—Regular Articles—

Step Nonisothermal Method in Kinetics Studies of Captopril Oxidation under Compressed Oxygen

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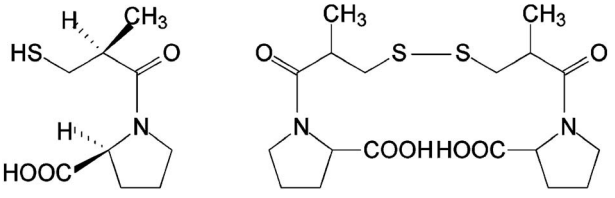
A step nonisothermal experiment under high oxygen pressure and a computation with optimization for a step nonisothermal experiment on a stability study of drugs are introduced. The kinetics parameters of captopril oxidation in aqueous solution were determined with this method. It is reported that the reaction of captopril solution occurs under either aerobic or anaerobic conditions, giving different products. Then the total rate constant k_{total} can be expressed as: $k_{total}=k_{anaerobic}+k_{aerobic}=A_{anaerobic}\exp(-E_{a,\,anaerobic}/RT)+A_{aerobic}\exp(-E_{a,\,aerobic}/RT)p_{O_2}$, where $k_{anaerobic}$ and $k_{aerobic}$ are the rate constants of anaerobic and aerobic degradation, respectively. The results indicate that the parameters obtained in the step nonisothermal experiment are comparable to those obtained in isothermal-isobaric experiments.

Key words——captopril; drug stability; step nonisothermal experiment; oxidative degradation; kinetics; optimization

INTRODUCTION

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 is 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 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, due to the complexity of experimental design. Many papers reported that some drugs are unstable (or stable) under aerobic (or anaerobic) conditions,[1–11] but few reported the oxidation rate.[12,13]

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[14,15] captopril disulfide (II).



I II

To quantify the influence of oxygen on the stability of captopril, it is necessary to determine its oxidation kinetics. Timmins *et al.*[16] determined the oxidation rate constants of captopril solution at 50°C. In their study, to 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 studied the stability of captopril solution at a fixed temperature and oxygen partial pressure (90–760 mmHg).[17] However, the dependence of the oxidation on temperature was not reported.

Shi *et al.* reported a drug stability experiment accelerated by compressed oxygen.[13] 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.[18–20] These experiments save time and labor;

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however, the use of sophisticated temperature-control devices limited their popularity. The step nonisothermal experiment[21,22] 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:

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

where c is the residual concentration at time t, c_0 the initial concentration, k_i the reaction rate constant at 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 Eq. (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:

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

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 Fig. 1: where T_0, T_1 $\ldots T_i$ are the stepping incubation temperatures, and Δt_0, $\Delta t_1 \ldots \Delta t_i$ are the incubation duration, respectively. 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 ($\sim$hours). Thus for the step nonisothermal method, Eq. (2) can be expressed as:

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

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 E_a value is assumed correctly, k_0 will be a constant and can be taken out of the sum, which then yields Eq. (4):

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

According to Eq. (4), a straight line can be obtained from a plot of the concentration function $f(c)$ *versus*

$$\sum_{i=0}^{i} \exp\left[\frac{E_a}{R}\left(\frac{1}{T_0} - \frac{1}{T_i}\right)\right] \Delta t_i$$

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 Eq. (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 Eq. (4), a group of regression lines with different correlation coefficients r can be obtained. The higher the correlation coefficient r is, the closer the assumed E_a will be 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 the present paper, 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 to those obtained using the isothermal-isobaric method, with the advantage of using only $\sim$1/4 the number of measurements. A computation method with optimization for the step nonisothermal experiment was introduced. Comparison of the optimization and conventional computation indicated that the limitations of the conventional computation had been overcome.

