# A New Kinetic Approach. Stability of Cefquinome Sulfate under Variable pH and Temperature

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## ABSTRACT

**Object:** This study was evaluation of Cefquinome Sulfate (CS) long-term stability under different conditions of pH and temperature, so as to suggest the best conditions to perform its clinical application. **Method:** The CS stability was investigated at different values of pH (2.0-9.0) and temperature ($4\text{-}37^{0}C$). Kinetic parameters of CS degradation in alkaline environment were calculated adopting a new model that took into consideration the equilibrium between the active and a reversibly inactivated form of CS after long-time degradation. **Result:** All temperatures show increased CS instability,and this effect decreased in following sequence: $37^{0}C > 25^{0}C > 15^{0}C > 4^{0}C$, which describe typical Arrhenius behaviors with good correlation for $K_d(R^2 = 0.941)$. **Conclusion:** The alkali-catalyzed degradation of CS was faster and stronger than the acidic one. The best conditions were met at a pH6.8 and $4^{0}C$ .

**Key words:** CS, Kinetic parameters, stability, Arrhenius.

## INTRODUCTION

Antibiotics of the β-lactam group are used intensely in dairy farming, particularly to combat mastitis, a serious disease that inflicts significant economic losses to the world's dairy industry.[1] The β-lactam family is the biggest and most important class of clinical antibiotics.[2] Their sales are estimated at 15 billion dollar, so they represent the major biotechnology products with worldwide dosage sales at around 65% of the total market of antibiotics.[3] β-lactams have a common β-lactam ring attached to different structures.[4] They were unstable in aqueous solution.

Cefquinome Sulfate (CS) is a fourth-generation cephalosporin, which has been developed solely for veterinary use.[5] It shows potent antibacterial activity against a broad spectrum of bacterial species. The antibiotic has better pharmacokinetic characteristic, and it can be absorbed quickly, and can reach the peak concentration ($C_{max}$) quickly, moreover, it can maintain a high concentration in animal's lungs and mammary glands. It's toxicity is low, and the residue in edible tissue is small.[6] Most important of all, the antibiotic has been extensive use for treatment of cattle and pig against bacterial infections of respiratory tract and the udder.[7]

However, like other β-lactam compounds, CS in its crude form is chemically unstable, due to susceptibly of the carbonyl group linked to the β-lactam ring to suffer an acidic $(H^+)$-or alkaline $(OH^-)$-catalyzed attack by water molecules.[8,9] However, in aqueous solution under certain condition, the CS degree of degradation is very low, and still be able to meet the clinical needs of the administration. But, not everyone knows that and there are no literatures on the stability of CS currently. Thus, in addition to the use of CS under different conditions in clinical applications, and provide guidance for the CS reasonable clinical application, studies covering its stability in a wide range of pH, temperature and plasma. The results of this study seldom compared with literature because of the new model proposed in this study and no literature to study the stability of CS.

This study aimed at evaluating the stability of CS at different pH and temperature. The

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kinetic parameters of its degradation in alkaline environment were then calculated adopting a new model that takes into consideration the equilibrium between the active form and a reversibly inactivated form of CS after long-time degradation at alkaline environment.

## MATERIALS AND METHODS

### Materials

Cefquinome Sulfate (CS) of pharmaceutical grade (87 purity) obtained from institute of biomedical products in Wuhan Chang Heng (Wuhan , China) was used in the stability tests, while the one used to prepare standard solutions was purchased from institute of biomedical products in Shanghai (Shanghai ,China).All the other reagents were of analytical grade and used as received. According to Chinese Pharmacopoeia, the solutions for the CS stability tests were prepared in phosphate buffer by adding disodium phosphate and sodium hydroxide at different molar concentrations depending on pH.

### Experimental procedure

All stability tests were performed in solutions containing an initial CS concentration of 40 mg/L. They were prepared by diluting a concentrated stock solution (250 mg/L) with phosphate buffer with the selected pH. The solutions were then homogenized in Water Bath Incubator at 15°C or at the desired temperature. Aliquots of the solutions were withdrawn at different times, and the concentration of undegraded CS was determined as described later. All experiments were performed in triplicate, and the standard deviations and confidence intervals calculated. The limit of significance for the statistical analysis was resulting in a confidence interval of 95 %.

