Compressed Oxygen in Drug Stability Experiments

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A drug stability experiment accelerated by compressed oxygen was established. The stability of 10% ascorbic acid solution as a model was studied and the kinetic parameters were obtained with the newly established experimental method. Because ascorbic acid degrades under both anaerobic and aerobic conditions, the total rate constant k_{total} can be expressed as: $k_{total}=k_{anaerobic}+k_{aerobic}$, where $k_{anaerobic}$ and $k_{aerobic}$ are the rate constants of anaerobic and aerobic degradations, respectively. The $k_{anaerobic}$ can be expressed as $k_{anaerobic}=A_{anaerobic}\cdot\exp(-E_{a,anaerobic}/RT)$ according to Arrhenius equation, and the $k_{aerobic}$ was found to be $k_{aerobic}=A_{aerobic}\cdot\exp(-E_{a,aerobic}/RT)\cdot p_{O_2}$ in our study.

Key words drug stability experiment; compressed oxygen; degradation kinetics; ascorbic acid

In oxido-stability studies, some drugs are unstable to oxygen, indicating that their stabilities depend mainly on the pressure of oxygen (or the concentration of dissolved oxygen in solutions). The drugs that are unstable to both oxygen and heat should obviously depend on both the pressure of oxygen and the storage temperature. In order to predict and improve the stability of these drugs, it is important to study their oxidation rates, but a quantitative study of such phenomena has seldom been found in the literature because of theoretical and technological limitations. It was reported that some drugs were unstable (or stable) under an aerobic (or anaerobic) condition,[1—11] however, the oxidation rate has not been found in the literature.

Antioxidant played a very important part in pharmaceutical preparations to protect drugs unstable to oxygen; however, the selection of antioxidants in pharmaceutical studies is considerably empirical.[12—17] The theoretics found in the literature was the comparison of the standard oxidation (or reduction) potentials of antioxidants and that of drugs.[18] According to the theoretics of physical chemistry, these standard potentials are related to ΔG^0 and K, the increment of standard Gibbs function and the equilibrium constant of the chemical reactions.[19,21] The criterion $dG_{T,p}\leqq 0$ carries over into chemistry as the remark that, at constant temperature and pressure, chemical reactions are spontaneous in the direction of decreasing Gibbs function.[20,22] Therefore, the comparison of the standard potentials can only demonstrate a possibility of the protection because the emphasis up to this point has been on the equilibrium properties of substances in pure or solution form.

A competitive oxidation reaction between the antioxidant and the protected drug occurred in a pharmaceutical preparation. Studying the rates of reactions is the practical importance of being able to predict how quickly a reaction mixture approaches equilibrium.[23] Therefore, to determine the actual priority of the competitive oxidations and compare the drug-protect capacity of antioxidants, it is important to determine their oxidation rate constant using chemical kinetics. A ratio of oxygen consumed by antioxidants to that by protected drugs can be calculated theoretically, provided the oxidation rate constants of both antioxidants and protected drugs were determined. Moreover, the amount of a drug protected by antioxidant can be therefore calculated. Such viewpoint and study have not been found in the literature.

In the present paper, kinetic studies were used to quantitate the influence of the pressure of oxygen on the oxidation rate of drugs. In order to save time, compressed oxygen were used in isothermal experiments. This kind of experiments has not been found in drug stability studies, except in some lipid oxidation in foods.[24—26] Ascorbic acid solution, used as a model drug, was incubated under a group of oxygen pressures and temperatures. The oxidation rate constants of the model drug at various temperatures were obtained and the relationship among pressure of oxygen, temperature and degradation rate constant was established. According to the relationship, the rate constant at room temperature and normal oxygen pressures was extrapolated.

In our other studies which will be reported in separated submissions, kinetics is used to quantitatively determine the oxidation rate constants of some widely used antioxidants.

These studies are benefit to improve drug stability, determine the shelf life of drugs, and reduce the harm resulted from excessive quantity of antioxidants.

