaction orders accompanied by change of mechanism which possibly occurs in the course of degradation cannot be adequately analyzed from the data obtained under non-isothermal conditions. Degradation mechanisms may be changed by accumulation of degradation products which can accelerate or inhibit the drug degradation. In addition, water adsorption or desorption which occurs in the course of degradation may affect the physical properties of drug products, resulting in acceleration or inhibition of drug degradation. Considering the possibility of such changes in degradation mechanisms, a shelf-life which depends on the initial degradation rate should be estimated based on the data observed in the initial stage of degradation.

Second, non-isothermal experiments tend to include larger errors in temperature control than isothermal experiments. Therefore, the effect of errors in temperature control should also be taken into account in addition to the errors in measurement of the drug contents when the reliability of the method is compared with that of the isothermal method. (In the previous study (Yoshioka et al., 1987), temperature was assumed to be well-controlled and to rise exactly as programmed.)

On the other hand, the kind of kinetic parameters which should be estimated in the treatment of accelerated stability data is an important point in obtaining practical information on the stability properties of drug products. A parameter set of shelf-life and activation energy, which can be estimated directly from the accelerated data by non-linear regression analysis, is considered to be a more practical set of kinetic parameters in indicating drug stability than a parameter set of frequency factor and activation energy as estimated by the classical linear regression analysis of the Arrhenius equation (King et al., 1984). For practical purposes, however, activation energy is less useful as a parameter in indicating drug stability, and can be replaced by the expiration dating period at 40 °C (the time period required for a drug to degrade to 90% of original content at 40 °C), which can provide more practical information about the stability properties.

The present work was undertaken to examine appropriate experimental designs for the non-isothermal method, considering the above-mentioned problems involved in non-isothermal methods, and introducing the expiration dating period at 40 °C as an alternative parameter to activation energy. The reliability of the proposed method is evaluated by the Monte Carlo method and compared with the isothermal method. The usefulness and limitations of the non-isothermal method in prediction of drug stability will be discussed.

Theory

Kinetic parameters, shelf-life ($t_{90(25)}$) and activation energy (E_a) for a first-order degradation under non-isothermal conditions can be estimated non-linearly by the following equations, as reported in the previous paper (Yoshioka et al., 1987),

$$C = C_0 \exp\left[\frac{-0.1054}{t_{90(25)}} \cdot \exp\left(\frac{E_a}{R \cdot 298}\right) \cdot I\right] \quad (1)$$

$$I = \int_0^t \exp\left(\frac{-E_a}{R \cdot T(t)}\right) dt \quad (2)$$

where C_0 and C are drug contents at time zero and time t, and $T(t)$ is the absolute temperature at time t. Since E_a is given by Eqn. 3 employing $t_{90(25)}$ and the expiration dating period at 40 °C

($t_{90(40)}$), Eqns. 1 and 2 can be expressed by Eqns. 4 and 5.

$$E_a = \frac{R \cdot \ln(t_{90(25)}/t_{90(40)})}{(1/298 - 1/313)} \quad (3)$$

$$C = C_0 \exp\left\{ \frac{-0.1054}{t_{90(25)}} \times \exp\left[\frac{\ln(t_{90(25)}/t_{90(40)})}{(1/298 - 1/313) \cdot 298} \right] \cdot I \right\} \quad (4)$$

$$I = \int_0^t \exp\left[\frac{-\ln(t_{90(25)}/t_{90(40)})}{(1/298 - 1/313) \cdot T(t)} \right] dt \quad (5)$$

Eqns. 4 and 5 can be used for direct non-linear estimation of $t_{90(25)}$ and $t_{90(40)}$. Much the same as in Eqn. 4, equations for zero and second-order degradations are derived by substituting Eqn. 3 for E_a in Eqns. 6 and 7, respectively.

$$C = C_0 \left[1 - \frac{0.1}{t_{90(25)}} \cdot \exp\left(\frac{E_a}{R \cdot 298} \right) \cdot I \right] \quad (6)$$

$$C = \frac{C_0}{1 + \frac{0.111}{t_{90(25)}} \cdot \exp\left(\frac{E_a}{R \cdot 298} \right) \cdot I} \quad (7)$$

On the other hand, equations for degradation under isothermal conditions have been reported by King et al. (1984). They developed Eqn. 8 for a first-order degradation.

$$C = C_0 \exp\left\{ \frac{-0.1054}{t_{90(25)}} \exp\left[\frac{E_a}{R} \left(\frac{1}{298} - \frac{1}{T} \right) \right] \cdot t \right\} \quad (8)$$

The estimates of $t_{90(25)}$ and $t_{90(40)}$ can be obtained directly according to the equation obtained by substituting Eqn. 3 for E_a in Eqn. 8.

Materials and Methods

Computer simulation of the Monte Carlo method was carried out in a manner similar to that described previously (Yoshioka, et al., 1987). For each experimental design, 300 sets of simulated data were generated by adding errors in experiments to the theoretical values of drug contents calculated according to Eqns. 4, 6, 7 or 8. Drug content was expressed as percent of the initial content which was assumed to be constant. Linear and non-linear temperature programs, $T(t)$ were employed for the non-isothermal experiment. Non-linear temperature programs were expressed by power functions of time ($T = 298 + kt^n$, where k and n are constants).

