—Regular Articles—

Single Time Point Isothermal Drug Stability Experiments at Constant Humidity

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A single time point isothermal drug stability experiments at constant humidity is introduced. In the new method, kinetic parameters related to both moisture and temperature were obtained by a single pair of experiments: these related to moisture by one with a group of testing humidities and a fixed temperature, those related to temperature by the other with a group of testing temperatures and a constant humidity. By a simulation, the estimates for the kinetic parameters (E_a, m, A) obtained by the proposed method and the reported programmed humidifying and heating method were statistically evaluated and were compared with those obtained by the isothermal measurements at constant humidity. Results indicated that under the same experimental conditions, the estimates obtained by the proposed method were significantly more precise than those obtained by the reported programmed humidifying and heating method. The estimates obtained by the isothermal method at constant humidity were somewhat more precise than those obtained by the proposed method. However, the experimental period needed by the isothermal method at constant humidity was greatly longer than that needed by the proposed method. The stability of dicloxacillin sodium, as a solid state model, was investigated by the single time point isothermal drug stability experiments at constant humidity. The results indicated that the kinetic parameters obtained by the proposed method were comparable to those from the reported.

Key words——single time point humidifying; dicloxacillin sodium; programmed humidifying; classical isothermal humidifying; simulation

INTRODUCTION

Conventionally, the stability of a solid drug that is unstable to both moisture and heat has been assessed by a set of experiments (usually 15～20 experiments) at constant humidity and temperature.[1–4] The standard methods are generally accurate and reliable, however, they waste time, labor and materials, and its application is limited.

Zhao *et al.*[5] and Yin *et al.*[6] reported a programmed humidity and temperature controlled method, using a pocket computer. The kinetic parameters related to both moisture and temperature were obtained by a single pair of programmed experiments: one with humidity control and the other non-isothermal. The programmed humidifying and heating experiments save time, labor and materials. However, the use of a self-made computer-controlled environmental chamber limited the popularity of the experiments.

Single time point method was used to determine degradation rates for a drug that is unstable to heat.[7] In this method, only one sample under each testing temperature is analyzed at a single time point. The reduced accuracy, resulting from the single time point measurement, was compensated for by increasing the number of test temperature. The accuracy obtained using this method is similar to the classical multi-time point isothermal method, with the advantage of using only about 1/5 the number of measurements.

In the light of Pang's report,[7] a single time point isothermal drug stability experiments at constant humidity was established to assess the stability of a solid drug that is unstable to both moisture and heat. Kinetic parameters related to both moisture and temperature were obtained by a single pair of experiments: these related to moisture by one with a group of testing humidities and a fixed temperature, those related to temperature by the other with a group of testing temperatures and a constant humidity. The proposed method saved time, labor and materials compared with isothermal experiments at constant humidity; and no self-made computer-controlled environmental chamber needed to be used in the experiments compared with programmed humidifying and heating experiments.

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THEORY

The degradation of dicloxacillin sodium at constant humidity and temperature did not obey the nonfractional order kinetics.[6] There was an initial induction period, an acceleratory period and a decelerated period in the degradation, the rate could be described as:

$$-\frac{dc}{dt}=k\cdot c(1-c) \qquad (1)$$

After integrating, Eq. (1) can be expressed as:

$$\ln\left(\frac{c}{1-c}\right)=-kt+\ln\left(\frac{c_0}{1-c_0}\right)$$

or

$$k=\frac{\ln\left(\frac{c_0}{1-c_0}\right)-\ln\left(\frac{c}{1-c}\right)}{t} \qquad (2)$$

where k is the observed rate constant, c_0 is the initial content, c is the residure content at the time t. In the equations, ''1'' is the content of pure dicloxacillin sodium with a unit of 1 gram per gram, and $(1-c)$ is the impurities in the dicloxacillin sodium. It is seen from Eq. (1), the degradation rate is proportional to both the content of dicloxacillin sodium and the impurities in it.

In 1977, Genton and Kesselring described the effect of temperature and humidity on the decomposition of nitrazepam using a linear relationship between the logarithm of the rate constant and relative humidity.[2] In 2005, Yin and Zhan *et al*. did that in the study on dicloxacillin sodium.[6] For a given temperature, a linear regression line was obtained from a plot of $\ln k$ *versus* H_r by Eq. (3) in which m is a parameter related to humidity.

$$\ln k=\ln A-\frac{E_a}{RT}+mH_r \qquad (3)$$

where H_r is the relative humidity, T is the temperature, A is the pre-exponential factor, E_a is the activation energy and m is the parameter related to humidity.

