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Step Nonisothermal Method to Study Stability of Drugs

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A step nonisothermal experiment under high oxygen pressure and a new computation with optimization for step nonisothermal experiment on stability study of drugs was introduced. The kinetics parameters of captopril oxidation in aqueous solution were determined by this method. It is reported that the reaction of captopril solution occurs under either aerobic or anaerobic condition, giving different products. Then the total rate constant k_{total} can be expressed as

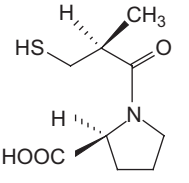
$$k_{total} = k_{anaerobic} + k_{aerobic} = A_{anaerobic}\exp\left(-\frac{E_{a,anaerobic}}{RT}\right) + A_{aerobic}\exp\left(-\frac{E_{a,aerobic}}{RT}\right)p_{O_2},$$

where $k_{anaerobic}$ and $k_{aerobic}$ are the rate constants of anaerobic and aerobic degradations, respectively. The results indicated that the parameters obtained in the step nonisothermal experiment were comparable with those obtained in the isothermal–isobaric experiments. By a computer simulation, the estimates for the kinetic parameters (E_a and k_0) obtained with step nonisothermal method were statistically evaluated. Results indicated that the estimates obtained with isothermal–isobaric method were somewhat more precise than those obtained with step nonisothermal method. However, the experimental period needed by isothermal–isobaric method was much longer than that needed by step nonisothermal method.

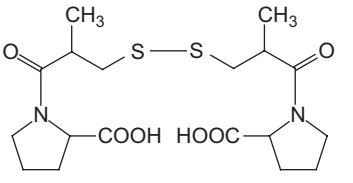
Keywords captopril; drug stability; step nonisothermal experiment; oxidative degradation kinetics; optimization; computer simulation

In stability studies, the influences of temperature, light, and humidity are often considered quantitatively, whereas the influence of oxygen is not. Some drugs are quite stable at high temperatures but unstable to oxygen, and then their stability will mainly depend on the ambient oxygen pressure. Furthermore, for drugs unstable to both oxygen and heat, the stability obviously depends on both the ambient oxygen pressure and the storage temperature. To predict and improve the stability of the oxygen-sensitive drugs, it is important to study their oxidation rates. However, a quantitative discussion of such phenomena has seldom been found in the literature, because of the complexity of experimental design. Many papers reported that some drugs are unstable (or stable) under aerobic (or anaerobic) conditions (Blaug & Hajratwala, 1972; Fatouros, Österberg, & Mikaelsson, 1997; Gallarate, Carlotti, Trotta, & Bovo, 1999; Hung, Lam, Perrier, & Souter, 1988; Jones, Crundwell, & Taylor, 1979; Michael, Thomas, Natarajan, Louis, & Larry, 1988; Pogocki & Scheich, 2000; Špiclin, Gašperlin, & Kmetec, 2001; Stevenson, Leonard, & Hall, 1999; Vermeire & Remon, 1999; Yeh & Lach, 1961), but few reported the oxidation rate (Gleditsch & Waaler, 2001; Shi et al., 2007).

Captopril (I), an orally active inhibitor of the angiotensin-converting enzyme, is used widely to treat hypertension and congestive heart failure. Like all thiols, captopril is subjected to oxidation to form the dimer (Ferguson, Brunner, Turini, Gavras, & McKinstry, 1977; Capozzi & Modena, 1974), captopril disulfide (II).



I



II

In order to quantify the influence of oxygen on the stability of captopril, it is necessary to determine its oxidation kinetics. Timmins, Jackson, and Wang (1982) determined the oxidation rate constants of captopril solution at 50°C. In their study, to

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ensure that oxygen was always in excess, the glass vials containing captopril solution were opened and air was bubbled through the solution once a day. However, the procedure is time-consuming. Lee and Notari (1987) studied the stability of captopril solution at a fixed temperature and oxygen partial pressure (90–760 mmHg) . However, the dependence of the oxidation on temperature was not reported.

Shi et al. (2007) reported a drug stability experiment accelerated by compressed oxygen. The stability of ascorbic acid solution as a model was studied, and the kinetic parameters were obtained with the method. Nevertheless, the disadvantage was still the requirement for a long experimental time and much effort.

Historically, nonisothermal experiments, which enable the stability of a drug to be estimated from a single experiment, were used in thermal stability studies (Eriksen & Stelmach, 1965; Rogers, 1963; Zhan, Yin, Wang, & Ma, 1997). These experiments save time and labor; however, the use of sophisticated temperature-control devices limited their popularity. The step nonisothermal experiment (Edel & Baltzer, 1980; Pang, Fan, & Hou, 1981) is a special nonisothermal type with all the above advantages; furthermore, only a normal thermostat is required instead of a sophisticated temperature-control device.

A nonfractional order nonisothermal chemical reaction can be described by some form of the general equation:

$$f(c) = -\int_0^t k_i \mathrm{d}t + f(c_0), \tag{1}$$

where c is the residual concentration at time t, c_0 the initial concentration, k_i the reaction rate constant at the temperature T_i, and $f(c)$ the concentration function, which depends on the reaction order. For zero-, first-, and second-order reactions, $f(c)$ is c, ln c, and $-1/c$, respectively. Combining Equation 1 with the Arrhenius equation $k_i = k_0 \exp\{E_a/R[(1/T_0)-(1/T_i)]\}$ and heating model $T_i = T(t)$ yields

$$f(c) = -\int_0^t k_0 \cdot \exp\left\{\frac{E_a}{R}\left[\frac{1}{T_0} - \frac{1}{T(t)}\right]\right\}\mathrm{d}t + f(c_0), \tag{2}$$

where T_0 is the initial reaction temperature and k_0 the rate constant at temperature T_0.