Random numbers for assay variance were selected from a normal distribution with a mean of zero and a prescribed standard deviation (1–3% of the initial drug content), and added to the theoretical values of drug content. Errors in temperature control were taken into account in addition to errors in measurement of drug contents. Random numbers selected from a normal distribution with a mean of zero and a prescribed standard deviation (0.2, 0.5 or 0.7 °C) were added to the theoretical values of temperature $T(t)$. Temperature was assumed to vary at the interval corresponding to 1/10 of the sampling interval. The errors of temperature both in non-isothermal and isothermal experiments were treated as cumulative errors in Eqn. 2 (Mandel, 1957). For isothermal experiments, T in Eqn. 8 was considered to be variable and drug content C was calculated using Eqns. 1 and 2.

We investigated also another type of temperature error. It was assumed that the rate of temperature increase is varied in each non-isothermal experiment. A single random number, expressed as percent, was selected from a normal distribution with a mean of zero and a prescribed S.D. (0.5–2%) for each experiment, multiplied by the difference between the theoretical value of temperature and the initial temperature, and added to theoretical value of temperature. In this case, isothermal experiments were assumed to include no temperature variance, and drug content was calculated by Eqn. 8.

Various types of drug degradation (zero, first- and second-order degradations) were simulated based on various theoretical values of shelf-life and activation energy, both of which have wide

ranges. Experimental data were generated until the drug contents decreased to 80% of the initial content.

Non-linear estimation of $t_{90(25)}$ and $t_{90(40)}$ was carried out by the iterative technique of the Simplex method according to Eqns. 4, 6, 7 or 8, as reported previously (Yoshioka et al., 1987). The BASIC program for a microcomputer (PC-9801 VMII) was used with some modifications. For each experimental design, 300 sets of estimates of $t_{90(25)}$ and $t_{90(40)}$ were obtained from 300 sets of simulated data, and the sampling distribution of the estimates was formed. The reliability of the estimates obtained for each experimental design was evaluated based on the mean and the S.D. of each sampling distribution.

Results and Discussion

Comparison of relatively long-term non-isothermal prediction with isothermal prediction at real temperature

The effect of temperature rise patterns on the accuracy and precision of the estimates was examined with linear and non-linear temperature programs, where temperature is expressed by power functions of time, $T = 298 + kt^n$ (k and n are constants). Since the experimental data obtained at temperatures close to room temperature (25°C) were found to contribute largely to the accuracy of the estimates of $t_{90(25)}$ in the previous study, the values of n were designed to be 1 or more.

Fig. 1 shows the temperature rise patterns for n equals 1–6 and the corresponding theoretical degradation curves of drug products, whose $t_{90(25)}$ and $t_{90(40)}$ are 156 and 20.6 weeks, respectively (i.e., E_a is 25 kcal/mol). The theoretical curves were calculated according to Eqns. 4 and 5, assuming the degradation is of first-order. The value of k was fixed so that the drug product degraded to 80% of the original content over 39 weeks. In the experiment, two samples were designed to be assayed every week.

The variance and the bias, defined as difference between the mean and the theoretical value, were

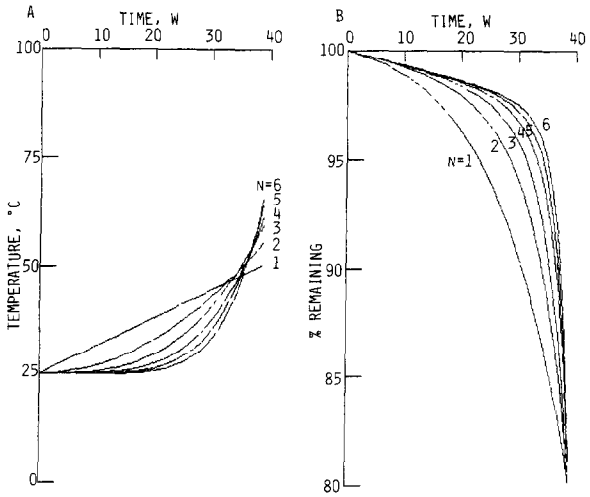

Fig. 1. Temperature programs (long-term) with various values of n ($T = 298 + kt^n$) (A) and the theoretical degradation curves for a drug product with 156 weeks of $t_{90(25)}$ and 20.6 weeks of $t_{90(40)}$ (B). The values of k were fixed so that the drug degraded to 80% remaining during 39 weeks.

calculated for each sampling distribution of the estimates of $t_{90(25)}$ and $t_{90(40)}$ obtained under each temperature condition, and are shown in Fig. 2. The variance of $t_{90(40)}$ increased with an increase of n, but that of $t_{90(25)}$ showed a minimum at