In one experiment with a group of testing humidities and a fixed temperature, $\ln A-E_a/RT$ is a constant. According to Eq. (3), a linear regression line can be obtained from a plot of $\ln k$ *versus* H_r, with slope and intercept equal to m and $\ln A-E_a/RT$, respectively.

In the other experiment with a group of testing temperatures and a constant humidity, $\ln A+mH_r$ is a constant. According to Eq. (3), a linear regression line can be obtained from a plot of $\ln k$ *versus* $1/T$, with slope and intercept equal to $-E_a/R$ and $\ln A+mH_r$, respectively.

A can then be obtained by either combining the values of E_a and $\ln A-E_a/RT$ or combining m and $\ln A+mH_r$.

COMPUTER SIMULATIONS

To evaluate the advantage and disadvantage of a method, it is better to use computer simulation instead of real experiment because a specific drug may disagree with the rate equation or Arrenius law. The computer simulations in our study were carried out with a computer and a MATHEMATICA program programmed. By a simulation of experimental data, the estimates for the kinetic parameters (E_a, m, A) obtained by the proposed method and the reported programmed humidifying and heating method were statistically evaluated and were compared with those obtained by the isothermal measurements at constant humidity.

In the experiment with a group of testing humidities and a fixed temperature, in the light of Eq. (3), the k could be obtained by assigning values to E_a, m, A, c_0, c_{final} and selecting $T=70$°C and eleven humidity data points from H_r 80～50% at the same intervals. Then, again based on Eq. (3), theoretical (errorless) drug content data, which were expressed as percents of the remaining drug, were generated by assigning values to k, c_0, and t and selecting $f(c)$.

In the experiment with a group of testing temperatures and a constant humidity, in the light of Eq. (3), the k could be obtained by assigning values to E_a, m, A, c_0, c_{final} and selecting $H_r=70\%$ and eleven temperature data points from 80～50°C at the same intervals. Then, again based on Eq. (3), theoretical (errorless) drug content data, which were expressed as percents of the remaining drug, were generated by assigning values to k, c_0, and t and selecting $f(c)$.

In our study, to simulate the degradation of dicloxacillin sodium, we let $f(c)=\text{In}\,[c/(1-c)]$, $E_a=167$ kJ·mol^{-1}, $m=15.5$, $A=2.4\times10^{20}$ h^{-1},[6] $c_0=0.99$, and $c_{\text{final}}=0.5$.

The estimates for the kinetic parameters (E_a, m, A) are obtained according to the method mentioned in the THEORY section and are listed in Table 1.

For comparison, a set of programmed humidifying and heating experiments′ data were generated by computer simulation according to the reported

Table 1. Results from Simulations of the Three Types of Experiments

Parameters	Theoretical value	Experiment Ⅰ[a]	Experiment Ⅱ[b]	Experiment Ⅲ[c]
m	15.5	15.5±0.13%	15.5±0.57%	15.4±4.70%
E_a (kJ·mol^{-1})	167.0	167.0±0.11%	167.0±0.48%	166.7±4.30%
A	2.4×10^{20}	$2.4\times10^{20}\pm6.50\%$	$2.5\times10^{20}\pm29\%$	$6.5\times10^{21}\pm586\%$

[a] isothermal experiments at constant humidity;[6] [b] single time point isothermal experiments at constant humidity; [c] programmed humidifying and heating experiments.[6] Results are presented as mean±RSD% of 500 sets of data.

programmed humidifying and heating method.[6] In the programmed humidifying experiments at constant temperature, the temperature was kept at 70℃ and the relative humidity was decreased continuously from 80% to 50%. In nonisothermal experiments at constant humidity, the relative humidity was kept at 70% and the temperature was decreased continuously from 80℃ to 50℃. All the other experimental conditions were the same as those just mentioned in the single time point isothermal drug stability experiments at constant humidity. The estimates for the kinetic parameters are also listed in Table 1.

Besides, a set of isothermal data at constant humidity was generated by computer simulation according to Eq. (3) and the rate equation of dicloxacillin sodium $-dc/dt=k\cdot c(1-c)$.[6] Estimations were carried out at temperature (50℃, 60℃, 70℃, and 80℃) and the relative humidity (50%, 60%, 70%, and 80%), respectively. All the other experimental conditions were the same as those just mentioned in the single time point isothermal drug stability experiments at constant humidity. The estimates for the kinetic parameters are also listed in Table 1.