A first set of experiments was performed where the CS stability was determined at pH 2.0, pH 6.8, pH 7.6 and pH 9.0. Furthermore, this part was also devoted to the determination of CS degradation kinetics at different pH for 144 h.

In a second set of experiments, the CS stability was determined at pH 6.8 but varying the temperature of 4°C, 15°C, 25°C and 37°C. In addition, the study was devoted to the determination of CS degradation kinetics at different temperatures (4°C, 15°C, 25°C and 37°C ) for 120 h as well as to the estimation of the related kinetics parameters making use of simple Arrhenius-type model.

### Analytical methods

According to the CS Concentration[10] was determined by ProStar HPLC system (LC–10A VP, Shimadzu liquid chromate graph, Kyoto, Japan) was composed of a quaternary pump (LC-20AT), a vacuum degasser, a thermostatied autosampler, a column thermostat (CTO–10A) and a RF–10AXL UV detector. Data collection and processing were performed using CLASS–VP Ver.6.1 workstation software (Shimadzu Corporation). The separations were performed on a Kromasil C18 (150 mm × 4.6 mm I.D., 5 μm particle size) reversed–phaseanalytical column (Dikma Technologies, Beijing, China), which was protected by a Shimadzu Shim–Pack guard column (C18, 10 mm×4.6 mm). The mobile phase consisted of a mixture of 0.83 % phosphate and acetonitrile (90:10, v/v), with a flow rate of 0.8 mL/min. The UV detection was operated at 270 nm and the column temperature was 25 °C. During the assay, 20 μL of samples were injected in duplicate into the analytical column. Standard solutions with different concentrations (in phosphate buffer and plasma) were used to get a calibration curve able to relate the peak area with the concentration.

## RESULTS AND DISCUSSION

### Study of CS stability different pH

The stability of CS in phosphate buffer at pH ranging from 2.0 to 9.0 was determined at 0.5 h、1 h、2 h、4 h、6 h、8 h、12 h、16 h、20 h、24 h、36 h、48 h、60 h、72 h、84 h、96 h、120 h and 144 h. The related results are presented in [Figure 1]. In order to contribute to the clinical application of CS, the above range of pH was selected .The pH 2.0, pH 6.8 and pH 7.6 were simulate the gastric, intestinal and tissue fluid environment respectively. And the pH 9.0 represent for the alkaline environment.

As expected, the longer the exposition time, the greater the stability loss under all conditions tested. Moreover, this loss of stability was more pronounced at extreme alkaline environment (pH 9.0).In particular, after 60 h at $2.0 \leq pH \leq 7.6$ no more than 6.55-14.24 % of CS was degraded in the study, whereas, the pH 9.0 have 83.84 % of CS was degraded. Furthermore, after 120 h at $2.0 \leq pH \leq 7.6$ no more than 14.3-21.3% of CS was degraded, but pH 9.0 loss of 95 % .The results showed that the alkaline environment exerted a significant influence on CS stability.

The observed CA instability could have been the result either of acid- or alkali-catalyzed degradation. From [Figure 1], it appears reasonable to conclude that the alkali-catalyzed degradation of CA was faster and stronger than the acidic one. This may be due to the presence in its structure of the carbonyl group of the β-lactam ring, which is susceptible to attack by hydroxide ions more than protons. These results as a whole demon-