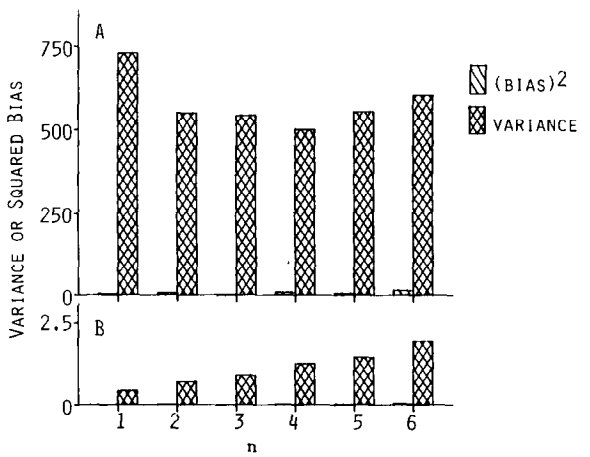

Fig. 2. Effect of temperature programs (long-term) on variance and squared bias of parameter estimates for $t_{90(25)}$ (A) and $t_{90(40)}$ (B). Theoretical values: $t_{90(25)} = 156$, $t_{90(40)} = 20.6$ weeks. Temperature program: $T = 298 + kt^n$. The values of k were fixed so that the drug degraded to 80% remaining during 39 weeks. Two samples were assayed every week. S.D. of assay: 2% of the initial content. S.D. of temperature: 0.5°C.

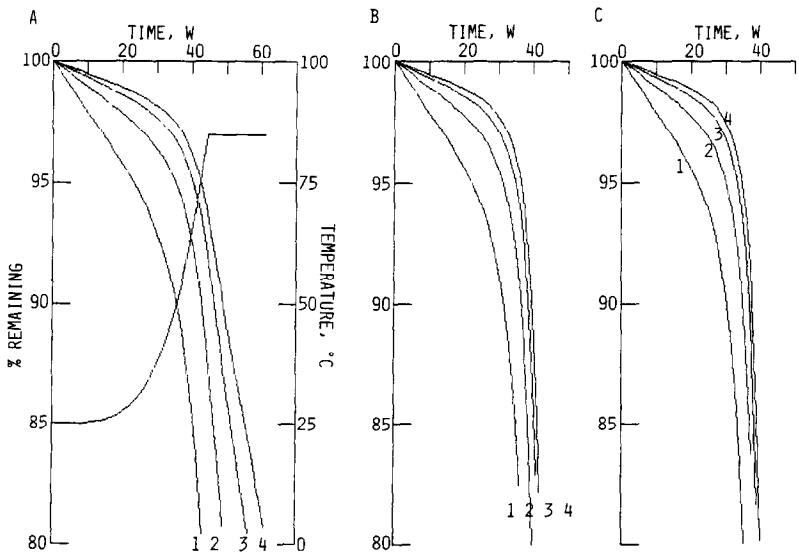

Fig. 3. Theoretical degradation curves under a temperature program proposed for long-term tests ($T = 298 + 1.56 \times 10^{-5} \cdot t^4$). Theoretical values: E_a = 10 (A), 20 (B) and 25 kcal/mol (C); $t_{90(25)}$ = 50 (1), 100 (2), 156 (3) and 200 weeks (4).

$n = 4$. Since the temperature program for $n = 4$ and $k = 1.56 \times 10^{-5}$ (deg/week^4) appeared to give relatively reliable estimates of $t_{90(25)}$ and $t_{90(40)}$, the program was chosen to apply to stability predictions of drug products which have various values of $t_{90(25)}$ and $t_{90(40)}$.

Fig. 3 shows the theoretical degradation curves simulated by assuming 50, 100, 156 and 200 weeks

TABLE 1

Comparison between the parameter estimates obtained by the relatively long-term non-isothermal method and those obtained by the isothermal method at real temperatures

Theoretical t_{90} (week)		E_a (kcal/mol)	Experimental period (week)	NONISO				ISO			
				$t_{90(25)}$		$t_{90(40)}$		$t_{90(25)}$		$t_{90(40)}$	
25 °C	40 °C			Mean (week)	RSD (%)	Mean	RSD	Mean	RSD	Mean	RSD
50	6.61	25	35	49.7	6.7	6.69	8.2	50.4	8.4	6.60	4.8
50	9.91	20	36	50.1	6.6	10.0	8.7	50.0	7.6	9.91	4.0
50	22.3	10	43	49.9	5.4	22.3	3.3	49.8	6.3	22.3	2.9
100	13.2	25	37	99.8	11.6	13.4	9.6	102.7	17.0	13.2	3.7
100	19.8	20	40	100.3	9.6	19.8	4.2	102.4	13.5	19.7	3.0
100	44.5	10	49	100.4	8.3	44.6	4.7	100.8	9.6	44.5	4.7
156	20.6	25	39	158.7	14.1	20.7	5.4	162.7	25.0	20.6	3.0
156	30.9	20	41	158.4	14.6	31.0	6.1	161.1	21.3	31.0	4.2
156	69.4	10	56	157.4	11.0	69.7	7.0	159.1	12.5	69.4	5.5
200	26.4	25	40	207.3	17.6	26.7	7.0	216.2	33.7	26.4	3.8
200	39.6	20	42	201.4	16.8	39.8	7.7	215.9	27.3	39.6	5.1
200	89.0	10	61	204.8	13.6	90.2	8.6	203.3	14.0	89.3	6.2

Parameters were estimated by the non-isothermal (NONISO) and the isothermal (ISO) method. Non-isothermal temperature program: $T = 298 + 1.56 \times 10^{-5} t^4$. Two samples were assayed every week. Standard deviation of assay: 2% of the initial content. Standard deviation of temperature: 0.5 °C.

