Accuracy Assessment of Classical Isothermal Experiment in Drug Storage Period Studies

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Abstract: The purpose of this research was to assessment the effect of change of dissolved oxygen concentration on accuracy of classical isothermal experiment in ascorbic acid solution storage period studies. The experiments were performed at temperatures 35 ˚C, 40 ˚C, 45 ˚C and 50 ˚C, the apparent rate constant (k_A) and the adjusted apparent rate constant ($k_{A,A}$) were determined, respectively. By plotting $\ln k_A$ and $\ln k_{A,A}$ against $1/T$ resulted two lines, respectively. Then the apparent rate constant at 25 ˚C, $k_{A,25^\circ C}$ =5.168×10^{-4}(mol·L^{-1}·h^{-1}) and the adjusted apparent rate constant at 25 ˚C, $k_{A,A,25^\circ C}$=5.157×10^{-4}(mol·L^{-1}·h^{-1}), was extrapolated, respectively. Both the calculated storage period of the experimented ascorbic acid solution were all 55h by $k_{A,25^\circ C}$ and $k_{A,A,25^\circ C}$, respectively. The results suggested that the change of dissolved oxygen concentration has no effect on the accuracy of classical isothermal experiment in ascorbic acid solution storage period studies.

Keywords: Classical isothermal experiment, accuracy, ascorbic acid, dissolved oxygen, storage period.

1. INTRODUCTION

The essential requirement of pharmaceutical preparation is safe, effective and stable product. Drug stability refers to the capacity of a drug substance or drug product to remain within the established specifications to maintain its identity, strength, quality and purity in a specified period of time. The stability of drug is of great importance since it becomes less effective as it undergoes degradation. Also drug decomposition may yield toxic by products that are harmful to the patient. So the stability data must be provided when new drug application according to the regulations of the State Food and Drug Administration [1]. Prediction of the storage period of pharmaceutical preparation is one of the important indexes to study its stability.

The classical isothermal experiment is used widely in predicting the storage period of pharmaceutical preparations. The theory of the classical isothermal experiment is based on the Arrhenius equation: $\ln k=\ln A - E_a / RT$(k: rate constant, A: frequency factor, E_a: activation energy, R: gas constant, T: absolute temperature). One straight line was obtained by plotting $\ln k$ against $1/T$ according to Arrhenius equation. And the rate constant at temperature 25 ˚C, $k_{25^\circ C}$, can be extrapolated by the straight line equation. Thus the storage period of pharmaceutical preparations can be calculated by $k_{25^\circ C}$.

The degradation rate of ascorbic acid is related to the oxygen solubility in solution in addition to temperature since it is unstable to oxygen. The oxygen solubility in solution decreased with the increase of temperature according to ideal gas law: $PV=nRT$. It was reported that the oxidation reaction was first order with respect to the dissolved oxygen concentration in the ascorbic acid solution [2-4], that is, the degradation rate decreased with the decrease of oxygen solubility. Therefore, the degradation rate increased with the increase of temperature by Arrhenius equation, on the other hand, the degradation rate decreased with the increase of temperature by $PV=nRT$. So, the effect of the change of dissolved oxygen concentration on the degradation rate was ignored if the classical isothermal experiment was used to predict the storage period of ascorbic acid solution. Thus the accuracy of the predicted storage period may be affected [2]. To make clear whether the accuracy of the predicted storage period of ascorbic acid solution by classical isothermal experiment will be affected, the experiments were conducted under high temperature conditions. And the apparent rate constants(k_A) at temperatures 35 ˚C, 40℃, 45 ˚C and 50 ˚C were obtained, respectively. Then the apparent rate constant at 25 ˚C ($k_{A,25^\circ C}$) was extrapolated. At the same time the apparent rate constants under high temperature conditions were adjusted, then the adjusted apparent rate constant at 25 ˚C ($k_{A,A,25^\circ C}$) was extrapolated by the adjusted apparent rate constants ($k_{A,A}$) too. Whether the accuracy of the predicted storage period of ascorbic acid solution by classical isothermal experiment will be affected was determined by comparing the storage period of the experimented ascorbic acid solution which calculated by $k_{A,25^\circ C}$ and $k_{A,A,25^\circ C}$, respectively. The significance of this work was to make clear whether the accuracy of the predicted storage period of ascorbic acid solution by classical isothermal experiment will be affected and provided a theory base for predicting the storage period of ascorbic acid solution more accurately.