In the step nonisothermal experiment, the temperature rises stepwise as shown in Figure 1, where T_0, T_1 . . . T_i are the stepping incubation temperatures and Δt_0, Δt_1 . . . Δt_i are the incubation durations. Each incubation stage can be regarded as a miniature isothermal kinetic model. The time required to raise the temperature between each stage (<5 min) is negligible because it is much shorter than the incubation duration (~hours). Thus, for the step nonisothermal method, Equation 2 can be expressed as follows:

$$f(c) = -\sum_{i=0}^{i} k_0 \cdot \exp\left[\frac{E_a}{R}\left(\frac{1}{T_0} - \frac{1}{T_i}\right)\right]\Delta t_i + f(c_0). \tag{3}$$

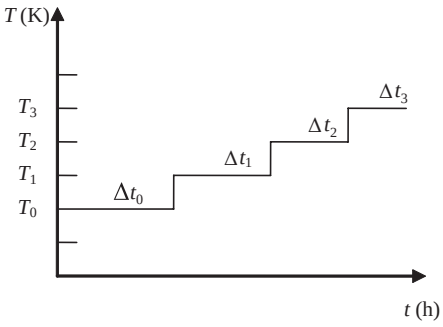


FIGURE 1. Time–temperature relationship for the step nonisothermal experiment.

Because E_a, the activation energy, is unknown, we need to assume an E_a value in a suitable range to carry out the calculation. If the E_a value is assumed correctly, k_0 will be a constant and can be taken out of the sum, which then yields Equation 4:

$$f(c) = -k_0 \sum_{i=0}^{i} \exp\left[\frac{E_a}{R}\left(\frac{1}{T_0} - \frac{1}{T_i}\right)\right]\Delta t_i + f(c_0). \tag{4}$$

According to Equation 4, a straight line can be obtained from a plot of the concentration function $f(c)$ versus $\sum_{i=0}^{i} \exp\{E_a/R[(1/T_0)-(1/T_i)]\}\Delta t_i$ with intercept $f(c_0)$ and slope equal to k_0. If the E_a value is assumed incorrectly, then k_0 will not be a constant and cannot be taken out of the sum and Equation 4 will be untenable; thus, the line will be curved and the correlation coefficient r will be reduced.If a group of different assumed E_a values within a definite range are evaluated using Equation 4, a group of regression lines with different correlation coefficients r can be obtained. The higher the correlation coefficient r, the closer the assumed E_a to the real E_a value. Therefore, the E_a which gives the highest r is the best estimate of the real E_a. In addition, the initial rate constant k_0 can be obtained from the slope of this regression.

To reduce the computation times, optimization was applied. Because the computation was too complex to be completed manually, a program was used for completing the whole computation automatically.

In this article, the stability of captopril solution under elevated oxygen pressures and temperatures was studied in the step nonisothermal experiment. The results obtained using this method were comparable with those obtained using the isothermal–isobaric method, with the advantage of using only approximately one fourth of the measurements. A computation method with optimization for the step nonisothermal experiment was introduced. Comparison of the optimization and conventional computation was discussed; the results indicated that the limitations of the conventional computation had been overcome.

Reliability of the step nonisothermal method for stability study of drugs was statistically evaluated by computer simulations. The effects of errors in measurement of drug contents, in temperature control, and in oxygen pressure control were taken into account.

MATERIALS AND METHODS

Materials

Captopril was provided by Changzhou Pharmacy Co., Ltd. and was of not less than 99.0% purity. Captopril reference substance was received from the National Institute for the Control of Pharmaceutical and Biological Products. Oxygen was of medical application grade and contained ≥99.0% (vol/vol) of O_2. The other reagents used were of analytical grade.

Instruments

Thirty pressurized stainless-steel containers, a highly accurate pressure meter (YB-150, the measurement range is 0–6 MPa, the accuracy of pressure is 0.25%; Yangquan, China), a Fortin-type mercurial barometer (the accuracy is ±0.2 mm Hg; Jiaxing, China), an isothermal heating oven with high precision (the accuracy, precision, reproducibility of temperature are ≤ ±0.5°C in the range of room temperature to 100°C; self-made) (Zhan, Yin, & Ma, 1995), and high-performance liquid chromatograph (HPLC) instrumentation (10A style, SPD-10AVP detector; Shimadzu Co., Japan) were used.

Analysis

The chromatographic separation and quantitative determination were performed on a HPLC. The HPLC assay for captopril was similar to that described by Lee and Notari (1987). A reversed-phase C-18 column was employed, and the eluent was a mixture of acetonitrile–phosphoric acid aqueous solution (0.05%) (25:75). The flow rate was 1 mL/min. UV detection was carried out at 210 nm.

Samples for analysis were prepared by diluting the test solution with HPLC solvent to give a captopril concentration of 0.2 mg/mL, and 20 μL was injected onto the column via a rotary valve loop injector. Peak areas were used for quantitation.

Measuring Oxygen Pressure

Because of the difficulty in measuring the concentration of dissolved oxygen in containers, p_{O_2}, the oxygen pressure in a state of equilibrium, was measured instead. The difference between the internal pressure of a container and the external barometric pressure was measured with a highly accurate pressure meter. The barometric pressure was measured with a Fortin-type mercurial barometer. Then the measured value of barometric pressure was corrected by the location-dependent latitude, height, and ambient temperature because they influence the specific gravity of mercury.

Step Nonisothermal Experiment at Constant Oxygen Pressure

An exact weight of approximately 4.5 g of captopril was dissolved in 800 mL distilled water. Then the solution was diluted with water to a total volume of 1,000 mL. An exact volume of 25 mL of the captopril solution was transferred to a 50-mL beaker. Then the beaker was placed into a pressurized stainless-steel container. About 21 such containers were pressurized with oxygen to a predetermined pressure and put into a thermostatic oven at the beginning of the experiment. Three containers were taken out of the thermostatic oven at each suitable interval, and the residual concentrations of the samples were measured with HPLC.