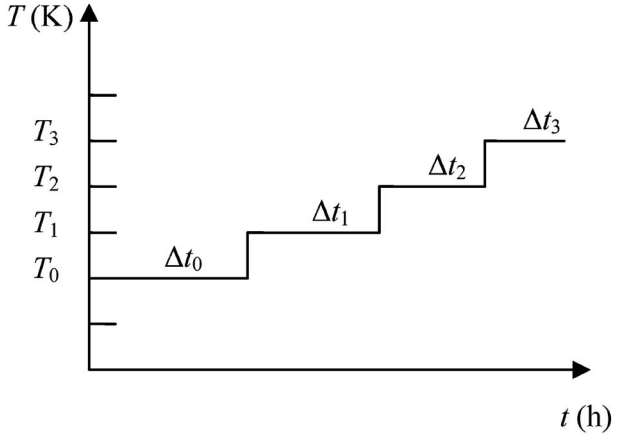


Fig. 1. Time-temperature Relationship for the Step Nonisothermal Experiment

MATERIALS AND METHODS

Materials Captopril was provided by Chan-

gzhou 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 $\geq$ 99.0% (v/v) of O_2. The other reagents used were of analytical grade.

Instruments Thirty oxygen bombs (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 $\pm$0.2 mmHg, Jiaxing, China), and an isothermal heating oven with high precision (the accuracy, precision, reproducibility of temperature are $\leq \pm 0.5$℃ in the range of room temperature to 100℃; self-made),[23] and HPLC instrumentation (Shimadzu Co., Japan, 10A style, SPD-10AVP detector) were used.

Analysis The chromatographic separation and quantitative determination were performed on a high-performance liquid chromatograph (HPLC). The HPLC assay for captopril was similar to that described by Lee and Notari.[17] A reverse-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^{-1}, 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 bombs, p_{O_2}, the oxygen pressure in a state of equilibrium, was measured instead. The difference between the internal pressure of a bomb 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 of water. Then the solution was diluted with water to a total volume of 1000 ml. An exact volume of 25 ml of the captopril solution was transferred to a 50-ml beaker. Then the beaker was placed into an oxygen bomb. About 21 such oxygen bombs were pressurized with oxygen to a predetermined pressure and put into a thermostatic oven at the beginning of the experiment. Three bombs 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℃. The temperature was increased by 5 ℃ (heating rate$>$1℃/min) at the beginning of each step and was kept constant for the remainder of the step time. The oxygen pressures were 0.595 MPa, 0.995 MPa, 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 of water. Then the solution was diluted with water to a total volume of 1000 ml. An exact volume of 25 ml of the captopril solution was transferred to a 50-ml beaker. Then the beaker was placed into an oxygen bomb. About 21 such oxygen bombs were pressurized with oxygen to a predetermined pressure and put into a thermostatic oven at the beginning of the experiment. Three bombs 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℃ and 0.595 MPa, 0.995 MPa, 1.395 MPa, and 1.795 MPa, respectively.

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 Eq. (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℃ of the degradation of captopril solution were obtained. Thus based on the Arrhenius equation $k_T = k_{30} \exp\left[E_{a,\,total}/R(1/303.15 - 1/T)\right]$, the total rate constants k_{total} at each experimental temperature and oxygen pressure could be calculated and are given in Table 2.

Table 1. Results of Step Nonisothermal Experiments of Captopril Solution at Constant Oxygen Pressure

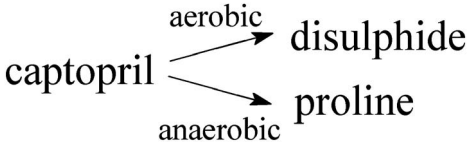
T (℃)	p_{O_2}=0.595 MPa		p_{O_2}=0.995 MPa		p_{O_2}=1.395 MPa	
	t (h)	c (mol·l^{-1})	t (h)	c (mol·l^{-1})	t (h)	c (mol·l^{-1})
	0	0.0200±0.0002*	0	0.0198±0.0001	0	0.0198±0.0001
30	16.50	0.0186±0.0002	9.00	0.0184±0.0002	6.50	0.0186±0.0001
35	13.00	0.0172±0.0001	7.50	0.0169±0.0001	6.00	0.0172±0.0001
40	9.00	0.0160±0.0002	7.00	0.0156±0.0002	5.50	0.0157±0.0002
45	7.50	0.0149±0.0001	6.00	0.0144±0.0003	5.00	0.0142±0.0002
50	7.00	0.0137±0.0003	5.00	0.0131±0.0004	4.50	0.0126±0.0003
55	6.00	0.0126±0.0004	4.00	0.0118±0.0003	3.00	0.0115±0.0004

Results are presented as mean±SD% of three samples.