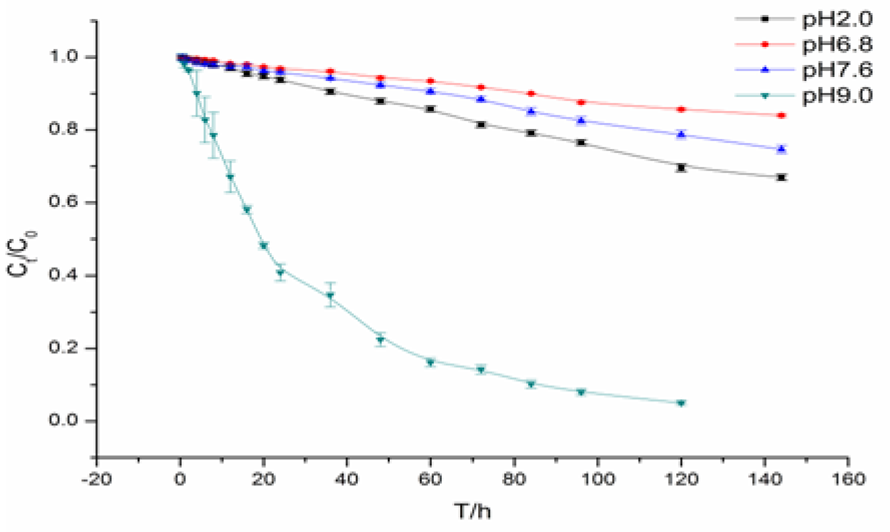

**Figure 1: Stability of CS in phosphate buffer at different values of pH from time 0 to144 h at 15°C. pH 2.0(■),6.8(◆),7.6(▲)9.0(▼). The symbol and vertical bar represent the mean and standard error of the mean (n=3).**

strate that the best conditions for CS stability are in the pH range of 2.0-7.6.

According to Chen, B. et al,[11] the exponential rate model can fit the degradation of compounds in a wide range. The degradation rate and no degradation percentage of expression as follows:

$$-\frac{dC}{dt} = K_dC^n \text{---------------------------}(1)$$

Where C is no degradation of drug percentage; $K_d$ is the degradation rate constant; n is the reaction order. The integral form as follows:

$$n=0\text{: } C_t\text{-}C_0 = -K_dt \text{--------------------}(2)$$

$$n=1\text{: } \ln\ln\frac{Ct}{C_0} = -K_dt \text{------------------}(3)$$

$$n=2\text{: } \frac{1}{C_t} - \frac{1}{C_0} = K_dt \text{-----------------------}(4)$$

Where $C_0$ is initial concentration of the drug and $C_t$ is the concentration of the drug at time t.

Basis (2)、(3) and (4)to fit the degradation of CS at different pHs . The results show that the first-order model successfully employed to describe the degradation of CS versus time with good correlation in both cases (Any one $R^2 > 0.99$). The concentration of CS measured at different pHs after 264 h is only 2-10 % higher or lower

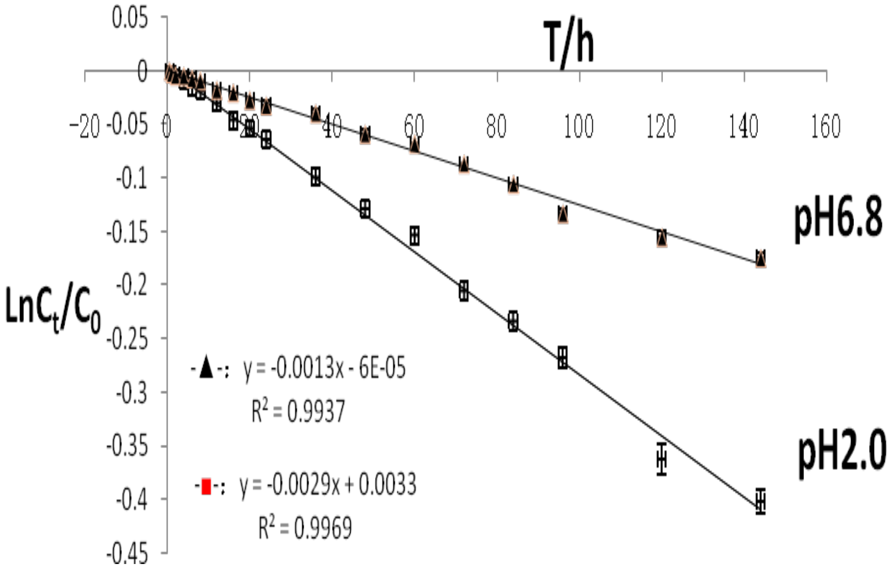

**Figure 2: The regression curve of degradation rate equation for CS at pH 2.0 and pH 6.8. pH 2.0(🟥)6.8(▲).The symbol and vertical bar represent the mean and standard error of the mean (n=3).**

than that concentration of CS calculated using the model. The related results are presented in [Figure 2-4] and [Table 1]