The experimental temperature was raised stepwise from 30 to 55°C. The temperature was increased by 5°C (heating rate > 1°C/min) at the beginning of each step and was kept constant for the remainder of the step time. The oxygen pressures were 0.595, 0.995, and 1.395 MPa in three experiments, respectively.

Isothermal–Isobaric Experiments

An exact weight of approximately 4.5 g of captopril was dissolved in 800 mL distilled water. Then the solution was diluted with water to a total volume of 1,000 mL. An exact volume of 25 mL of the captopril solution was transferred to a 50-mL beaker. Then the beaker was placed into a pressurized stainless-steel container. About 21 such containers were pressurized with oxygen to a predetermined pressure and put into a thermostatic oven at the beginning of the experiment. Three containers were taken out of the thermostatic oven at each suitable interval and the residual concentrations of the samples were measured with HPLC.

The experimental temperatures and oxygen pressures were 25, 35, 45, and 55°C and 0.595, 0.995, 1.395, and 1.795 MPa, respectively.

Computer Simulations

The computer simulations were carried out with a MATHEMATICA program programmed by us. In light of Equation 4, the k_0 could be obtained by assigning values to E_a, c_0, c_{final}, T_0, T_{final}, t_{final}, and selecting a $f(c)$ based on the order of the reaction. Then, again based on Equation 4, theoretical (errorless) drug content data were generated by assigning values to k_0, E_a, c_0, T_0, and t and selecting a $f(c)$. In our study, $f(c)$ was kept at c (zero-order reaction), E_a and A were kept at 24,121.0 J/mol and 0.2387, respectively. The estimates for E_a were optimized by a golden section approach; then the values of k_0 were obtained from the slope of the regressions with best E_a. The estimates for E_a were explored under the following conditions: (a) temperature range 25–55°C, oxygen pressure 0.995 MPa, final degradation 60%, and six steps with ±1 and ± 2% random errors in content; (b) temperature range 25–55°C, oxygen pressure 0.995 MPa, final degradation 60%, and six steps with ± 0.5 and ± 1°C random errors in temperature; (c) temperature range 25–55°C, oxygen pressure 0.995 MPa, final degradation 60%, and six steps with ± 0.01 and ± 0.02 MPa random errors in oxygen pressure. For each model, 500 sets of data were generated.

For comparison, 500 sets of isothermal–isobaric data were also generated by computer simulation according to zero-order

kinetics. Estimations were carried out at temperatures 25, 35, 45, and 55°C, oxygen pressure 0.995 MPa, final degradation 60% with ±1 and ±2% random errors in contents.

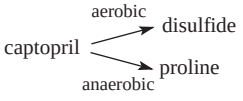
RESULTS

Step NonIsothermal Experiment at Constant Oxygen Pressure

The experimental results are tabulated in Table 1. Our experiments indicated that the degradation of captopril solution at constant temperature and oxygen pressure obeyed zero-order kinetics (see "Isothermal–Isobaric Experiments"). Therefore, the data listed in Table 1 were treated with Equation 4 according to zero-order kinetics. The total activation energy $E_{a,total}$ at each experimental oxygen pressure and total rate constants $k_{total,30}$ at 30°C of the degradation of captopril solution were obtained and tabulated in Table 2.

Thus, based on the Arrhenius equation $k_T = k_{30}\exp[E_a/R[(1/303.15) - (1/T)]$, the total rate constants k_{total} at each experimental temperature and oxygen pressure could be calculated and are given in Table 3.

It is reported that the degradation of captopril solution occurs under either aerobic or anaerobic conditions, giving different degradants (Timmins et al., 1982); disulfide is the major degradation product under aerobic conditions. However, under anaerobic conditions, it undergoes hydrolysis to give praline.



It is suggested that the total degradation of captopril solution can be divided into two parallel reactions: the anaerobic and the aerobic reactions. Therefore, the total degradation rate constant k_{total} can be expressed as follows:

$$k_{total} = k_{anaerobic} + k_{aerobic}, \quad (5)$$

TABLE 2
Values of $E_{a,total}$ and Rate Constant $k_{total,30}$ at 30°C Calculated by Optimization

p_{O_2} (MPa)	$E_{a,total}$ (kJ mol^{-1})	$k_{total,30} \times 10^3$ (mol L^{-1} h^{-1})
0.595	22.48	0.097
0.995	21.90	0.149
1.395	20.45	0.208

where $k_{anaerobic}$ is the rate constant under anaerobic conditions and $k_{aerobic}$ is the rate constant under aerobic conditions.

From the data in Table 3, six linear regression lines, with the correlation coefficients $r > .994$, shown in Figure 2, could be obtained by plotting k_{total} versus p_{O_2} for each experimental temperature. The rate constants under anaerobic conditions $k_{anaerobic}$ were the intercepts of these lines and are also listed in Table 3.

Based on the Arrhenius equation, $k_{anaerobic}$ can be expressed as follows:

$$\ln k_{anaerobic} = \ln A_{anaerobic} - \frac{E_{a,anaerobic}}{RT}, \quad (6)$$

where $E_{a,\ anaerobic}$ and $A_{anaerobic}$ are the activation energy and pre-exponential factor of the degradation under anaerobic conditions, respectively. Therefore, a straight line, with the correlation coefficient $r = .997$, would be obtained by plotting $\ln k_{anaerobic}$ versus $1/T$ (shown in Figure 3). From the slope and the intercept of the $\ln k_{anaerobic} - 1/T$ line, $E_{a,anaerobic}$ and $A_{anaerobic}$, respectively, could be determined and are tabulated in Table 9.