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2. MATERIALS AND METHODS

2.1. Apparatus and Reagents

An electronic analytical balance (FA2004B, Shanghai, China), a pH meter (PHS-3C, Shanghai, China), an ampoule sealing lamp (RF-1, Shanghai, China) and an electrothermostat (Wujiang, China) were used.

Ascorbic acid (it contains ≥99.7% of $C_6H_8O_6$, Chengdu Tianhua Co. Ltd., China) was used. The other reagents were all analytical grade.

2.2. Methods

2.2.1. Preparation of 5% Ascorbic Acid Solution

A 50-g quantity of ascorbic acid was dissolved in distilled water. The solution was adjusted with saturated NaOH to pH6.8 and then was diluted with distilled water to a total volume of 1000 ml.

2.2.2. Measurement of Ascorbic Acid [5]

A 5 ml aliquot of ascorbic acid solution was placed in a 100 ml stoppered flask. Then 15 ml distilled water and 4 ml 0.6% diluted acetic acid were added, then titrated with 0.05865 mol·l^{-1} iodine solution using starch as indicator. A blank titration was run under identical conditions.

3. RESULTS AND DISCUSSION

3.1. Establishment of Degradation Rate Equation of Ascorbic Acid

The oxidative degradation rate equation of ascorbic acid under aerobic condition can be written as:

$$\frac{\mathrm{d}\Delta c}{\mathrm{d}t} = k_{\mathrm{T}} c^{\alpha} c^{\beta}_{\mathrm{O_2,d}} \quad (1)$$

Where Δc is concentration drops of ascorbic acid within time t; k_T is the rate constant of ascorbic acid react with oxygen; c and $c_{\mathrm{O_2,d}}$ are the residual concentrations of ascorbic acid and the dissolved oxygen at time t, respectively; α and β are the reaction orders with respect to ascorbic acid and oxygen, respectively.

It is reported that the reaction order with respect to ascorbic acid and oxygen is zero and first, respectively [2-4]. So Eq. (1) can be rewritten as:

$$\frac{\mathrm{d}\Delta c}{\mathrm{d}t} = k_{\mathrm{T}} c_{\mathrm{O_2,d}} \quad (2)$$

Since the dissolve concentration of oxygen under given temperature is constant. Let $k_{\mathrm{T}} c_{\mathrm{O_2,d}} = k_{\mathrm{A}}$, Eq. (2) can be expressed as:

$$\frac{\mathrm{d}\Delta c}{\mathrm{d}t} = k_{\mathrm{A}} \quad (3)$$

Where k_A is the apparent rate constant of ascorbic acid oxidative degradation. Integrating Eq. (3) yields:

$$\Delta c = k_{\mathrm{A}} t \quad (4)$$

3.2. Determination of Apparent Rate Constants of Ascorbic Acid Under Different Temperatures

A 10 ml of 5% ascorbic acid solution was filled into 20-ml (labeled) ampoules and the ampoules were sealed then incubated at temperatures 35 ˚C, 40 ˚C, 45 ˚C and 50 ˚C, respectively. Three exact volumes of 5 ml of the solution were taken out of the oven at intervals of time, and the residual concentration of the ascorbic acid was measured iodometrically. The results are listed in Table **1**. By plotting Δc against t, four straight lines were obtained as shown in Figure **1**. And the values of the apparent rate constants(k_A) were extrapolated from the lines. The results are listed in Table **2**. By plotting $\ln k_A$ against 1/T, a straight line with the equation: $\ln k_{\mathrm{A}} = -2444/\mathrm{T} + 0.6335$ was obtained as shown in Figure **2**. The calculated value of $k_{A,25^{\circ}C}$=5.168×10^{-4}(mol·L^{-1}·h^{-1}) was obtained by

Table 1: The Relationship Between *c* and *t* Under Different Temperatures

35°C		40°C		45°C		50°C	
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	0.2836±0.0012	0	0.2843±0.0010	0	0.2830±0.0006	0	0.2833±0.0010
12	0.2761±0.0027	12	0.2754±0.0006	12	0.2717±0.0006	12	0.2713±0.0006
24	0.2676±0.0016	24	0.2641±0.0006	24	0.2645±0.0006	24	0.2590±0.0006
36	0.2611±0.0021	36	0.2546±0.0010	36	0.2535±0.0010	36	0.2494±0.0010
48	0.2515±0.0010	48	0.2487±0.0006	48	0.2426±0.0006	48	0.2354±0.0016
60	0.2429±0.0012	60	0.2371±0.0010	60	0.2310±0.0010	60	0.2255±0.0006