The slopes of the straight lines as that in **[**Figure 2] and the half-life ($t_{1/2}$) is numerically equal to ln 2/K allowed estimating both the value of $K_d$ (0.0029 $h^{-1}$ and 0.0013 $h^{-1}$ respectively) and the value of $t_{1/2}$(239 h and 533 h respectively) . However, the results of [Figure] 3 and [Figure 4], taken as an example, show that first-order model is able to effectively describe the kinetics of the system only at the beginning of the exposition, whereas

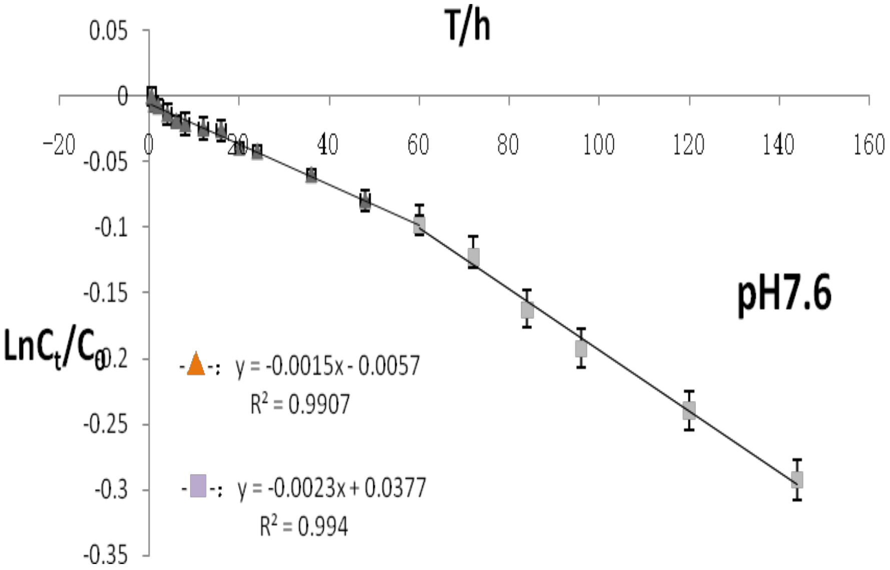

**Figure 3: The regression curve of degradation rate equation for CS at pH 7.6. The symbol and vertical bar represent the mean and standard error of the mean (n=3).**

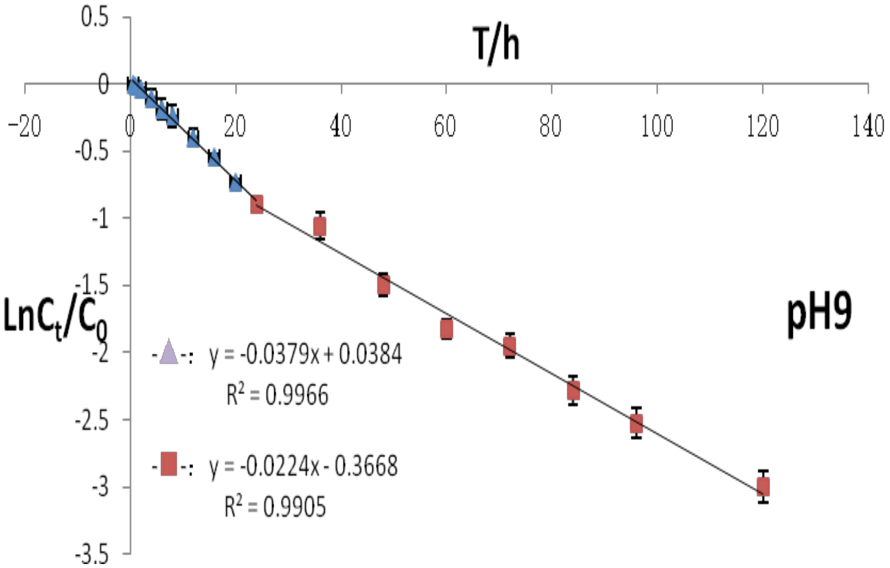

**Figure 4: The regression curve of degradation rate equation for CS at pH 9.0. The symbol and vertical bar represent the mean and standard error of the mean (n=3).**

**Table 1: The concentration of CS measured at different pH after 264h compare with the concentration of CS calculated using the degradation model, respectively.**

| The concentration of CS (mg/L) | pH 2.0 | pH 6.8 | pH 7.6 | pH 9.0 |
|---|---|---|---|---|
| **measured** | 19.32 | 28.37 | 22.91 | 0.057 |
| **calculated** | 18.66 | 27.80 | 23.38 | 0.052 |
| **The difference** | +3.5 % | +2.1% | -2% | +10% |

a accelerate (In pH 7.6) or deceleration (In pH 9.0) of the degradation took place in a second phase.