It was found in both Zhao's[5] and Yin's[6] studies that the amount of scatter in the content data increased gradually as the residual penicillin potassium decreased and can be described approximately as $\pm(0.102-0.1\times c)$. To obtain simulated experimental data, random numbers with a mean zero and maximum deviations of $\pm(0.102-0.1\times c)$ were added to the errorless data. For each model, 500 sets of data were generated. The mean and RSD of the estimates for the kinetic parameters (E_a, m, A) are also listed in Table 1.

The results indicate that under the same experimental conditions, the estimates obtained by our single time point isothermal drug stability experiments at constant humidity were more accurate (closer to the theoretical value) and significantly more precise (the RSD is significantly smaller) than those obtained by the reported programmed humidifying and heating method. The estimates obtained by the isothermal method at constant humidity were somewhat more accurate and precise than those obtained by our single time point isothermal drug stability experiments at constant humidity. However, to get the same extent of drug degradation, the experimental period needed by the isothermal method at constant humidity was greatly longer than that needed by the proposed method.

EXPERIMENTAL

Dicloxacillin Sodium Stability Prediction by Single Time Point Isothermal Experiments at Constant Humidity

Drugs and Instruments Dicloxacillin sodium and its reference substance were provided by Hainan Haisheng Pharmaceuticals Co., Ltd., P. R. China. An instrument of HPLC and an environmental chamber were used. The methanol used was HPLC grade and the water was freshly distilled. The HPLC system consisted of a constant flow pump (Shimadzu, type 10AT), a rotatory valve injector (Microliter, type 702) equipped with a 25 μl loop, an ultraviolet (UV) detector (Shimadz, type SPD-10AVP) and a custom-made environmental chamber were used.

Determination of Dicloxacillin Sodium

Chromatographic Conditions The chromatographic separation and quantitative determination were performed on a high-performance liquid chromatograph (HPLC).[6] The column (10 cm×4.6 mm) used was packed with silica particles bonded with octadecylsilane. The mobile phase consisted of methol-monobasic sodium phosphate (0.01 mol/l, adjusted to pH 4.5 with phosphoric acid and diluted with water) (55 : 45). The flow rate was 1 ml/min. UV detection was carried out at 225 nm.

Assay Procedure For assay preparation, an exact amount of approximately 0.1 g of dicloxacillin sodium was transferred to a 50-ml volumetric flask and

diluted with water to volume; then an exact amount of 5 ml of this solution was transferred to another 50-ml volumetric flask and diluted with water to volume. For standard preparation, an exact amount of approximately 10 mg of dicloxacillin sodium was transferred to a 50-ml volumetric flask and diluted with water to volume. A new standard was generated for each HPLC run. Equal volumes (10 μl) of the standard preparation and the assay preparation were injected into the chromatograph and the chromatograms were recorded. The responses (peak areas) for the major peaks were measured and the quantity of dicloxacillin sodium in the assay solution was calculated according to external reference method.

Experiment with a Group of Testing Humidities and a Fixed Temperature An exact amount of approximately 0.1 g of dicloxacillin sodium was spread evenly onto the bottom of a 50-ml beaker. A group of incubation humidities was used: 65～50% at 1.5% intervals, with a fixed temperature 70℃. A total of 3 beakers were taken out of the environmental chamber after each incubation for three measurements of the residual dicloxacillin sodium concentration. The predefined time to end each incubation was when the concentration decreased to about 0.8.

According to Eq. (3), a linear regression line was obtained from a plot of ln k *versus* H_r (Fig. 1), with correlation coefficient r=0.9974. From the slope and intercept equal of the regression line in Fig. 1, m= 15.41, $A\cdot\exp(-E_a/RT)=1.0055\times10^{-5}$.

Experiment with a Group of Testing Temperatures and a Constant Humidity An exact amount of approximately 0.1 g of dicloxacillin sodium was spread evenly onto the bottom of a 50-ml beaker. A group of incubation temperature was used: 70～55℃ at 1.5℃ intervals, with a constant humidity 65%. A total of 3 beakers were taken out of the environmental chamber after each incubation for three measurements of the residual dicloxacillin sodium concentration. The predefined time to end each incubation was when the concentration decreased to about 0.8.

According to Eq. (3), a linear regression line was obtained from a plot of ln k *versus* $1/T$ (Fig. 2), with correlation coefficient $r=-0.9968$. From the slope and intercept equal of the regression line in Fig. 2, E_a $=165.51$ kJ·mol^{-1}, $A\cdot\exp(mH_r)=3.5077\times10^{24}$.

Calculation of A The results obtained from the single time point isothermal experiments at constant humidity are listed in Table 2.