According to Equation 5, $k_{aerobic}$ could be calculated by $k_{aerobic} = k_{total} - k_{anaerobic}$. The values of $k_{aerobic}$ are also tabulated in Table 3.

TABLE 1
Results of the Step Nonisothermal Experiments of Captopril Solution at Constant Oxygen Pressure

	p_{O_2} = 0.595 MPa		p_{O_2} = 0.995 MPa		p_{O_2} = 1.395 MPa	
T (°C)	t (h)	c (mol/L)	t (h)	c (mol/L)	t (h)	c (mol/L)
	0.00	0.0200 ± 0.9	0.00	0.0198 ± 0.7	0.00	0.0198 ± 0.5
30	16.50	0.0186 ± 1.3	9.00	0.0184 ± 0.2	6.50	0.0186 ± 0.5
35	13.00	0.0172 ± 0.6	7.50	0.0169 ± 0.6	6.00	0.0172 ± 0.6
40	9.00	0.0160 ± 1.0	7.00	0.0156 ± 1.0	5.50	0.0157 ± 1.1
45	7.50	0.0149 ± 0.9	6.00	0.0144 ± 1.9	5.00	0.0142 ± 1.7
50	7.00	0.0137 ± 2.1	5.00	0.0131 ± 2.7	4.50	0.0126 ± 2.2
55	6.00	0.0126 ± 2.8	4.00	0.0118 ± 2.9	3.00	0.0115 ± 3.1

Results are presented as $M \pm RSD$% of three samples.

TABLE 3
Rate Constants of Captopril Solution Obtained from the Step Nonisothermal Experimental

T (°C)	p_{O_2} (MPa)	$k_{total} \times 10^3$ (mol/L/h)	$k_{anaerobic} \times 10^3$ (mol/L/h)	$k_{aerobic} \times 10^3$ (mol/L/h)
30	0.595	0.097	0.012	0.085
	0.995	0.149		0.137
	1.395	0.208		0.196
35	0.595	0.112	0.017	0.095
	0.995	0.172		0.155
	1.395	0.237		0.220
40	0.595	0.129	0.023	0.106
	0.995	0.197		0.174
	1.395	0.269		0.246
45	0.595	0.147	0.029	0.118
	0.995	0.225		0.196
	1.395	0.305		0.276
50	0.595	0.168	0.036	0.132
	0.995	0.256		0.220
	1.395	0.343		0.307
55	0.595	0.191	0.045	0.146
	0.995	0.289		0.244
	1.395	0.385		0.340

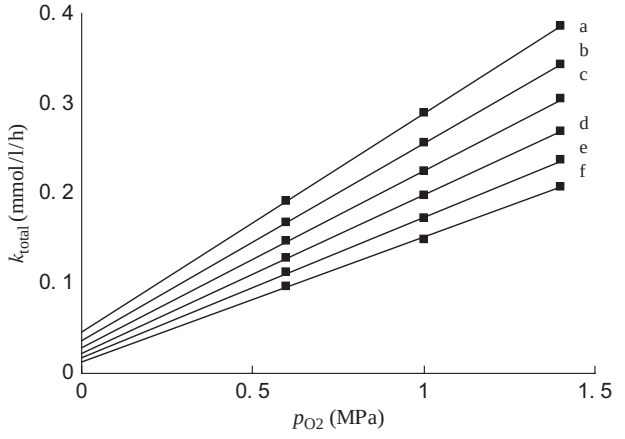


FIGURE 2. The relationship between k_{total} and oxygen pressure p_{O_2} in the step nonisothermal experiment: (a) 55°C, (b) 50°C, (c) 45°C, (d) 40°C, (e) 35°C, and (f) 30°C.

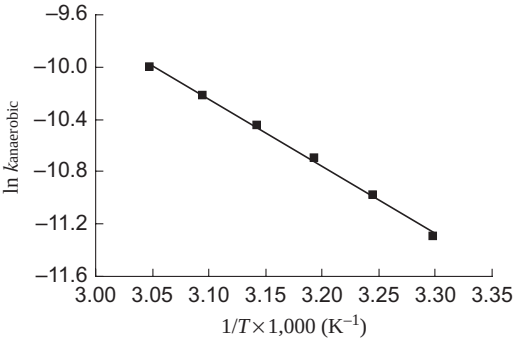


FIGURE 3. The linear relationship between ln $k_{anaerobic}$ and $1/T$ in the step nonisothermal experiment.

Because of the linear relationship between k_{total} and p_{O_2} (shown in Figure 2), $k_{aerobic}$ was proportional to p_{O_2}. The relationship between $k_{aerobic}$ and oxygen pressure was expressed as Equation 7 by Shi et al. (2007)

$$k_{aerobic} = A_{aerobic} \exp\left(-\frac{E_{a,\ aerobic}}{RT}\right) \cdot p_{O_2}, \qquad (7)$$

where p_{O_2} is the oxygen pressure and $E_{a,\ aerobic}$ and $A_{aerobic}$ are the activation energy and pre-exponential factor of the degradation under aerobic conditions, respectively. Our experiments indicated that the aerobic rate constant $k_{aerobic}$ of the degradation of captopril solution also coincided with Equation 7. Taking the natural logarithm of both sides of Equation 7 yields

$$\ln k_{aerobic} = \ln (A_{aerobic} p_{O_2}) - \frac{E_{a,\ aerobic}}{RT}. \qquad (8)$$

According to Equation 8, three straight lines, with correlation coefficient $r > .990$, were obtained by plotting ln $k_{aerobic}$ versus $1/T$ (Figure 4). Thus, $E_{a,aerobic}$ and $A_{aerobic}$ could be determined from the slope and the intercept, respectively. The values are also listed in Table 9.