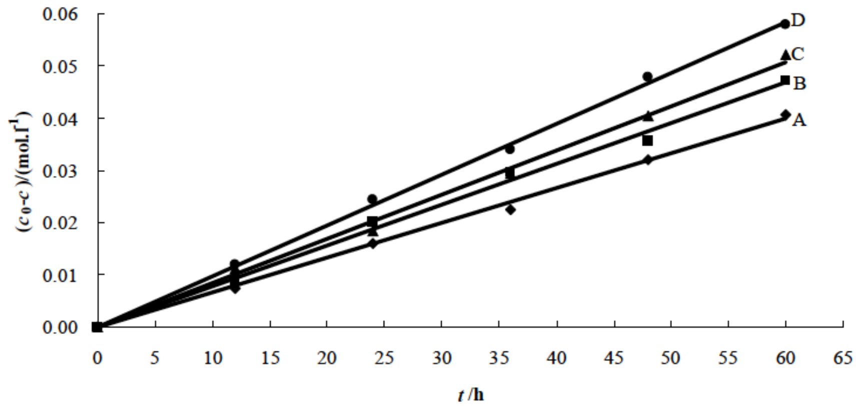

Figure 1: The relationship between *c* and *t* under different temperatures (A) 35 °C, (B) 40 °C, (C) 45 °C and (D) 50 °C.

Table 2: The Relationship Between Rate Constant and *T*

T(˚C)	$k_A\times10^4$(mol·L^{-1}·h^{-1})	$c_{O2}\times10^4$(mol·L^{-1})	k_T(mol·L^{-1}·h^{-1})	$k_{A,A}\times10^4$ (mol·L^{-1}·h^{-1})
35	6.650	2.0547	3.236	7.91
40	7.826	1.8978	4.124	10.08
45	8.463	1.7527	4.829	11.80
50	9.739	1.6195	6.014	14.70

substituting *T*=298 into equation $\ln k_A = -2444/T + 0.6335$.

2.3. Determination of k_T and $k_{A,A}$ Under Different Temperatures

To eliminating the effect of change of dissolved oxygen concentration resulted from temperature changing on degradation rate constant of ascorbic acid, let $k_{A,A}= k_T\times c_{O2,25^\circ C}$, where $k_{A,A}$ was adjusted apparent rate constant. The values of the k_T at temperatures 35 ˚C, 40 ˚C, 45 ˚C and 50 ˚C can be calculated according to equation $k_T c_{O_2,d} = k_A$. At the same time the adjusted apparent rate constants ($k_{A,A}$) were calculated by $k_{A,A} = k_T c_{O_2,25^\circ C}$. The results are listed in Table **2**. By plotting ln$k_{A,A}$ against 1/T, a straight line with the equation: $\ln k_{A,A} = -4028.8/T + 15.16$ was obtained as shown in Figure **3**. The calculated value of $k_{A,A,25^\circ C}$=5.158×10^{-4}(mol·L^{-1}·h^{-1}) was obtained by substituting *T*=298 into equation $\ln k_{A,A} = -4028.8/T + 15.16$.

3. CONCLUSIONS

The storage period of pharmaceutical preparations is the length of time that the degradation amount is 10% of the initial concentration. So Δ*c*=0.02841 for 5% ascorbic acid solution according to Δ*c*=c_0×10% (where