Table 2. Rate Constants of Captopril Solution Obtained from the Step Nonisothermal Experiment

p_{O_2} (MPa)	T (℃)	$k_{total}\times10^3$ (mol·l^{-1}·h^{-1})	$k_{anaerobic}\times10^3$ (mol·l^{-1}·h^{-1})	$k_{aerobic}\times10^3$ (mol·l^{-1}·h^{-1})
0.595	30	0.097	0.012	0.085
	35	0.112	0.017	0.095
	40	0.129	0.023	0.106
	45	0.147	0.029	0.118
	50	0.168	0.036	0.132
	55	0.191	0.045	0.146
0.995	30	0.149	0.012	0.137
	35	0.172	0.017	0.155
	40	0.197	0.023	0.174
	45	0.225	0.029	0.196
	50	0.256	0.036	0.220
	55	0.289	0.045	0.244
1.395	30	0.208	0.012	0.196
	35	0.237	0.017	0.220
	40	0.269	0.023	0.246
	45	0.305	0.029	0.276
	50	0.343	0.036	0.307
	55	0.385	0.045	0.340

It is reported that the degradation of captopril solution occurs under either aerobic or anaerobic conditions, giving different degradants.[16] Disulphide 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 aerobic reaction. Therefore the total degradation rate constant k_{total} can be expressed as:

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

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 2, six linear regression lines, with the correlation coefficients $r>0.994$, shown in Fig. 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 2.

Based on the Arrhenius equation, $k_{anaerobic}$ can be

expressed as:

$$\ln k_{\text{anaerobic}} = \ln A_{\text{anaerobic}} - \frac{E_{\text{a, anaerobic}}}{RT}$$

where $E_{\text{a, anaerobic}}$ and $A_{\text{anaerobic}}$ are the activation energy and preexponential factor of the degradation under anaerobic conditions, respectively. Therefore, a straight line, with the correlation coefficient $r=0.997$, would be obtained by plotting $\ln k_{\text{anaerobic}}$ *versus* $1/T$ (shown in Fig. 3). From the slope and the intercept of the $\ln k_{\text{anaerobic}} \sim 1/T$ line, $E_{\text{a, anaerobic}}$ and $A_{\text{anaerobic}}$, respectively, could be determined and are given in Table 3.

According to Eq. (5), k_{aerobic} could be calculated by $k_{\text{aerobic}} = k_{\text{total}} - k_{\text{anaerobic}}$. The values of k_{aerobic} are also shown in Table 2.

Because of the linear relationship between k_{total} and p_{O_2} (shown in Fig. 2), k_{aerobic} was proportional to p_{O_2}. The relationship between k_{aerobic} and oxygen pressure was expressed as Eq. (6) by Shi *et al.*[13]

$$k_{\text{aerobic}} = A_{\text{aerobic}} \exp\left(-\frac{E_{\text{a, aerobic}}}{RT}\right) \cdot p_{O_2} \qquad (6)$$

where p_{O_2} is the oxygen pressure, and $E_{\text{a, aerobic}}$ and A_{aerobic} are the activation energy and preexponential 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 Eq. (6). Taking the natural logarithm of both sides of Eq. (6) yields:

$$\ln k_{\text{aerobic}} = \ln (A_{\text{aerobic}} p_{O_2}) - \frac{E_{\text{a, aerobic}}}{RT} \qquad (7)$$

According to Eq. (7), three straight lines, with correlation coefficient $r > 0.990$, were obtained by plotting $\ln k_{\text{aerobic}}$ *versus* $1/T$ (Fig. 4). Thus $E_{\text{a, aerobic}}$ and A_{aerobic} could be determined from the slope and the intercept, respectively. The values are also listed in Ta-

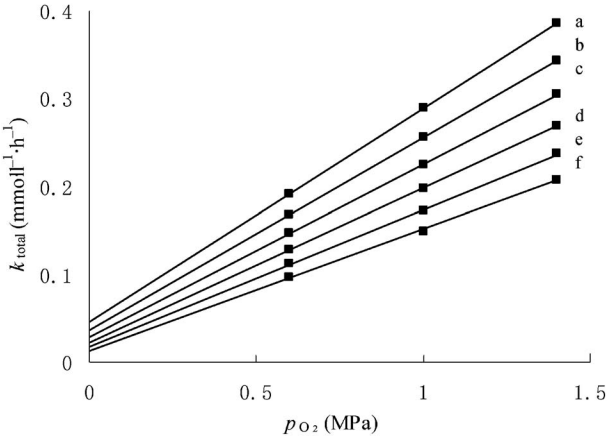


Fig. 2. Relationship between k_{total} and Oxygen Pressure p_{O_2} in the Step Nonisothermal Experiment
(a) 55℃; (b) 50℃; (c) 45℃; (d) 40℃; (e) 35℃; (f) 30℃.