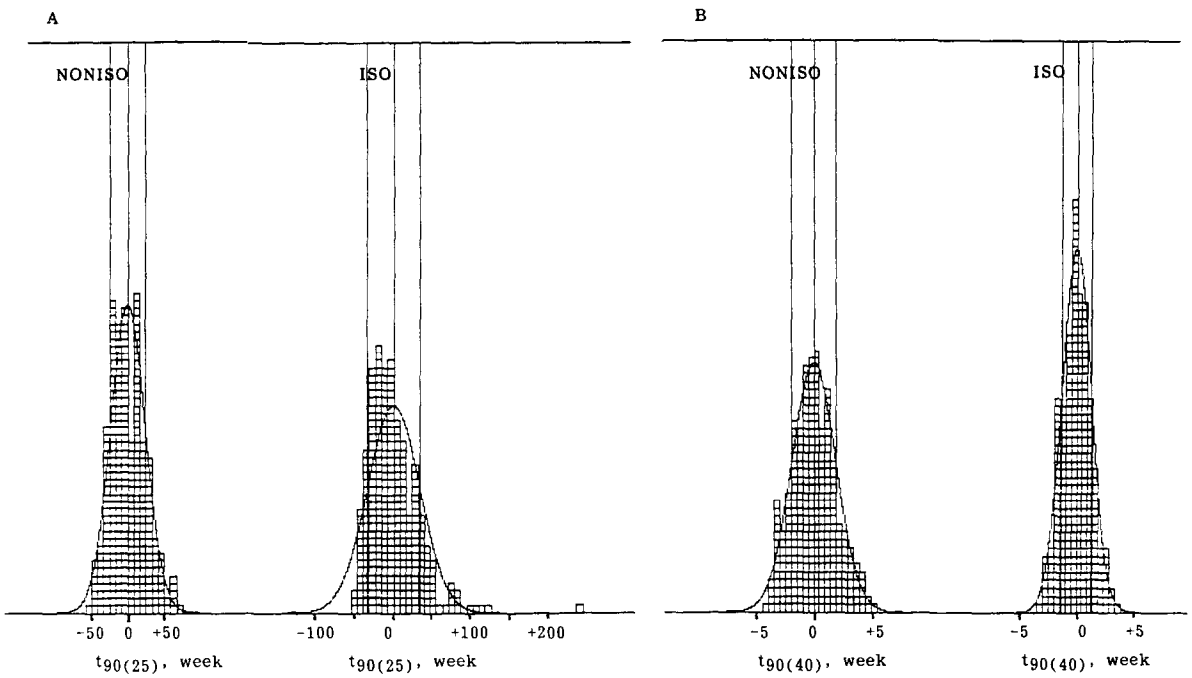

Fig. 4. Typical sampling distributions for the estimates of $t_{90(25)}$ (A) and $t_{90(40)}$ (B) obtained by the non-isothermal method (NONISO) and the isothermal method (ISO) for a drug product with 156 weeks of $t_{90(25)}$ and 30.9 weeks of $t_{90(40)}$. The mean of the estimates was taken to be zero. Temperature program: $T = 298 + 1.56 \times 10^{-5} t^4$. Two samples were assayed every week. S.D. of assay: 2% of the initial content. S.D. of temperature: 0.5 °C.

for $t_{90(25)}$, and 10, 20 and 25 kcal/mol (41.8, 83.7 and 104.6 kJ/mol) for E_a. Degradation for each case was followed up to 20% degradation. Temperature was designed to be constant after it had reached 85 °C. The estimates of $t_{90(25)}$ and $t_{90(40)}$ obtained for each drug product are listed in Table 1 and compared with those obtained under isothermal conditions. Isothermal estimation of $t_{90(25)}$ and $t_{90(40)}$ was carried out at real temperatures (i.e., 25 and 40 °C), but the Arrhenius relationship was not employed to obtain the estimates. The experimental period and the number of samples

TABLE 2

Parameter estimates obtained by the non-isothermal method for relatively unstable drugs

Theoretical t_{90} (week)		E_a (kcal/mol)	Period (week)	Estimate			
				$t_{90(25)}$		$t_{90(40)}$	
25 °C	40 °C			Mean (week)	RSD	Mean	RSD
10	1.98	20	8.8	10.0	4.7	2.01	9.2
20	2.64	25	9.2	20.0	7.9	2.66	5.8
20	8.90	10	11.6	20.0	6.3	8.90	3.4
52.1	6.89	25	10.0	53.2	14.4	6.94	5.9
52.1	23.2	10	15.8	52.8	11.1	23.4	7.2
75	9.93	25	10.2	77.3	19.8	10.0	8.8
75	33.4	10	18.6	77.4	16.8	34.0	11.1

Non-isothermal temperature program: $T = 298 + 0.004\ t^4$. Two samples were assayed every 0.2 weeks. S.D. of assay: 2% of the initial content. S.D. of temperature: 0.5 °C.

assayed at each sampling time were designed to be the same as in the non-isothermal method. In both cases, the relative standard deviation of the estimates obtained increased with an increase of the theoretical values of $t_{90(25)}$. The large relative standard deviation observed in the estimate for stable drug products, especially in the $t_{90(25)}$, suggests a difficulty in estimation of $t_{90(25)}$ for stable products. Though the estimates of $t_{90(40)}$ obtained under isothermal conditions showed smaller variance than those obtained under non-isothermal conditions, the isothermal method provided less reliable estimates of $t_{90(25)}$ with larger variance in all the cases studied. This is because the extent of drug degradation achieved at 25 °C during the experimental period was not sufficient to estimate $t_{90(25)}$. Typical sampling distributions for the estimates of $t_{90(25)}$ and $t_{90(40)}$ obtained by the isothermal and non-isothermal methods are shown in Fig. 4.