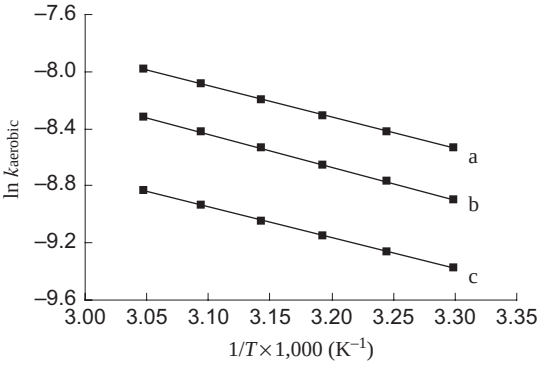


FIGURE 4. The linear relationship between ln $k_{aerobic}$ and $1/T$ in the step nonisothermal experiment: (a) p_{O_2} = 1.395 MPa, (b) p_{O_2} = 0.995 MPa, and (c) p_{O_2} = 0.595 MPa.

Isothermal–Isobaric Experiments

The experimental results are shown in Tables 4–7 and Figures 5–8. As seen from the lines in Figures 5–8, the degradation of captopril solution at constant temperature and oxygen pressure obeyed zero-order kinetics:

$$c = c_0 - k_{total}\, t$$

Therefore, the k_{total} at each experimental temperature and oxygen pressure was obtained from slope of each line in Figures 5–8 and was listed in Table 8.

From the data in Table 8, four linear regression lines, with the correlation coefficients $r > .980$, shown in Figure 9, could be obtained by plotting k_{total} versus p_{O_2} for each experimental temperature. The rate constants under anaerobic condition $k_{anaerobic}$ were the intercepts of these lines and are also listed in Table 8.

By plotting ln $k_{anaerobic}$ versus the reciprocal of temperature $1/T$, a straight regression line was obtained ($r = .970$), shown in Figure 10, with slope and intercept equal to $E_{a,anaerobic}/R$ and ln $A_{anaerobic}$, respectively. The values of $E_{a,anaerobic}$ and $A_{anaerobic}$ are listed in Table 9.

The values of $k_{aerobic}$ were calculated using Equation 5 and listed in Table 8. Four straight lines, with correlation coefficient $r > .975$, were obtained by plotting ln $k_{aerobic}$ versus $1/T$ (shown in Figure 11). $E_{a,aerobic}$ and $A_{aerobic}$ could be determined

TABLE 4
Results of the Isothermal–Isobaric Experiments of Captopril Solution at 25°C

0.595 MPa		0.995 MPa		1.395 MPa		1.795 MPa	
t (h)	c (mol L^{-1})	t (h)	c (mol L^{-1})	t (h)	c (mol L^{-1})	t (h)	c (mol L^{-1})
00.0	0.0195 ± 0.6	00.0	0.0199 ± 0.5	00.0	0.0196 ± 0.5	00.0	0.0194 ± 0.7
24.5	0.0183 ± 0.9	20.0	0.0182 ± 0.8	10.0	0.0177 ± 0.7	8.0	0.0179 ± 0.9
48.0	0.0164 ± 1.0	40.0	0.0162 ± 0.9	24.0	0.0156 ± 1.0	16.0	0.0167 ± 1.1
72.0	0.0150 ± 0.8	58.0	0.0146 ± 1.1	34.0	0.0133 ± 0.9	24.0	0.0149 ± 0.9
96.0	0.0128 ± 2.1	74.0	0.0121 ± 1.9	45.0	0.0121 ± 2.2	30.0	0.0140 ± 2.6
116.0	0.0108 ± 2.4	86.0	0.0113 ± 2.7	56.0	0.0112 ± 2.5	36.0	0.0122 ± 2.8
				64.0	0.0100 ± 2.9	42.0	0.0114 ± 2.8

Results are presented as $M \pm RSD$% of three samples.

TABLE 5
Results of the Isothermal–Isobaric Experiments of Captopril Solution at 35°C

0.595 MPa		0.995 MPa		1.395 MPa		1.795 MPa	
t (h)	c (mol L^{-1})	t (h)	c (mol L^{-1})	t (h)	c (mol L^{-1})	t (h)	c (mol L^{-1})
00.0	0.0196 ± 0.9	00.0	0.0199 ± 0.7	00.0	0.0195 ± 0.6	00.0	0.0202 ± 0.8
12.0	0.0188 ± 1.1	10.0	0.0180 ± 1.0	07.0	0.0177 ± 0.9	07.0	0.0183 ± 0.9
24.0	0.0176 ± 0.8	20.0	0.0164 ± 1.2	15.0	0.0159 ± 0.8	13.0	0.0160 ± 1.0
36.0	0.0156 ± 1.1	28.0	0.0149 ± 0.9	26.0	0.0133 ± 1.6	21.5	0.0142 ± 1.1
48.0	0.0150 ± 1.3	35.5	0.0137 ± 1.6	31.0	0.0121 ± 1.2	23.0	0.0135 ± 1.5
60.0	0.0131 ± 1.6	45.0	0.0118 ± 2.4	37.0	0.0108 ± 2.8	25.0	0.0128 ± 2.5
72.0	0.0120 ± 2.7	55.0	0.0114 ± 2.7	41.0	0.0100 ± 3.0	27.0	0.0126 ± 2.9

Results are presented as $M \pm RSD$% of three samples.