Theoretical

According to the basement of physical chemistry, the oxidation of drugs can be described by some form of the following general equation:

$$\frac{-dc_{Drug}}{dt}=k'c_{Drug}^{n}p_{O_2}^{m} \quad \text{or} \quad \frac{-dc_{Drug}}{dt}=k'c_{Drug}^{n}c_{O_2}^{m} \tag{1}$$

where c_{Drug} is the concentration of drug, p_{O_2} the pressure of oxygen, c_{O_2} the concentration of oxygen, t the time, k' the reaction rate constant, n the reaction order of the drug, and m that of oxygen. According to Eq. 1, drugs will in general show a rate dependence on c_{Drug}, p_{O_2} or c_{O_2}. The p_{O_2} dependence was reported in studies of lipid oxidation in food.[24—26]

When p_{O_2} or c_{O_2} keeps constant, Eq. 1 can be expressed as:

$$\frac{-dc_{Drug}}{dt}=kc_{Drug}^{n} \tag{2}$$

where k is the apparent reaction rate constant. After integrating, Eq. 2 can be expressed as follows:

$$f(c)=f(c_0)-kt \tag{3}$$

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where c is the residual concentration at time t, c_0 the initial concentration, 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.

According to Eq. 3, the rate constant k can be obtained from the slope of a plot of $f(c)$ *versus* t. In experiments, we can place samples into oxygen bombs and pressurize the bombs with oxygen to a specific pressure. Then incubating the bombs isothermally and measuring the samples at each suitable interval, respectively.

For a drug that is unstable to both oxygen and heat, the k is the function of oxygen pressure and temperature:

$$k=f(p_{O_2}, T) \quad (4)$$

The relationship between rate constant and oxygen pressure and temperature can be obtained by repeating the above incubating experiment under a group of oxygen pressures and temperatures.

Experimental and Results

Instruments and Drugs Thirty oxygen bombs (pressurized-stainless-steel-container) were used and are shown in Fig. 1.

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 mmHg, Jiaxing, China), a pH Meter (Delta-320, Shanghai, China), and an isothermal heating oven with high precision (the accuracy, precision, and reproducibility of temperature are ≤±0.5 °C in the range of room temperature to 100 °C; self-made)[27] were used.

Ascorbic acid was received from Chengdu Chemical Company and of analytical grade. It contains ≥99.7% of $C_6H_8O_6$. Oxygen was of medical application grade. It contains ≥99.0% of O_2. The Other reagents used were of analytical grade.

Preparation of 10% (0.567 mol/l) Ascorbic Acid Solution A 50 g quantity of ascorbic acid was dissolved in 450 ml water. The pH was adjusted to 6.8 with NaOH. Then the solution was diluted with water to a total volume of 500 ml. Because ascorbic acid and ascorbate is a good pair of buffer, the pH change is ≤0.2 pH after each incubation. So no other buffer was used in our experiment.

It is reported that the degradation of high concentration ascorbic acid solution is little ionic strength dependent,[28] and, it is difficult to control the ionic strength in such high concentration solution. Therefore, the ionic strength was not controlled in our experiments.

Measuring Oxygen Pressure Because of the difficulty in measuring the concentration of dissolved oxygen in bombs, p_{O_2}, the pressure of oxygen in a state of equilibrium, was measured instead.

The difference between the internal pressure of a bomb and the external barometric pressure was measured by 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 the ambient temperature because they influenced the specific gravity of mercury.

Isothermal Experiments under Compressed Oxygen The oxidation rate of ascorbic acid is catalyzed by metal ions.[29] In order to avoid the contact of metal ions, an exact volume of 2 ml of the 10% ascorbic acid solution was transferred to a 50-ml beaker. Then the beaker was placed into an oxygen bomb. About 30 such bombs were pressurized with oxygen to a specific 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 iodometrically.[30,31]

The experimental temperatures and oxygen pressures were 15 °C, 25 °C, 35 °C, 45 °C (or 288.15, 298.15, 308.15, 318.15 K) and 0.597 MPa, 1.097 MPa, 1.597 MPa, 2.097 MPa, respectively. The experimental results are shown in Figs. 2—5.

Ascorbic acid is unstable in aqueous solutions. It is reported that the degradation of ascorbic acid obeys apparent zero-order kinetics in high concentration, such as 10% or

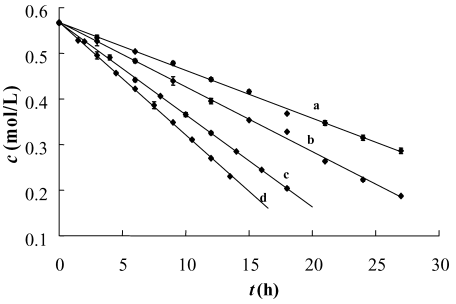


Fig. 2. The Residual Concentrations of Ascorbic Acid Solutions at 15 °C

The oxygen pressures were 0.597 MPa (a), 1.097 MPa (b), 1.597 MPa (c), 2.097 MPa (d), respectively.