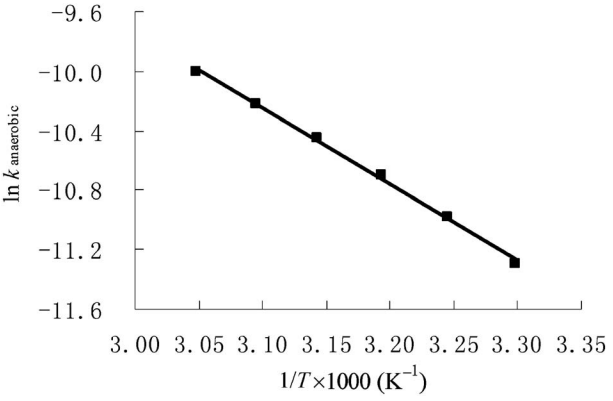


Fig. 3. Linear Relationship between $\ln k_{\text{anaerobic}}$ and $1/T$ in the Step Nonisothermal Experiment

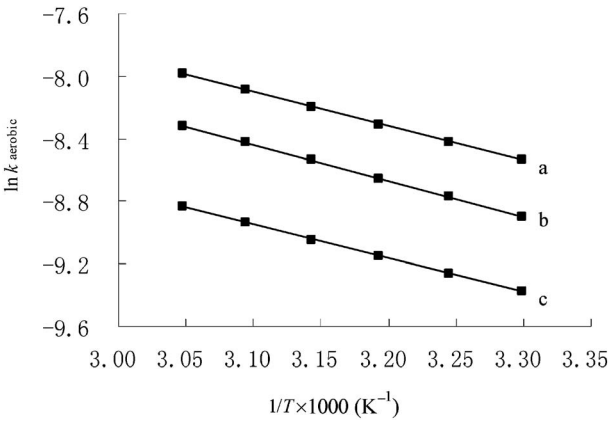


Fig. 4. Linear Relationship between $\ln k_{\text{aerobic}}$ and $1/T$ in the Step Nonisothermal Experiment
(a) p_{O_2}=1.395 MPa, (b) p_{O_2}=0.995 MPa and (c) p_{O_2}=0.595 MPa.

Table 3. Kinetic Parameters Obtained from the Step Nonisothermal Experiments and the Isothermal-isobaric Experiment

Kinetic Parameters	$E_{\text{a,anaerobic}}$ (kJ·mol^{-1})	$A_{\text{anaerobic}}$	$E_{\text{a,aerobic}}$ (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

ble 3.

Isothermal-isobaric Experiments As seen from the lines in Fig. 5, 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 Fig. 5 and listed in Table 4.