TABLE 6
Results of the Isothermal–Isobaric Experiments of Captopril Solution at 45°C

0.595 MPa		0.995 MPa		1.395 MPa		1.795 MPa	
t (h)	c (mol L^{-1})	t (h)	c (mol L^{-1})	t (h)	c (mol L^{-1})	t (h)	c (mol L^{-1})
0.0	0.0196 ± 0.5	00.0	0.0198 ± 0.7	00.0	0.0192 ± 0.9	00.0	0.0196 ± 1.0
12.0	0.0180 ± 0.9	08.0	0.0173 ± 0.8	07.0	0.0172 ± 0.8	08.3	0.0160 ± 0.7
22.0	0.0168 ± 0.9	16.0	0.0158 ± 1.0	14.0	0.0154 ± 1.1	13.0	0.0146 ± 1.3
32.0	0.0148 ± 0.8	24.0	0.0140 ± 1.3	21.0	0.0130 ± 1.5	17.0	0.0130 ± 1.4
44.0	0.0126 ± 1.0	32.0	0.0124 ± 1.6	25.0	0.0122 ± 1.7	21.0	0.0114 ± 2.5
58.0	0.0114 ± 2.4	39.0	0.0105 ± 2.7	28.0	0.0110 ± 2.4	23.0	0.0107 ± 3.1
70.0	0.0102 ± 3.3	42.0	0.0101 ± 3.3	32.0	0.0103 ± 3.0		

Results are presented as $M \pm RSD$% of three samples.

TABLE 7
Results of the Isothermal–Isobaric Experiments of Captopril Solution at 55°C

0.595 MPa		0.995 MPa		1.395 MPa		1.795 MPa	
t (h)	c (mol L^{-1})	t (h)	c (mol L^{-1})	t (h)	c (mol L^{-1})	t (h)	c (mol L^{-1})
00.0	0.0196 ± 0.6	00.0	0.0196 ± 0.9	00.0	0.0196 ± 1.0	00.0	0.0195 ± 0.3
06.0	0.0187 ± 0.8	06.0	0.0178 ± 0.5	05.0	0.0178 ± 0.7	03.0	0.0180 ± 0.6
11.0	0.0177 ± 0.2	12.0	0.0158 ± 0.7	10.0	0.0162 ± 0.8	05.0	0.0175 ± 1.0
16.0	0.0165 ± 0.9	18.0	0.0141 ± 1.1	15.0	0.0140 ± 1.7	10.0	0.0144 ± 1.5
21.0	0.0158 ± 1.7	24.0	0.0122 ± 2.5	20.0	0.0115 ± 2.8	16.0	0.0120 ± 2.9
28.0	0.0149 ± 2.2	30.0	0.0108 ± 3.0	24.5	0.0102 ± 3.8	19.0	0.0106 ± 3.9
36.0	0.0125 ± 2.9						

Results are presented as $M \pm RSD$% of three samples.

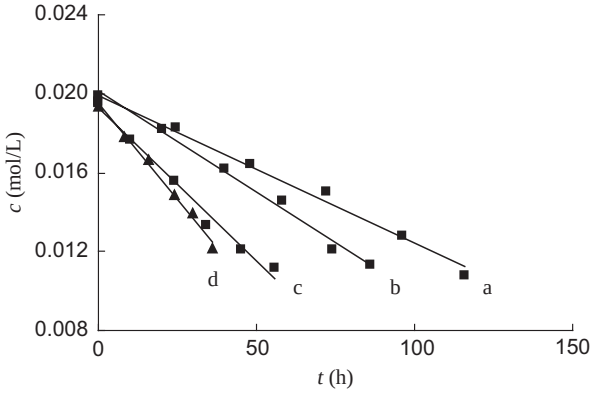


FIGURE 5. The relationship between c and t at 25°C: (a) p_{O_2} = 0.595 MPa, (b) p_{O_2} = 0.995 MPa, (c) p_{O_2} = 1.395 MPa, and (d) p_{O_2} = 1.795 MPa.

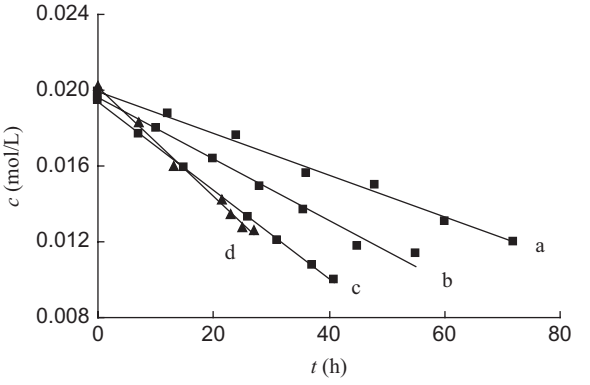


FIGURE 6. The relationship between c and t at 35°C: (a) p_{O_2} = 0.595 MPa, (b) p_{O_2} = 0.995 MPa, (c) p_{O_2} = 1.395 MPa, and (d) p_{O_2} = 1.795 MPa.

from the slope and the intercept (values also in Table 9), respectively.

Finally, the total rate constant k_{total} of the degradation of captopril solution can be rearranged as follows:

$$k_{total} = k_{anaerobic} + k_{aerobic} = A_{anaerobic}\exp\left(-\frac{E_{a,anaerobic}}{RT}\right) + A_{aerobic}\exp\left(-\frac{E_{a,aerobic}}{RT}\right)p_{O_2}. \quad (9)$$

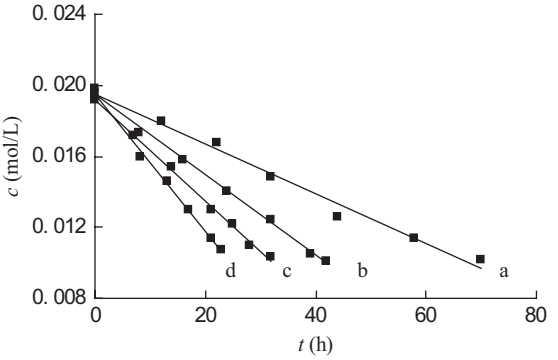


FIGURE 7. The relationship between c and t at 45°C: (a) p_{O_2} = 0.595 MPa, (b) p_{O_2} = 0.995 MPa, (c) p_{O_2} = 1.395 MPa, and (d) p_{O_2} = 1.795 MPa.