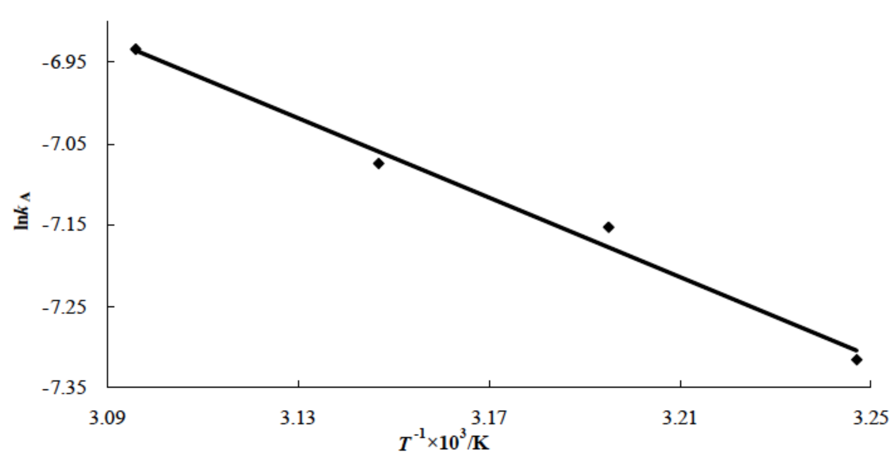

Figure 2: The relationship between lnk_A and 1/T.

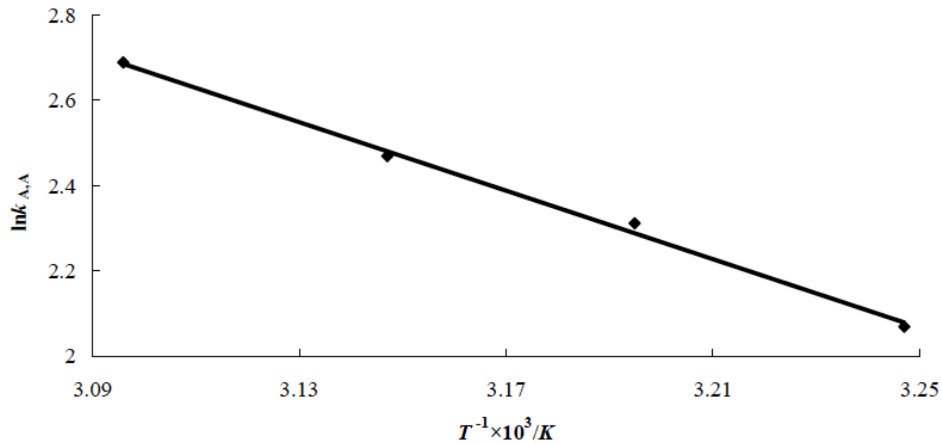

Figure 3: the relationship between ln$k_{A,A}$ and 1/T.

c_0 is the initial concentration of ascorbic acid). The storage period of the experimented ascorbic acid solution is 55h by substituting $k_{A,25^\circ C}$=5.168×10^{-4}(mol·L^{-1}·h^{-1}) into equation (4). Similarly the storage period of the experimented ascorbic acid solution is 55h by substituting $k_{A,A,25^\circ C}$=5.158×10^{-4} into equation (4), too. This indicated that the calculated storage period of the experimented ascorbic acid by two methods is equal. That is, the change of dissolved oxygen concentration has no effect on prediction accuracy of storage period of ascorbic acid solution by classical isothermal experiment.

The research group in this study found that the determined rate constant by classical isothermal experiment is apparent rate constant. So the research group concluded that the conditions (specification, concentration, formula, etc.) of the samples used to experiment should be the same as that of the products. Otherwise the accuracy of the predicted storage period will be affected. About this the research group is doing further research.

REFERENCES

[1] Cui FD. Pharmaceutics. 7th ed. Beijing: People's Medical Publishing House 2011; p. 66.

[2] Feng J, Zhan X, Qiao S, *et al*. A mathematical model for calculating the shelf life of ascorbic acid solution under given conditions[J]. Drug Dev Ind Pharm 2012; 38: 264-70. http://dx.doi.org/10.3109/03639045.2011.598537

[3] Zhan XC, Shi Y, Ma L, *et al*. Compressed oxygen in drug stability experiments[J]. Chem Pharm Bull 2007; 55: 87-91. http://dx.doi.org/10.1248/cpb.55.87

[4] Wilson RJ, Beezer AE, Mitchell JC. A kinetic study of the oxidation of L-ascorbic acid (vitamin C) in solution using an isothermal microcalorimeter[J]. Thermochim Acta 1995; 264: 27-40. http://dx.doi.org/10.1016/0040-6031(95)02373-A

[5] Shao ML. Pharmacopoeia of the people's Republic of China: Part II. Beijing: Chinese medicine science and Technology Press 2010; p. 904.

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