# Isothermal and Nonisothermal Decomposition of Famotidine in Aqueous Solution

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The kinetics of hydrolysis of famotidine in aqueous solution was studied by isothermal and nonisothermal method over the pH range of 1.71 to 10.0. Nonisothermal kinetics was studied with the purpose of determining its use in the establishment of the expiration date of pharmaceutical preparations, particularly drugs in solutions and for assessment of stability characteristics of pharmaceutical formulations during the development stage. A comparison of isothermal (55, 70 and 85°C) and nonisothermal kinetics was performed. Aqueous solutions of famotidine were buffered at pH 1.71, 2.24, 2.66, 4.0, 8.5, 9.0 and 10.0 were used. In the nonisothermal studies, the temperature rate of the reaction was continuously varied throughout the experiment. The energies of activation were found to be in close agreement for isothermal and nonisothermal studies, indicating that nonisothermal studies may save considerable amount of time in the early stages of drug development and stability testing. Logk-pH profiles were constructed for 55, 70 and 85°C from the first-order rate constants obtained from isothermal studies at pH values ranging from 1.71 to 10.00. The pH-rate profile indicated that famotidine undergoes specific acid catalysis in the acidic region and general base catalysis in the alkaline region. Hydrolysis in the acidic and alkaline media resulted in the formation of four and five degradation products, respectively. A possible degradation pathway for the acidic and alkaline hydrolysis was discussed.

KEY WORDS: famotidine; degradation; isothermal; nonisothermal; kinetics; stability.

## INTRODUCTION

Nonisothermal kinetic studies, as the name indicates, are carried out with continuous change in the temperature over the period of study. Nonisothermal kinetic methods enable drug stability to be estimated at any temperature from a single test rather than multiple experiments as required by the classical isothermal technique. As it becomes possible to determine the expiration date of the pharmaceutical preparation from one single experiment, this approach can bring about considerable savings of time and money. Also, it can be useful for quick assessment of stability characteristics of pharmaceutical prototypes during the developmental stage. The use of nonisothermal methods dates back to 1926, when Audibert used the method to study thermal decomposition of coal.[1] Baur and Bridges and Kofstad used this technique to study the oxidation of metals and estimate the values of the pre-exponential factor and the activation energy.[2,3] Since then, the method has been used extensively in thermogravimetric studies of solid samples. In the pharmaceutical area this method was first introduced by Rogers.[4] In the pharmaceutical field, work has predominantly been carried out on solution kinetics,[5–12] except for one study on solid state[13] and one on suspension kinetics.[14] Nonisothermal methods are applicable only for those systems which obey the Arrhenius relationship. In addition, the stability kinetics of stable formulations, requires a temperature programmer which raises the temperature at a very slow rate.

Famotidine is a potent $H_2$ receptor antagonist and is used in the healing of duodenal ulcers, gastric ulcers, prevention of ulcer recurrence, treatment of gastritis, gastroesophageal reflux disease, Zollinger-Ellison syndrome, acute upper gastrointestinal hemorrhage and protection against pulmonary aspiration of acid during anesthesia. In humans, famotidine selectively inhibits basal and simulated gastric acid secretion and has no clinically significant activity at histamine $H_2$ receptor sites outside the gastrointestinal tract. The objective of the present study was to explore the use of isothermal and nonisothermal techniques with the purpose of determining the kinetic parameters involved in the decomposition of famotidine in aqueous solution.

## MATERIALS AND METHODS

### Materials

Famotidine was supplied by Merck Sharp & Dohme, USA, and by Torcan-Delmar Chemicals, Canada. The three degradates of famotidine were supplied by Merck Sharp & Dohme (West Point, PA). Sulfacetamide, the internal standard was from Sigma Chemical Company. HPLC grade methanol and potassium phosphate monobasic were used. Acetonitrile was analytical grade and glacial acetic acid of reagent grade. Distilled-deionized water was used for the preparation of all the solutions. TLC plates were Baker Analyzed.