In these calculations, when E_a in the equation $A\cdot\exp(-E_a/RT)=1.0055\times10^{-5}$ was 165.51 kJ/mol, we got $A=1.575\times10^{20}$ h^{-1}; when m in the equation $A\cdot\exp(mH_r)=3.5077\times10^{24}$ was 15.41, we got $A=1.566\times10^{20}$ h^{-1}. Then the mean value of A was 1.6×10^{20} h^{-1}. The kinetic parameters obtained from our experiments are all listed in Table 3.

The kinetic parameters reported by Yin[6] are also listed in Table 3. Data in Table 3 indicated that these obtained by ours were comparable to those from the report.[6] The RSD values for Experiment V are calculated by using the data from three repetition experiments by the proposed method. The data of Experiment IV and VI were reported by Yin.[6] In his report, there were no RSD values.

As mentiond above, the results of computer simu-

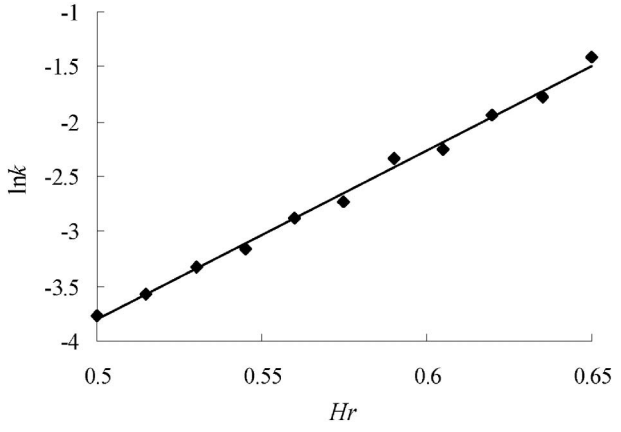

Fig. 1. The Plot of ln k *versus* Relative Humidity at 70℃ and a Humidity Range of 65～50%

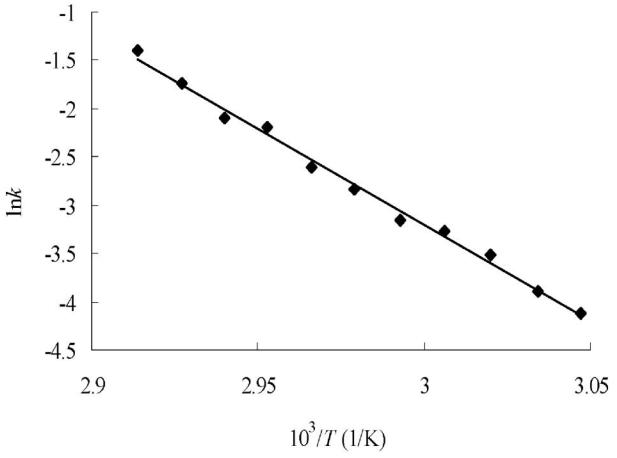

Fig. 2. Arrhenius Plot of the Degradation Rate Constant of Dicloxacillin Sodium in Solid State at H_r=65% and a Temperature Range of 70～55℃

Table 2. Results Obtained from the Single Time Point Isothermal Experiments at Constant Humidity

Experiment at 70℃ and a group of humidities	Experiment at H_r=65% and a group of temperatures
m=15.41	E_a=165.51 kJ·mol^{-1}
$A\cdot\exp\left(\frac{E_a}{RT}\right)=1.0055\times10^{-5}$	$A\cdot\exp(mH_r)=3.5077\times10^{24}$

Table 3. Results Obtained from the Three Types of Experiments

Parameters	Experiment Ⅳ[a]	Experiment Ⅴ[b]	Experiment Ⅵ[c]
m	15.52	15.41±2.21%	15.77
E_a (kJ·mol^{-1})	166.75	165.51±1.73%	167.41
A	2.4×10^{20}	$1.6\times10^{20}\pm105\%$	2.5×10^{20}

[a] isothermal experiments at constant humidity;[6] [b] single time point isothermal experiments at constant humidity; [c] programmed humidifying and heating experiments.[6]

lation indicate that the estimates obtained by our proposed method were more accurate and significantly more precise than those obtained by the reported programmed humidifying and heating method; and were somewhat less accurate and precise than those obtained by the isothermal method at constant humidity. Unfortunately, we can not see the above results in the dicloxacillin sodium study simply because there is no RSD value reported by Yin.

Even so, in our proposed method, we can still find that the RSD value for A is much greater than those for E_a and m in both the simulation (Table 1, Experiment II) and real experiments (Table 3, Experiment V). (Because of the very few repetion times in the real experiments and the random error we used in the simulation may not the same as that in the real experiments, we can not expect the RSD values in simulation are very close to those in real experiments.)

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