This behavior, which can be recognized also under some of the conditions utilized in the work,[12] is typical of equilibrium reactions or simultaneous opposite reactions[13,14] rather than of first-order irreversible reaction. So this study first hypothesize the preliminary occurrence of an equilibrium between the stable CS configuration (Cs) and a configuration more sensitive todegradation ($Cs_2$), as the likely result of the alkali-catalyzed complexation of CS, followed by a first-order reaction leading to the formation of the products of CS degradation ($C_d$). The proposed phenomenological situation can be described by the reaction sequence:

$$Cs \underset{}{\overset{k}{\rightleftarrows}} Cs_2 \xrightarrow{k_d} C_d \text{-------------}(5)$$

Where K is the constant of the complexation–decomplexation equilibrium and $k_d$ is the first-order constant of the irreversible degradation reaction.

At the start of the exposition, when the $Cs_2$ concentration is almost negligible, the former equilibrium can be regarded as totally shifted towards $Cs_2$ formation; therefore, the traditional first-order model approach can be applied successfully, because the degradation of $Cs_2$ to $C_d$ becomes the limiting stage. On the other hand, after this time, the concentration of $Cs_2$can not be neglected anymore, and the preliminary equilibrium reaches a condition where $Cs_2$ is shifted to Cs formation; therefore, it becomes the limiting step of the degradation, and the first-order reaction can be neglected. After the achievement of the equilibrium we can write:

$$K = \frac{Cs_2}{Cs} \text{-----------------------}(6)$$

Where:

$$Cs_2 = Cs_0 - C_s \text{-------------}(7)$$

Being $Cs_0$ the concentration of Cs at the start of each run.

Therefore:

$$\frac{Cs}{Cs_2} = \frac{1}{K+1} \text{------------}(8)$$

The slopes of the straight lines as that in Figure 3 and [Figure 4] allowed estimating the values of K (0.0023 and 0.0224 respectively) and $K_d$ (0.0015 $h^{-1}$ and 0.0379 $h^{-1}$ respectively).In addition, the results demonstrate that the K and $K_d$ values vary with the different alkaline environment (pH 7.6 and pH 9.0). The values of K and $K_d$ at pH 7.6 significantly less than at pH 9, which meaning the loss of stability of CS was more pronounced at pH 9.0. These results are in good agreement with the literaturel,[15] who investigated the degradation of amide bond have two steps. The first step is amide with $OH^-$ interactions to generate an intermediate. The second step is the intermediate degradation to hydrolyzate. The structure of CS has an amide bond and a β-lactam ring. It appear reasonable to speculate that the $OH^-$ only activated the β-lactam ring at pH 7.6 (weak base conditions), whereas, the $OH^-$ can activated both β-lactam ring and amide bond to accelerating CS's degration. Perhaps this is the reason why the values of K and $K_d$ at pH 7.6 significantly less than at pH 9.0.