### HPLC Analysis

The HPLC system consisted of solvent delivery pump (Beckman 100A), a 250 mm × 4.6 mm column packed with 5 μm octadecylsilane (Beckman), an UV detector (Hitachi Model 100-40), and a computing integrator (Shimadzu CR501 Chromatopac). The mobile phase consisted of Methanol:Acetonitrile:Glacial Acetic Acid:0.01M Potassium Phosphate Monobasic (12:3:0.1:84.9) at a pH of 5.0. The mobile phase mixture was filtered through 0.45 micron pore nylon membrane (Millipore Corp.) and deaerated by sonication under reduced pressure. The flow rate was maintained at 1.0 ml/min. The detection wavelength was set at 266 nm.

### Thin Layer Chromatography of Degradation Products

A thin layer ascending chromatographic technique (TLC) was used as a qualitative method to determine the purity of famotidine and its degradates and to identify the degradates formed due to acidic and alkaline hydrolysis of famotidine. A TLC developing tank lined with filter paper and a short wavelength UV light (Chromatovue Cabinet, Model CC-60) were used. The solvent system used was ethyl acetate:methanol:toluene:ammonia (40:25:20:2). The sol-

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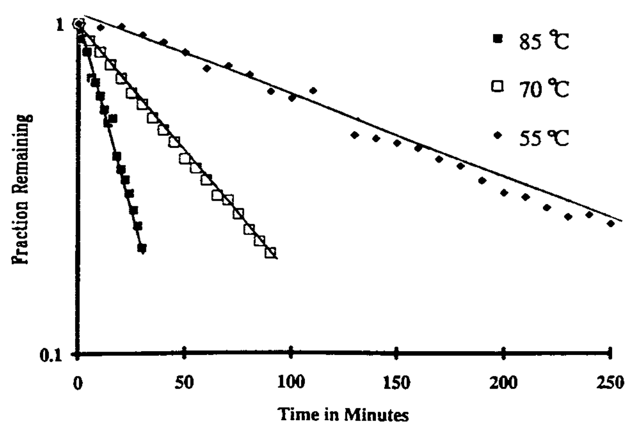



Fig. 1. Semilogarithmic plot showing influence of temperature on degradation of famotidine.

vent was added to the TLC chamber and was allowed to equilibrate for 45 minutes prior to use. TLC plates (20 × 20 cm) precoated with silica gel $GF_{254}$ and 250 micrometer thickness were used (Baker Analyzed -'Baker' Si250F-PA(19C)). Standard solutions were prepared by dissolving famotidine, degradate 1 and degradate 2 in glacial acetic acid, distilled-deionized water and glacial acetic acid, pH 1.5 Sörenson buffer (1:1) respectively. Degradation of famotidine was carried out in pH 2.0 and 10.0 Sörenson buffers at a temperature of 85°C. Samples were withdrawn at times 0, 0.5 and 3 hours. These samples obtained by degradation of famotidine were spotted along with the standard samples on the TLC plate. Standards and samples were applied to the TLC plate and dried under a stream of air at room temperature. The plate was placed in the TLC chamber containing the solvent system and allowed to develop. When the solvent front had migrated to 3/4th height of the plate, the plate was removed and dried at room temperature. The plate was observed under short wavelength UV light to detect the spots.

### Isothermal Kinetic Method

Eighty (80) mg of famotidine was accurately weighed and placed in a 100 ml volumetric flask. This was dissolved in 10 ml methanol and brought up to volume with distilled-deionized water to yield a stock solution having a concentration of 0.8 mg/ml.

Forty (40) ml of Sörenson buffer solution at pH 1.5, 2.0, 2.5, 4.0, 8.5, 9.0 and 10.0 was taken in 100 ml volumetric flask. All the buffers were adjusted to same ionic strength (0.2 M) using potassium chloride. These flasks were kept in waterbath maintained at 85°C. Similarly, two more sets of buffer solutions were placed in two other waterbaths maintained at 70 and 55°C. These flasks were allowed to equilibrate with the bath for 30 minutes. Later 10 ml of the famotidine solution (0.8 mg/ml) was added to these flasks. The flasks were shaken to mix the buffer solution with the famotidine solution. The pH of the resultant solution was determined. Samples of 1.0 ml were drawn at appropriate time intervals. The withdrawn samples were immediately placed in ice-cold waterbath to quench the reaction. The samples were frozen and kept at −20°C until the analysis was performed.