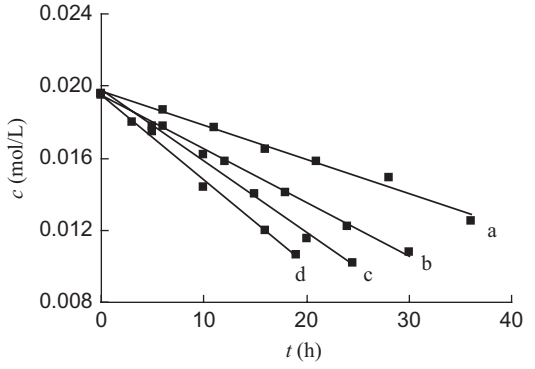


FIGURE 8. The relationship between c and t at 55°C: (a) p_{O_2} = 0.595 MPa, (b) p_{O_2} = 0.995 MPa, (c) p_{O_2} = 1.395 MPa, and (d) p_{O_2} = 1.795 MPa.

According to Equation 9, the rate constant k_{total} of degradation of captopril solution at specific temperature and oxygen pressure can be calculated. It is seen from the data in Table 9 that the results obtained from the step nonisothermal experiments are in agreement with those obtained from the isothermal–isobaric experiments. The value of k_{total} calculated using Equation 9 at 32°C and 1 atm oxygen pressure is 2.9×10^{-5} mol/L/h, which is comparable with the value of 1.7×10^{-5} mol/L/h reported in the literature (Lee & Notari, 1987).

Computer Simulations

Effects of contents errors on accuracy and precision of the estimates for E_a and k_{298} were listed in Table 10. The results in Table 10 indicate that with the same extent of drug degradation and in the same range of temperature change, the estimates obtained by isothermal–isobaric method are somewhat more accurate and precise than those obtained by step nonisothermal method. However, the experimental period needed by the isothermal–isobaric method is much longer (about four times) than that needed by the step nonisothermal method.

Effects of temperature and oxygen pressure errors on accuracy and precision of the estimates for E_a and k_{298} were listed in Table 11. Comparing the data in Table 11 with that in Table 10, we find that the step nonisothermal method is still reliable at ±1.0°C temperature errors or ±0.02 MPa pressure errors.

DISCUSSION

In the conventional computation of the step nonisothermal method (Pang, Fan, & Hou, 1981), the rate constants are calculated with the following equation:

$$\frac{[f(c_i) - f(c_{i+1})]}{t_i} = k_i, \tag{10}$$

where $f(c_i)$ and $f(c_{i+1})$ are the initial and final concentration functions of the ith incubation stage, respectively, i is the number of incubation stage being considered, k_i the rate constant at each incubation temperature, and t_i the incubation time. According to Equation 10, k_i can be obtained in a single-step nonisothermal experiment. Usually in drug stability studies, the entire experiment is in the initial stage of the degradation. If a large number of experimental points are arranged in this stage, the time interval will be very small and the concentration data will be very close together, especially at the beginning of the experiment while the temperature is low. Therefore, the relative error of the concentration function difference $[f(c_i)–f(c_{i+1})]$ will be very large even if the measurements of the concentrations are quite accurate. That will reduce the correlativity of the data and the correlation coefficient r of the straight line.

As a comparison, the data in Table 1 were treated with the conventional computation and the results were shown in Figure 12. The activation energy $E_{a,anaerobic}$ and pre-exponential factor $A_{anaerobic}$ under anaerobic conditions obtained from the conventional computation were 35.23 kJ/mol and 10.6, which differed significantly from those of isothermal–isobaric experiments. Furthermore, they were much less reliable than those of optimization because of their low correlativity of data and low coefficient (r = .787).

CONCLUSION

The use of nonisothermal experiments in drug stability studies began in the 1960s. In comparison with the earlier classic isothermal experiments, nonisothermal method saves time, labor, and drugs. However, with the conventional nonisothermal heating controller, the accuracy and the regulation of temperature can hardly be very satisfactory because of its use of the motor, gears, cam, and mercury thermoregulator. The main advantage of the step nonisothermal method is that the experimental temperature can be changed manually, no complicated temperature-control device is required, and it could therefore be widely applied in almost every laboratory. In this article, the kinetics parameters of captopril oxidation in aqueous solution

TABLE 8
Rate Constants of Captopril Solution Obtained from the Isothermal–Isobaric Experiments

T (°C)	p_{O_2} (MPa)	$k_{total} \times 10^3$ (mol/L/h)	R	$k_{anaerobic} \times 10^3$ (mol/L/h)	$k_{aerobic} \times 10^3$ (mol/L//h)
25	0.595	0.0765	0.974	0.0106	0.0659
	0.995	0.1034	0.964		0.0928
	1.395	0.1566	0.987		0.1460
	1.795	0.1945	0.992		0.1839
35	0.595	0.1160	0.981	0.0186	0.0974
	0.995	0.1504	0.989		0.1318
	1.395	0.2318	0.997		0.2132
	1.795	0.2861	0.996		0.2675
45	0.595	0.1401	0.986	0.0253	0.1148
	0.995	0.2265	0.996		0.2012
	1.395	0.2849	0.997		0.2596
	1.795	0.3838	0.998		0.3585
55	0.595	0.1900	0.994	0.0512	0.1388
	0.995	0.2976	0.998		0.2464
	1.395	0.3920	0.995		0.3408
	1.795	0.4790	0.995		0.4278

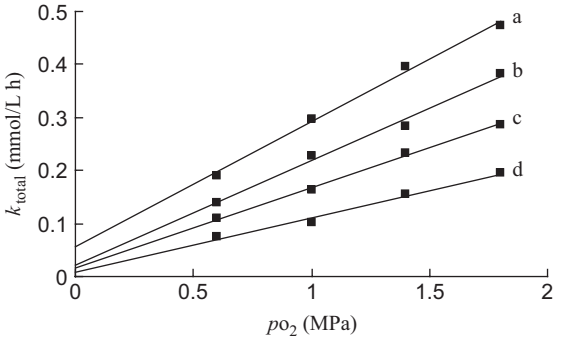


FIGURE 9. The relationship between k_{total} and oxygen pressure p_{O_2} in the isothermal–isobaric experiments: (a) 55°C, (b) 45°C, (c) 35°C, and (d) 25°C.