The temperature program with $n = 4$ can also be applied to stability predictions for relatively unstable drug products whose expiration dates are supposed to be 1 year. In this case, the coefficient k was fixed to be 0.004 so that the drug product, whose $t_{90(25)}$ and $t_{90(40)}$ are 52.1 weeks (1 year) and 6.89 weeks, respectively (i.e., E_a is 25 kcal/mol), degraded to 80% of the original content over 10 weeks. Table 2 shows the characteristics of sampling distribution for the estimates of $t_{90(25)}$ and $t_{90(40)}$ obtained for drug products whose theoretical $t_{90(25)}$ ranges from 10 to 75 weeks. In the experiment, two samples were assayed every 0.2 weeks up to 20% degradation. Fig. 5 shows the theoretical degradation curves simulated for each product, as well as the temperature rise program used.

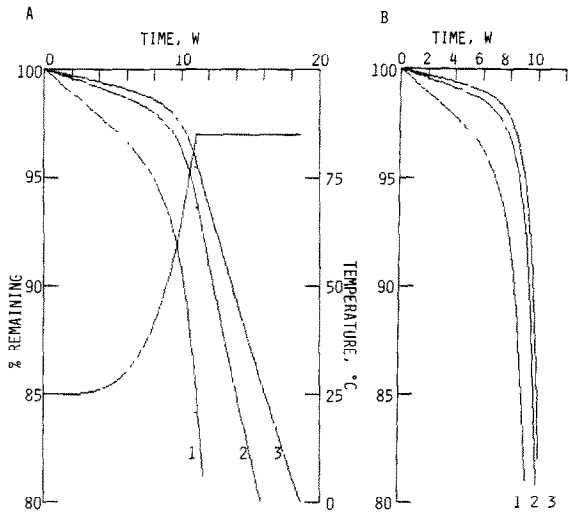

Fig. 5. Theoretical degradation curves under a temperature program proposed for relatively unstable drugs ($T = 298 + 0.004\,t^4$). Theoretical values: $E_a = 10$ (A) and 25 kcal/mol (B); $t_{90(25)} = 20$ (1), 52.1 (2) and 75 weeks (3).

Comparison of short-term non-isothermal prediction with isothermal prediction under elevated temperature conditions

As mentioned above, the non-isothermal method provided more reliable estimates of $t_{90(25)}$

TABLE 3

Comparison between the parameter estimates obtained by the short-term non-isothermal method and those obtained by the isothermal method at 4 levels of elevated temperature

Theoretical t_{90} (week)		Experimental period (week)	NONISO (4 samples) *				NONISO (8 samples) *				ISO (4 samples) *			
			$t_{90(25)}$		$t_{90(40)}$		$t_{90(25)}$		$t_{90(40)}$		$t_{90(25)}$		$t_{90(40)}$	
25 °C	40 °C		Mean (week)	RSD (%)	Mean	RSD	Mean	RSD	Mean	RSD	Mean	RSD	Mean	RSD
50	6.61	7.8	50.8	14.0	6.62	4.2	50.2	10.3	6.61	2.9	51.3	15.9	6.67	8.0
50	22.3	15.8	50.7	8.4	22.5	4.8	50.0	6.1	22.3	3.5	50.2	6.8	22.3	4.2
100	13.2	9.2	101.3	16.6	13.2	6.7	100.6	12.4	13.2	4.9	100.7	11.1	13.2	5.6
100	44.5	22.0	100.6	10.1	44.6	6.6	100.2	8.5	44.5	5.6	100.2	6.9	44.5	4.4
156	20.6	10.0	159.4	18.3	20.7	8.2	157.8	12.7	20.6	5.8	155.3	10.5	20.5	5.6
156	69.4	29.0	158.7	14.4	70.1	9.8	156.5	9.3	69.5	6.3	156.1	6.6	69.4	4.3

Non-isothermal temperature program: $T = 298 + 4t$. S.D. of assay: 2% of the initial content. S.D. of temperature: 0.5 °C.
* Four or 8 samples were assayed every 0.2 weeks.

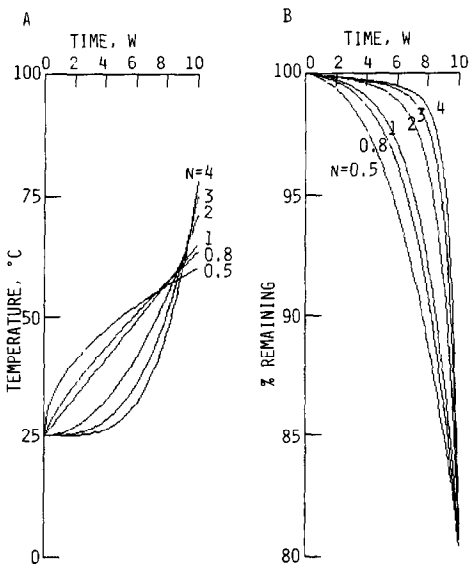

Fig. 6. Temperature programs (short-term) with various values of n ($T = 298 + kt^n$) (A) and the theoretical degradation curves for a drug product with 156 weeks of $t_{90(25)}$ and 20.6 weeks of $t_{90(40)}$ (B). The values of k were fixed so that the drug degraded to 80% remaining during 10 weeks.