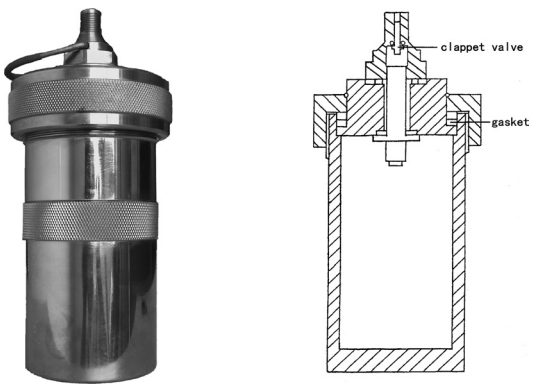


Fig. 1. The Outside Drawing and Profile Chart of the Oxygen Bomb

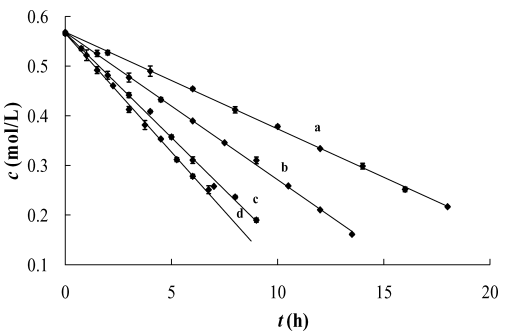


Fig. 3. The Residual Concentrations of Ascorbic Acid Solutions at 25 °C

The oxygen pressures were 0.597 MPa (a), 1.097 MPa (b), 1.597 MPa (c), 2.097 MPa (d), respectively.

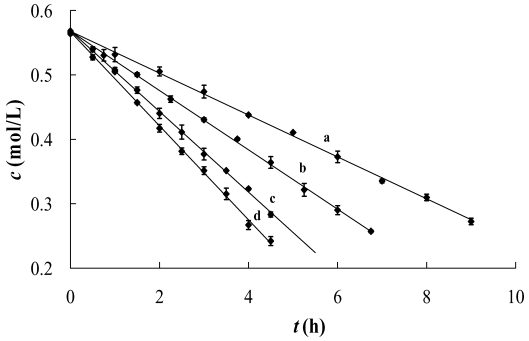


Fig. 4. The Residual Concentrations of Ascorbic Acid Solutions at 35 °C

The oxygen pressures were 0.597 MPa (a), 1.097 MPa (b), 1.597 MPa (c), 2.097 MPa (d), respectively.

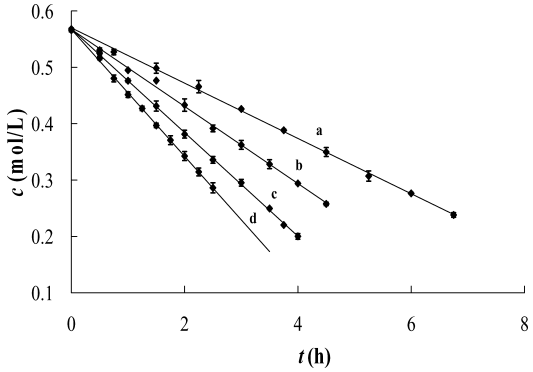


Fig. 5. The Residual Concentrations of Ascorbic Acid Solutions at 45 °C

The oxygen pressures were 0.597 MPa (a), 1.097 MPa (b), 1.597 MPa (c), 2.097 MPa (d), respectively.

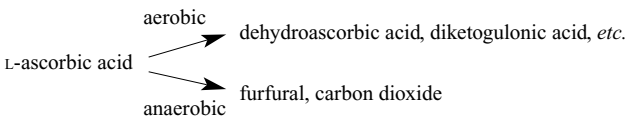
Table 1. The Degradation Rate Constants of Ascorbic Acid Solution at Experimental Temperatures and Pressures of Oxygen

T (K)	p_{O_2} (MPa)	k_{total} [mol/(l·h)]	$k_{anaerobic}$ [mol/(l·h)]	$k_{aerobic}$ [mol/(l·h)]	$\|r\|$
288.15	0.597	0.0105	0.0043	0.0062	0.9988
	1.097	0.0142		0.0099	0.9989
	1.597	0.0202		0.0159	0.9998
	2.097	0.0247		0.0204	0.9997
298.15	0.597	0.0195	0.0086	0.0109	0.9997
	1.097	0.0297		0.0211	0.9995
	1.597	0.0421		0.0335	0.9987
	2.097	0.0477		0.0391	0.9988
308.15	0.597	0.0325	0.0164	0.0161	0.9994
	1.097	0.0460		0.0296	0.9996
	1.597	0.0626		0.0462	0.9995
	2.097	0.0729		0.0565	0.9987
318.15	0.597	0.0490	0.0228	0.0262	0.9994
	1.097	0.0684		0.0456	0.9989
	1.597	0.0919		0.0691	0.9998
	2.097	0.1126		0.0898	0.9996