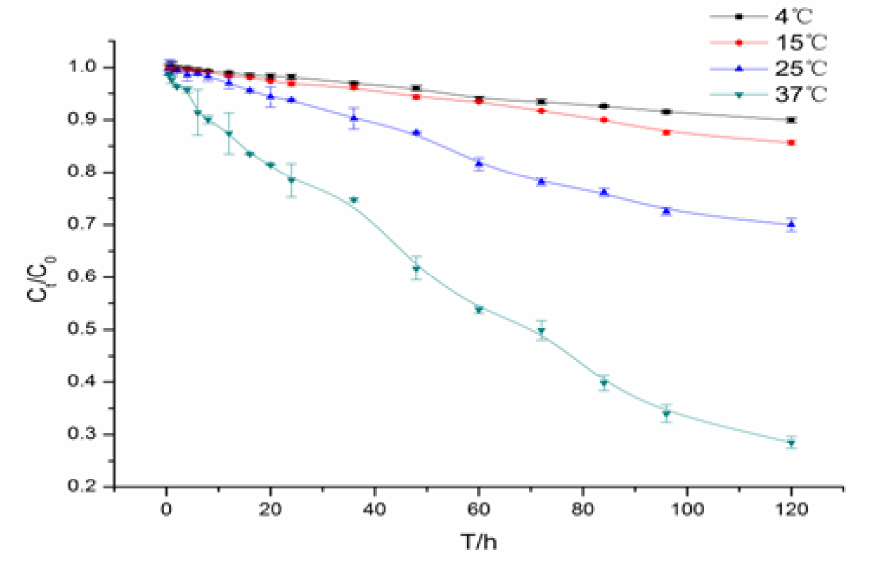

**Figure 5: Stability of CS in phosphate buffer at pH 6.8 and different temperature (4°C,15°C,25°C and 37°C) from time 0 to 120 h. °C :4(■),15(◆),25(▲)37(▼). The symbol and vertical bar represent the mean and standard error of the mean (n=3).**

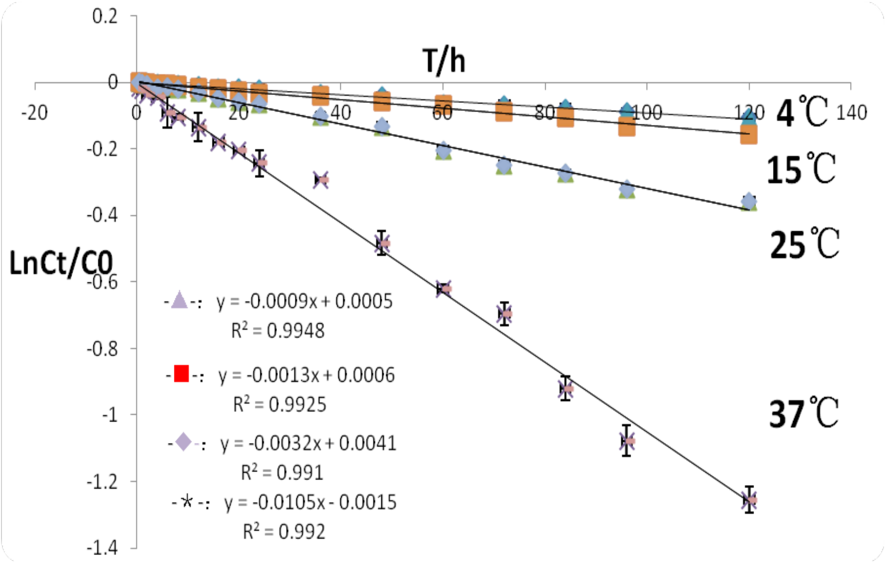

**Figure 6: The regression curve of degradation rate equation for CS at different temperature (4$^0$C,15$^0$C,25$^0$C and 37$^0$C) from time 0 to 120 h. $^0$C :44$^0$C (▲),15$^0$C (🟥),25$^0$C (◆)37$^0$C (*). The symbol and vertical bar represent the mean and standard error of the mean (n=3).**

**Table 2: The concentration of CS measured at different temperatures after 240h compare with the concentration of CS calculated using the degradation model, respectively.**

| The concentration of CS (mg/L) | 4$^0$C | 15$^0$C | 25$^0$C | 37$^0$C |
|---|---|---|---|---|
| measured | 33.96 | 27.84 | 19.91 | 3.574 |
| calculated | 32.25 | 29.30 | 18.63 | 3.22 |
| The difference | +5.35% | -4.98% | +6.8% | +11% |