than the isothermal method carried out at real temperatures (i.e., 25 and 40 °C), because the extent of drug degradation achieved under the isothermal condition (25 °C) was often too small to estimate the kinetic parameters. Isothermal stability study, however, can bring about a sufficient extent of degradation when it is carried out under elevated temperature conditions. For example, when isothermal study is carried out at 50, 60, 70 and 80 °C, the drug product, whose $t_{90(25)}$ is 156 and $t_{90(40)}$ is 20.6 weeks, degrades to 80% over 12.4, 3.8, 1.2 and 0.4 weeks, respectively. Therefore, the non-isothermal method was compared with the isothermal accelerated method under these higher temperature conditions. The experimental period for the non-isothermal method was normalized to the isothermal accelerated method. Fig. 6 shows the temperature rise patterns employed in the non-isothermal method and the corresponding theoretical degradation curves of a representative product whose $t_{90(25)}$ and $t_{90(40)}$ are 156 and 20.6 weeks, respectively. The temperature program with $n = 1$ and $k = 4$ was employed for the non-isothermal method because the estimates obtained by the program were found to be the most reliable among the programs studied, as shown in Fig. 7. Fig. 8 shows the theoretical degradation curves of the drug products having various theoretical values of $t_{90(25)}$ and $t_{90(40)}$ under this temperature condition. In the simulated experiments, 4 or 8 samples were assayed every 0.2 weeks until the drug had degraded to 80%. The isothermal experiment was carried out at 4 temperature levels (50, 60, 70 and 80 °C) and a sample was assayed every 0.2 weeks at each temperature. Table 3 shows the estimates of $t_{90(25)}$ and $t_{90(40)}$ obtained by the non-isothermal method in comparison with those by the isothermal method. The former showed larger variance. Twice as many samples (i.e., 8 samples) seemed to be required to obtain estimates with the same level of S.D. as those by the isothermal method, except for the drug product which was

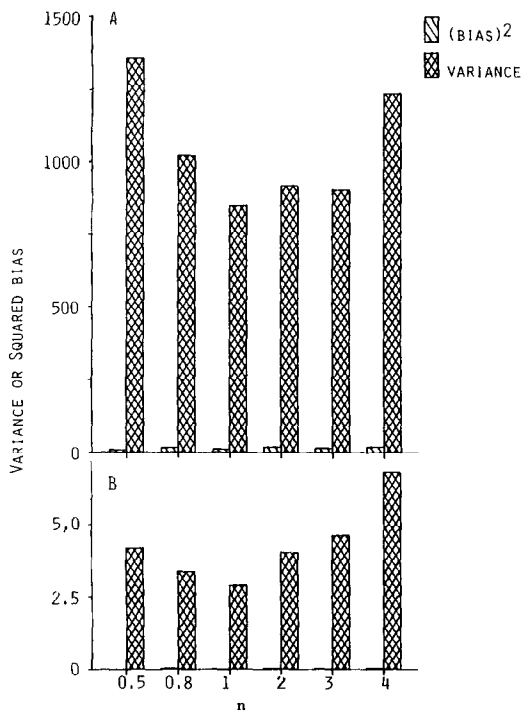

Fig. 7. Effect of temperature programs (short-term) on variance and squared bias of parameter estimates for $t_{90(25)}$ (A) and $t_{90(40)}$ (B). Theoretical values: $t_{90(25)} = 156$; $t_{90(40)} = 20.6$ weeks. Temperature program: $T = 298 + kt^n$. The values of k were fixed so that the drug degraded to 80% remaining during 10 weeks. Four samples were assayed every 0.2 week. S.D. of assay: 2% of the initial content. S.D. of temperature: 0.5 °C.

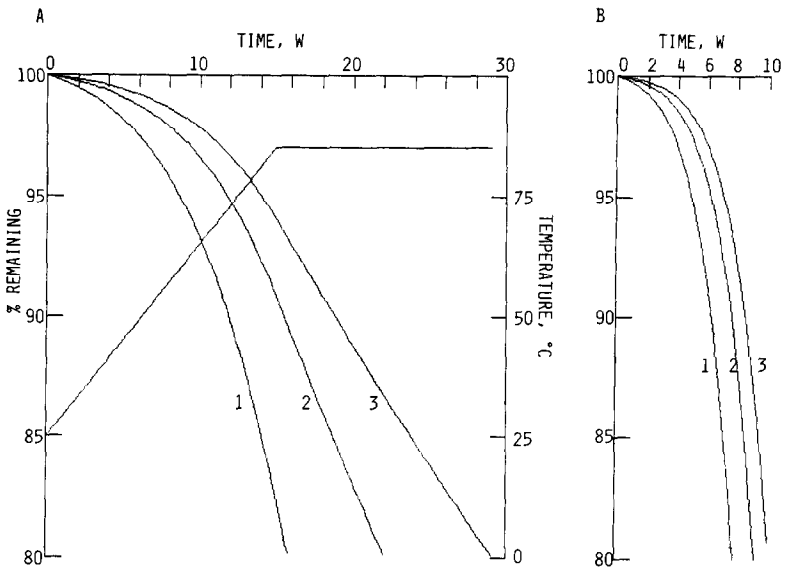

Fig. 8. Theoretical degradation curves under a temperature program proposed for short-term tests ($T = 298 + 4t$). Theoretical values: $E_a = 10$ (A) and 25 kcal/mol (B); $t_{90(25)} = 50$ (1), 100 (2) and 156 weeks (3).

the most unstable among the products studied (whose $t_{90(25)}$ is 50 and $t_{90(40)}$ is 6.61 weeks).