25%,[32] and obeys apparent first-order kinetics in low concentration, such as 2.5%, 1.5%, 0.5% *etc.*[29] From the lines in Figs. 2—5, the degradation of 10% ascorbic acid solution obeys apparent zero-order kinetics in our experiments:

$$c = c_0 - kt \tag{5}$$

It is reported that the degradation of ascorbic acid occurs under either aerobic or anaerobic condition, giving different degradants.[29] Under aerobic condition the ascorbic acid is oxidized to dehydroascorbic acid followed by hydrolysis and oxidation to give diketogulonic acid and oxalic acid. Under anaerobic condition, it undergoes dehydration and hydrolysis to give furfural and carbon dioxide.[29]



So it is suggested that the total degradation can be divided into two parallel reactions: the anaerobic and aerobic reaction. Therefore, the total degradation rate constant k_{total} can be expressed as:

$$k_{total} = k_{anaerobic} + k_{aerobic} \tag{6}$$

The k_{total} at each experimental temperature and oxygen pressure was obtained from slope of each line in Figs. 2—5 and is listed in Table 1.

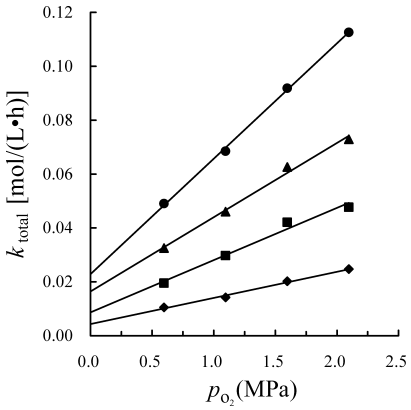


Fig. 6. Relationships between k_{total} and p_{O_2}

◆: 15 °C, ■: 25 °C, ▲: 35 °C, ●: 45 °C.

Kinetic Parameters of Ascorbic Acid Solution under Anaerobic Condition From the data in Table 1, four linear regression lines, shown in Fig. 6, can be obtained by plotting k_{total} *versus* p_{O_2} for each experimental temperature, respectively. The rate constants under anaerobic condition $k_{anaerobic}$ are the intercepts of these lines and are also listed in Table 1.

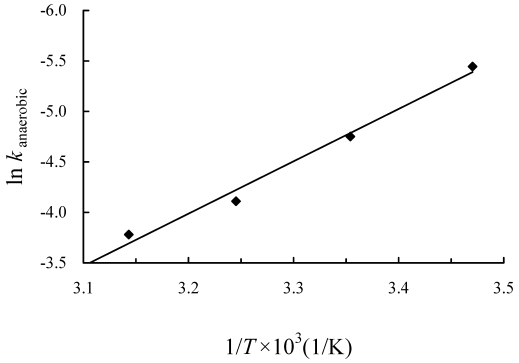


Fig. 7. Relationship between $\ln k_{anaerobic}$ and $1/T$

Based on the Arrhenius equation, a straight line was obtained by plotting $\ln k_{anaerobic}$ *versus* $1/T$ (shown in Fig. 7). Therefore, $k_{anaerobic}$ can be expressed as:

$$\ln k_{anaerobic} = \ln A_{anaerobic} - \frac{E_{a,anaerobic}}{RT} \tag{7}$$

where $E_{a,anaerobic}$ and $A_{anaerobic}$ are the activation energy and pre-exponential factor of the degradation under anaerobic conditions, respectively. From the slope and the intercept of the $\ln k_{anaerobic} \sim 1/T$ line, $E_{a,anaerobic}$ and $A_{anaerobic}$, respectively, can be determined (values listed in Table 2).

Kinetic Parameters of Ascorbic Acid Solution under Aerobic Condition According to Eq. 6 $k_{aerobic}$ can be calculated as:

$$k_{aerobic} = k_{total} - k_{anaerobic} \tag{8}$$

The values of $k_{aerobic}$ are also listed in Table 1. Because of the linear relationship between k_{total} and p_{O_2} (shown in Fig. 6), $k_{aerobic}$ is proportional to p_{O_2}:

$$k_{aerobic} = B \cdot p_{O_2} \tag{9}$$

where B is the slope of each line in Fig. 6 and listed in Table 3. It is evident that the higher the temperature, the larger the value of B; therefore, B is also a function of temperature.