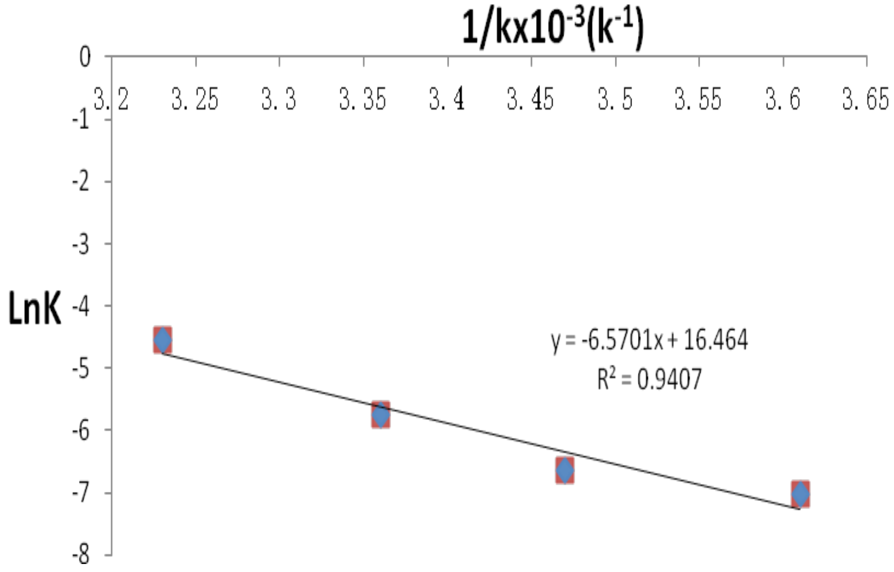

**Figure 7 : Semi-log plots of Kd versus the reciprocal absolute temperature**

## Stability of Cefquinome Sulfate at different temperatures

The stability of Cefquinome Sulfate (CS) in phosphate buffer at pH 6.8 and temperature ranging from 4 to 37°C was determined at 0.5 h、1 h、2 h、4 h、6 h、8 h、12 h、16 h、20 h、24 h、36 h、48 h、60 h、72 h、84 h、96 h and 120 h. The related results are presented in [Figure 5].

As expected, the higher the exposition time, the greater the stability loss in tested. After 60 h at 4°C no more than 6.05 % of CS was degraded in the study, whereas, at 37°C loss 46.23 % of CS was degraded, Furthermore, after 120 h at 4 °C no more than 10 % of CS was degraded, but at 37°C loss 71.5 % .The results showed that the temperatures exerted a significant influence on CS stability.

The used of first-order model to fit the degradation of CS at different temperatures. The results have a good correlation in both cases (Any one $R^2 > 0.99$). The concentration of CS measured at different temperatures after 240 h is only 5-11 % higher than that concentration of CS calculated using the model. The related results are presented in [Figure 6] and [Table 2].

The slopes of the straight lines as that in [Figure] 6] allowed estimating the value of $K_d$(0.0009 $h^{-1}$、 0.0013 $h^{-1}$、 0.0032 $h^{-1}$ and 0.0105 $h^{-1}$ respectively)and $t_{1/2}$( 770 h、 533 h、 216 h and 66 h respectively). It is noteworthy that the values of $K_d$ increased with temperature, which suggested Arrhenius-type behaviors. Plotting these data in semi-log plots versus the reciprocal absolute temperature, the straight lines illustrated in [Figure 7] were obtained, which describe typical Arrhenius behaviors with good correlation ($R^2= 0.941$ for $K_d$).

## CONCLUSION

In view of future development of the clinical application of CS, the long-term stability of this cephalosporin antibiotics has been investigated under different conditions of pH and temperature. The longer the exposition time, the greater its instability under all the conditions tests, especially at alkaline environment (pH 9.0). The effect of pH was stronger than that of temperature. About pH, the effect of plasma and alkaline environment (pH 9.0) was stronger than that of acidic environment. Moreover, all temperatures show increased CS instability,and this effect decreased in following sequence: 37°C＞25°C＞15°C ＞4°C, which suggested Arrhenius-type behaviors($R^2= 0.941$). The all results showed that the best conditions were met at a pH 6.8 and 4°C.

Finally, a new kinetic model has been proposed for CS degradation at alkaline environment, which hypothesizes equilibrium between the active form and a reversibly form of CS and subsequent first-order irreversible degradation. The resulting kinetic results have been utilized to estimate the $K_d$ and $t_{1/2}$.

## CONFLICT OF INTEREST

We declare that we have no conflict of interest.

## ACKNOWLEDGEMENTS

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