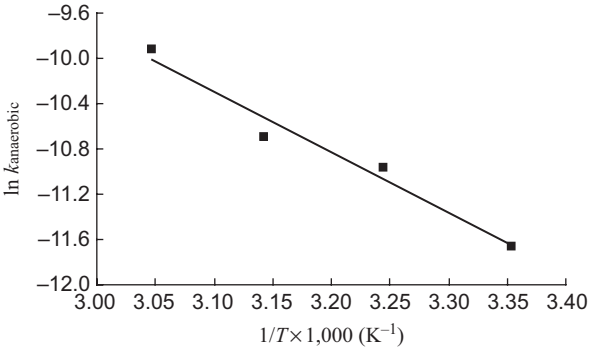


FIGURE 10. The linear relationship between ln $k_{anaerobic}$ and $1/T$ in the isothermal–isobaric experiments.

under compressed oxygen were determined with this method. The results indicated that the parameters obtained in the step nonisothermal experiment were comparable with those obtained in the isothermal–isobaric experiments. Computer simulation showed that the estimates for the kinetic parameters obtained with isothermal–isobaric method were somewhat more precise than those obtained with step nonisothermal method. However, the experimental period needed by isothermal–isobaric method was much longer than that needed by step nonisothermal method.

TABLE 9
Kinetic Parameters Obtained from the Step Nonisothermal Experiments and the Isothermal–Isobaric Experiment

Kinetic Parameters	$E_{a,\ anaerobic}$ (kJ mol^{-1})	$A_{anaerobic}$	$E_{a,aerobic}$ (kJ mol^{-1})	$A_{aerobic}$
Step nonisothermal	42.60	280.1	18.47	0.216
Isothermal–isobaric	44.62	572.0	22.44	1.39

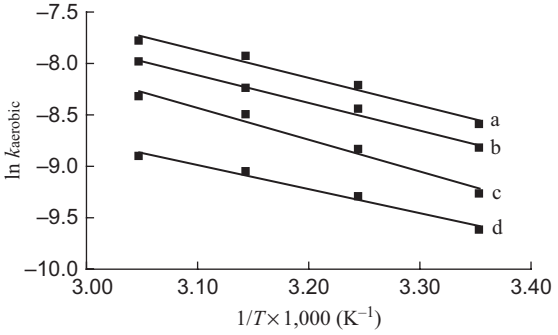


FIGURE 11. The linear relationship between ln $k_{aerobic}$ and $1/T$ in the isothermal–isobaric experiments: (a) 1.795 MPa, (b) 1.395 MPa, (c) 0.995 MPa, and (d) 0.595 MPa.

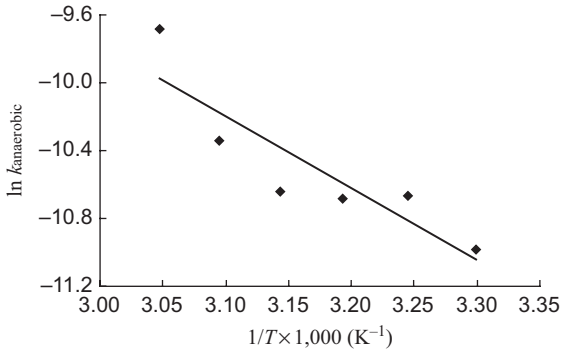


FIGURE 12. The relationship between ln $k_{anaerobic}$ and $1/T$ of ordinary computation.

TABLE 10
Results from Zero-Order Simulations of the Step Nonisothermal and Isothermal–Isobaric Experiments with Temperatures of 25–55°C, Oxygen Pressure 0.995 MPa, and Drug Degradation 100–60% with ±1 and ±2% Random Contents Errors in Each Program

Heating Model	Parameter	Theoretical Values from Errorless Data	Estimates from Data with ±1% Random Content Error	Estimates from Data with ±2% Random Content Error
Step nonisothermal	E_a (kJ/mol)	24121.0	24238.2 ± 10.9%[a]	24376.9 ± 23.9%
	$k_{298} \times 10^4$ (mol/L/h)	1.418	1.529 ± 12.6%	1.621 ± 15.0%
Isothermal–isobaric	E_a (kJ/mol)	24121.0	24134.8 ± 2.0%	24115.1 ± 3.7%
	$k_{298} \times 10^4$ (mol L^{-1} h^{-1})	1.418	1.442 ± 6.5%	1.503 ± 9.4%

[a]$M \pm RSD$% of 500 sets of data.

TABLE 11
Effects of Temperature and Oxygen Pressure Errors on Accuracy and Precision of the Estimates for E_a and k_{298} in Step Nonisothermal Experiments

Parameter	E_a (kJ/mol)	$k_{298} \times 10^4$ (mol/L/h)
Theoretical values from errorless data	24121.0	1.418
Estimates from data with ±0.5°C random temperature error	24153.8 ± 1.40%*	1.429 ± 5.0%
Estimates from data with ±1.0°C random temperature error	24126.3 ± 2.83%	1.466 ± 8.8%
Estimates from data with ±0.01 MPa random temperature error	24037.8 ± 4.09%	1.529 ± 13.4%
Estimates from data with ±0.02 MPa random temperature error	26475.9 ± 6.69%	1.778 ± 24.5%

*$M \pm RSD$% of 500 sets of data.

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