Then, we supposed the temperature dependence of B might be expressed as the following form, similar to the Arrhenius equation:

$$B = A_{aerobic} \cdot \exp\left(-\frac{E_{a,aerobic}}{RT}\right) \quad \text{or} \quad \ln B = \ln A_{aerobic} - \frac{E_{a,aerobic}}{RT} \tag{10}$$

Thus, $k_{aerobic}$ can be expressed as:

$$k_{aerobic} = A_{aerobic} \cdot \exp\left(-\frac{E_{a,aerobic}}{RT}\right) \cdot p_{O_2} \tag{11}$$

where $E_{a,aerobic}$ and $A_{aerobic}$ are the activation energy and pre-exponential factor of the degradation under aerobic conditions, respectively.

According to Eq. 10, a straight line, with correlation coefficient $r=0.9924$, was obtained by plotting $\ln B$ *versus* $1/T$ (shown in Fig. 8). From the slope and the intercept of the $\ln B \sim 1/T$ line, $E_{a,aerobic}$ and $A_{aerobic}$, respectively, were determined (values also listed in Table 2).

Because the degradation of ascorbic acid is very pH dependent, the estimates for the activation energies obtained by our experiments are comparable with the reported ones at the similar pH value: $E_{a,anaerobic}=49.51$ kJ/mol (pH=6.85)[32] and $E_{a,aerobic}=32.64$ kJ/mol (pH=6.6).[29]

Table 2. Kinetic Parameters of Ascorbic Acid Solution

$A_{anaerobic}$ [mol/(l·h)]	$E_{a,anaerobic}$ (kJ/mol)	$A_{aerobic}$ [mol/(l·h·MPa)]	$E_{a,aerobic}$ (kJ/mol)
2.97×10^5	43.10	4.67×10^4	36.70

Table 3. The Values of B at Various Temperatures

T (K)	B mol/(l·h·MPa)
288.15	0.0097
298.15	0.0194
308.15	0.0276
318.15	0.0428

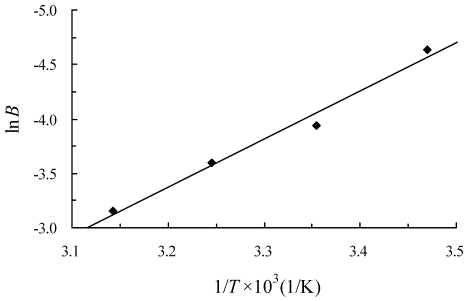


Fig. 8. Relationship between $\ln B$ and $1/T$

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

$$k_{total} = A_{anaerobic} \cdot \exp\left(-\frac{E_{a,anaerobic}}{RT}\right) + A_{aerobic} \cdot \exp\left(-\frac{E_{a,aerobic}}{RT}\right) \cdot p_{O_2} \tag{12}$$

According to Eq. 12, $k_{0.9,298\,K,0.0204\,MPa}$, the degradation rate constant of ascorbic acid solution at room temperature (25 °C) and normal pressure of oxygen ($0.097\times21\%=0.02037$ MPa) can be obtained, and therefore the $t_{0.9}$, the time for the c to decrease to 90% at 25 °C and 0.02037 MPa, can be estimated.

$$\begin{aligned} k_{298\,K,0.0204\,MPa} &= A_{anaerobic} \cdot \exp\left(-\frac{E_{a,anaerobic}}{RT}\right) + A_{aerobic} \cdot \exp\left(-\frac{E_{a,aerobic}}{RT}\right) \cdot p_{O_2} \\ &= 2.97\times10^5 \cdot \exp\left(\frac{-43100}{8.314\times298.15}\right) \\ &\quad + 4.67\times10^4 \cdot \exp\left(\frac{-36700}{8.314\times298.15}\right)\times0.02037 \\ &= 8.70\times10^{-3}\,\text{mol/(l·h)} \end{aligned}$$

$$t_{0.9} = \frac{c_0 - 0.9\cdot c_0}{k} = \frac{0.1\cdot c_0}{k} = \frac{0.1\times0.567}{8.70\times10^{-3}} = 6.52\ \text{h}$$

This calculated $t_{0.9}$ was confirmed to be 7.5 h by a room temperature storage testing.

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