When shelf-lives are predicted based on accelerated stability data, the constancy of activation energy is basically required. Practically, however, E_a in the range of room temperature often differs from that in the temperature range of the accelerated study. When the Arrhenius plots are not linear, the estimates of $t_{90(25)}$ and $t_{90(40)}$ obtained from the accelerated stability data should differ from the theoretical value. In order to examine this, 4 non-linear types of the Arrhenius plots shown in Fig. 9 were simulated; the theoretical values of E_a at a temperature range of 40–50 °C were assumed to be 90, 95, 105 and 110% of E_a at a temperature range of 25–40 °C, and E_a at a temperature range of 50–85 °C were 90, 95, 105 and 110% of E_a at a temperature range of 40–50 °C, respectively. Curve 3 in Fig. 9 shows linearity in the Arrhenius plots. Table 4 shows the estimates obtained neglecting the non-linear dependence of E_a on temperature for each of the E_a patterns. The isothermal method provided the estimates with significantly larger bias (the dif-

TABLE 4

Effect of uncertainty of activation energy on the parameter estimates

Type of Arrhenius plot *	Period (week)	NONISO				ISO			
		$t_{90(25)}$		$t_{90(40)}$		$t_{90(25)}$		$t_{90(40)}$	
		Mean (week)	RSD (%)	Mean	RSD	Mean	RSD	Mean	RSD
1	9.6	129.9	21.2	13.1	8.0	176.2	15.0	15.1	6.9
2	9.4	115.3	20.0	13.2	7.7	132.3	13.5	14.1	6.6
3	9.2	101.3	16.6	13.2	6.7	100.7	11.1	13.2	5.6
4	8.8	90.9	15.9	13.4	6.4	77.1	9.1	12.4	4.9
5	8.6	79.5	15.9	13.4	6.4	60.6	8.5	11.8	4.6

Theoretical values: $t_{90(25)} = 100$; $t_{90(40)} = 13.2$ weeks. Non-isothermal temperature program: $T = 298 + 4t$. Four samples were assayed at each sampling time. S.D. of assay: 2% of the initial content. S.D. of temperature: 0.5 °C.

* See Fig. 7.

TABLE 5

Effect of temperature variance on the parameter estimates

Errors in temperature control	Theoretical t_{90} (week)		Estimate			
			$t_{90(25)}$		$t_{90(40)}$	
	25 °C	40 °C	Mean (w)	RSD (%)	Mean	RSD
SD of temperature (°C)						
0.2	100	13.2	102.2	11.9	13.3	4.8
0.5	100	13.2	100.6	12.4	13.2	4.9
0.7	100	13.2	100.2	11.5	13.2	4.6
0.2	100	44.5	100.1	7.1	44.5	4.6
0.5	100	44.5	100.2	8.5	44.5	5.6
0.7	100	44.5	100.2	7.3	44.5	4.8
SD of temperature increase (%)						
0.5	100	13.2	99.5	10.6	13.2	4.5
1.0	100	13.2	102.0	11.9	13.3	5.0
2.0	100	13.2	101.1	12.0	13.3	6.4
0.5	100	44.5	99.8	6.4	44.4	4.1
1.0	100	44.5	99.7	6.4	44.4	4.2
2.0	100	44.5	100.0	6.4	44.5	4.6

Temperature program: $T = 298 + 4t$. Eight samples were assayed at each sampling time. Standard deviation of assay: 2% of the initial content.

ference between the mean of the estimates and the theoretical values) than the non-isothermal method.

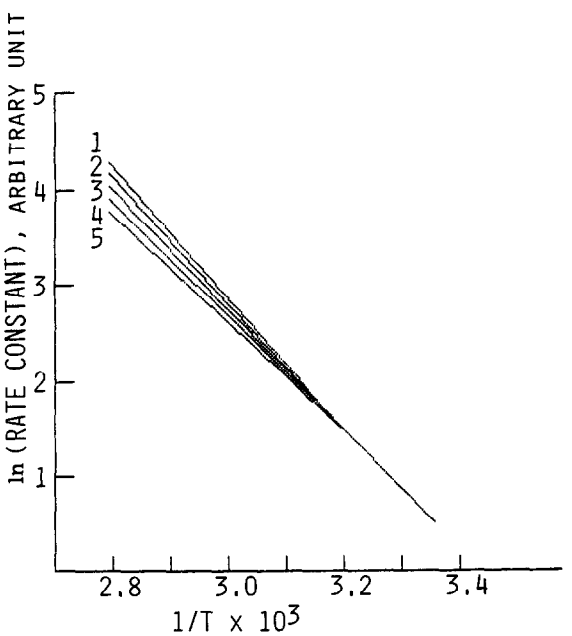

Fig. 9. Models of non-linear Arrhenius plots.