From the data in Table 4, four linear regression

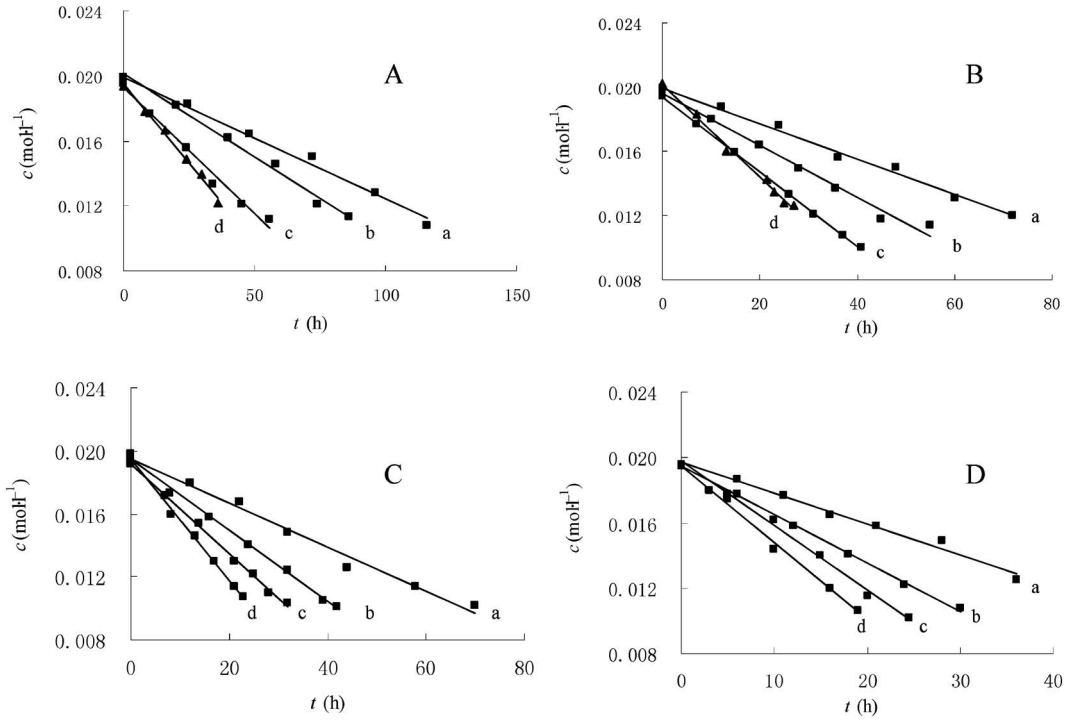


Fig. 5. Relationship between c and t at 25℃ (A), 35℃ (B), 45℃ (C) and 55℃ (D)
(a) p_{O_2}=0.595 MPa, (b) p_{O_2}=0.995 MPa, (c) p_{O_2}=1.395 MPa and (d) p_{O_2}=1.795 MPa.

Table 4. Rate Constants of Captopril Solution Obtained from the Isothermal–isobaric Experiments

T (℃)	p_{O_2} (MPa)	$k_{total}\times10^3$ (mol·l^{-1}·h^{-1})	r	$k_{anaerobic}\times10^3$ (mol·l^{-1}·h^{-1})	$k_{aerobic}\times10^3$ (mol·l^{-1}·h^{-1})
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

lines, with the correlation coefficients $r>0.980$, 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 4.

By plotting ln $k_{anaerobic}$ *versus* the reciprocal of temperature $1/T$, a straight regression line was obtained ($r=0.970$), 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 3.

The values of $k_{aerobic}$ were calculated using Eq. (5) and listed in Table 4. Four straight lines, with correlation coefficient $r>0.975$, were obtained by plotting ln $k_{aerobic}$ *versus* $1/T$. $E_{a,\,aerobic}$ and $A_{aerobic}$ could be determined from the slope and the intercept (values also in Table 3), respectively.

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

$$k_{total}=k_{anaerobic}+k_{aerobic}$$
$$=A_{anaerobic}\exp\left(-\frac{E_{a,\,anaerobic}}{RT}\right)$$
$$+A_{aerobic}\exp\left(-\frac{E_{a,\,aerobic}}{RT}\right)p_{O_2} \qquad (8)$$

According to Eq. (8), the rate constant k_{total} of the degradation of captopril solution at specific temperature and oxygen pressure can be calculated. It is seen from the data in Table 3 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 Eq. (8) at 32℃ and 1 atm oxygen pressure is 2.9×10^{-5} mol·l^{-1}·h^{-1}, which is comparable to the value of 1.7×10^{-5} mol·l^{-1}·h^{-1} reported in the literature.[17]

DISCUSSION

In the conventional computation of the step nonisothermal method,[22] the rate constants are calculated with the following equation:

$$\frac{[f(c_i)-f(c_{i+1})]}{t_i}=k_i \qquad (9)$$

where $f(c_i)$ and $f(c_{i+1})$ are the initial and final concentration function of the ith incubation stage, respectively; i is the number of incubation stage being considered, k_i represents the rate constant at each incubation temperature; and t_i is incubation time. According to Eq. (9), 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 is 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 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. The activation energy $E_{a,\,anaerobic}$ and preexponential factor $A_{anaerobic}$ under anaerobic conditions obtained from the conventional computation were 35.23 kJ·mol^{-1} and 10.6, which were 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 ($r=0.787$).

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