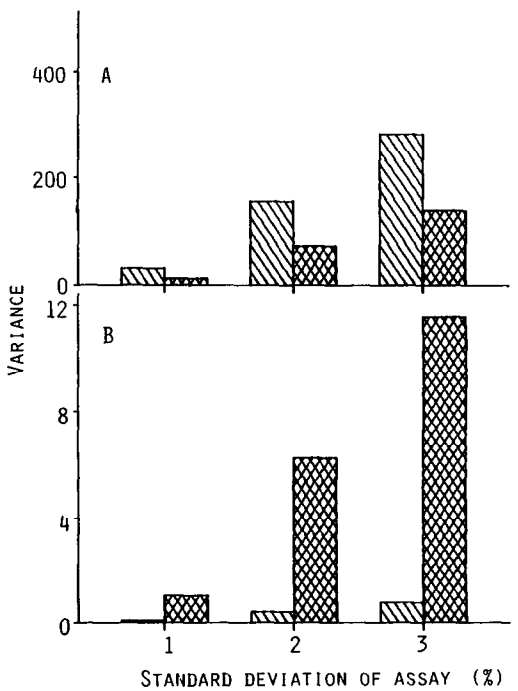

Fig. 10. Effect of assay variance on parameter estimates for $t_{90(25)}$ (A) and $t_{90(40)}$ (B). Theoretical values: $t_{90(25)} = 100$ weeks; $t_{90(40)} = 13.2$ (▨); 44.5 weeks (■). Temperature program: $T = 298 + 4t$. Eight samples were assayed at each sampling time. S.D. of temperature: 0.5 °C.

TABLE 6

Effect of different orders of degradation on the parameter estimates

Reaction order	Theoretical t_{90} (week)		Period (week)	NONISO				ISO			
				$t_{90(25)}$		$t_{90(40)}$		$t_{90(25)}$		$t_{90(40)}$	
	25 °C	40 °C		Mean (w)	RSD (%)	Mean	RSD	Mean	RSD	Mean	RSD
0	100	13.2	9.0	120.3	17.8	13.9	7.0	97.1	12.4	12.8	6.3
1	100	13.2	9.2	101.3	16.6	13.2	6.7	100.7	11.1	13.2	5.6
2	100	13.2	9.2	86.6	17.9	12.6	7.0	103.2	10.6	13.6	5.3
0	100	44.5	21.4	119.6	10.5	49.5	6.8	107.0	7.5	46.1	4.8
1	100	44.5	22.0	100.6	10.1	44.6	6.6	100.2	6.9	44.5	4.4
2	100	44.5	22.8	86.0	9.0	40.7	5.9	93.9	6.5	43.1	4.2

Non-isothermal temperature program: $T = 298 + 4t$. Four samples were assayed at each sampling time. S.D. of assay: 2% of the initial content. S.D. of temperature: 0.5 °C.

The effects of temperature variance and assay variance on the estimates obtained by the non-isothermal method are shown in Table 5 and Fig. 10, respectively. The precision of the estimates was found to depend largely on assay variance and less on temperature variance. This suggests that accuracy in assay of drugs is required in order to obtain reliable estimates.

The effects of various orders of reaction on the parameter estimates were examined and are shown in Table 6. When the degradation order for the drug product of interest was unknown and treated as first-order, the non-isothermal method appeared to provide less reliable estimates of $t_{90(25)}$ and $t_{90(40)}$ because of larger values of bias compared to the isothermal method. When the degradation order is unknown, a temperature rise program should be chosen for the non-isothermal method in such a way that the theoretical degradation curves may not depend largely on the degradation order.

Conclusion

Reliability of the non-isothermal method was found to depend largely on temperature rise programs. For a relatively long-term test, the temperature program expressed by a power function of time ($T = 298 + kt^n$; $n = 4$; $k = 1.56 \times 10^{-5}$ or 0.004 deg/week^4) could be applied to stability prediction for practical drug products, whose shelf-lives have a wide range. A linear temperature program ($n = 1$; $k = 4$ deg/week), on the other hand, was found to be suitable for a short-term test. The non-isothermal method with the proposed programs was evaluated by the Monte Carlo method and compared with the isothermal method for the same experiment period and number of samples. The accuracy of the estimates obtained was found to depend on assay variance rather than on temperature variance. Thus, accuracy in assay of drugs is found to be important in the experiments.

For a relatively long-term test, the proposed non-isothermal method can provide more reliable estimates of $t_{90(25)}$ and $t_{90(40)}$ than the isothermal method carried out at real temperatures (25 and 40 °C) where the Arrhenius relationship is not employed to obtain the estimates. For a short-term test, however, the non-isothermal method was found to require larger numbers of samples to obtain the same S.D. of the estimates as the isothermal accelerated study carried out under elevated temperature conditions, in the case of constant activation energy in the temperature range employed for the accelerated study. A great advantage of the non-isothermal method, on the other hand, was demonstrated for non-linear Arrhenius plots, which are often the case for drug degradation. Errors of the estimates brought about by the non-linearity of the Arrhenius plots were found to be smaller in the non-isothermal method compared to the isothermal method.

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