### Nonisothermal Kinetic Method

Famotidine solution (0.8 mg/ml) was prepared in methanol:distilled water (1:9). Forty (40) ml of appropriate buffer solution was placed in a 100 ml volumetric flask. These flasks were placed in a water-bath, whose temperature was programmed to rise at the rate of 0.56°C/min. The flasks were placed in the water-bath for 15 minutes and the solution in the flask was allowed to equilibrate with the surroundings. 10 ml of famotidine solution was added. The temperature programmer was started simultaneously and the temperature was increased at the above mentioned rate. Samples of 1 ml were drawn every 10 minutes and were immediately placed in ice-cold water-bath to quench the reaction. The corresponding temperature, at the time of sample withdrawal, was noted. The samples were frozen and kept at −20°C until analysis was performed.

## RESULTS AND DISCUSSION

### Rate Constants

For all the kinetic experiments the first order plots of famotidine decomposition were linear. Typical first order plot is shown in Figure 1. The apparent first order rate con-

**Table I.** Apparent First Order Decomposition Rate Constants in $min^{-1}$ for Famotidine at Various pH Values and Temperatures

| | pH | | | | | | |
|---|---|---|---|---|---|---|---|
| Temperature | 1.71 | 2.24 | 2.66 | 4.00 | 8.50 | 9.00 | 10.00 |
| 55°C | 0.00594* (0.00010) | 0.00154 (0.00004) | 0.00057 (0.00000) | 0.000044 (0.000002) | 0.000047 (0.000008) | 0.00014 (0.00000) | 0.00056 (0.00005) |
| 70°C | 0.01775 (0.00021) | 0.00549 (0.00001) | 0.00176 (0.00000) | 0.00012 (0.00001) | 0.00034 (0.00000) | 0.00105 (0.00005) | 0.00469 (0.00054) |
| 85°C | 0.05170 (0.00255) | 0.01435 (0.00035) | 0.00526 (0.00059) | 0.00035 (0.00001) | 0.00207 (0.00010) | 0.00596 (0.00012) | 0.02580 (0.00042) |
| E[a] (kcal/mole) | 16.815 | 17.563 | 17.260 | 16.000 | 29.480 | 29.410 | 30.810 |

* N = 2 ( ) = Standard Deviation.
[a] E = Energy of Activation.

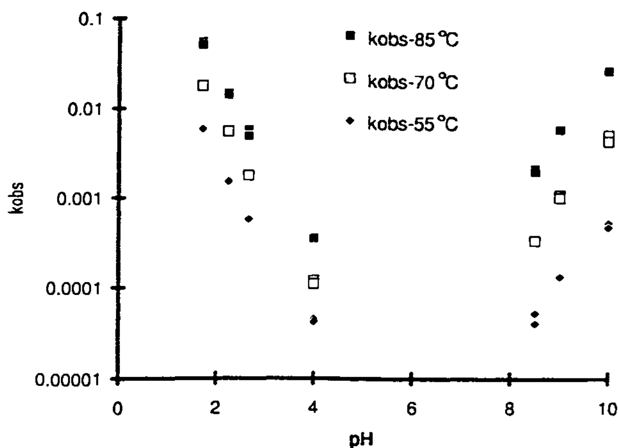



Fig. 2. Overall pH-rate profile for degradation of famotidine.

stants (k) were obtained from slopes in accordance with equation (1)

$$\ln A = \ln A_o - kt \quad (1)$$

where $A$ and $A_o$ are the concentrations at time $t$ and zero, respectively. The rate constants are given in Table I.

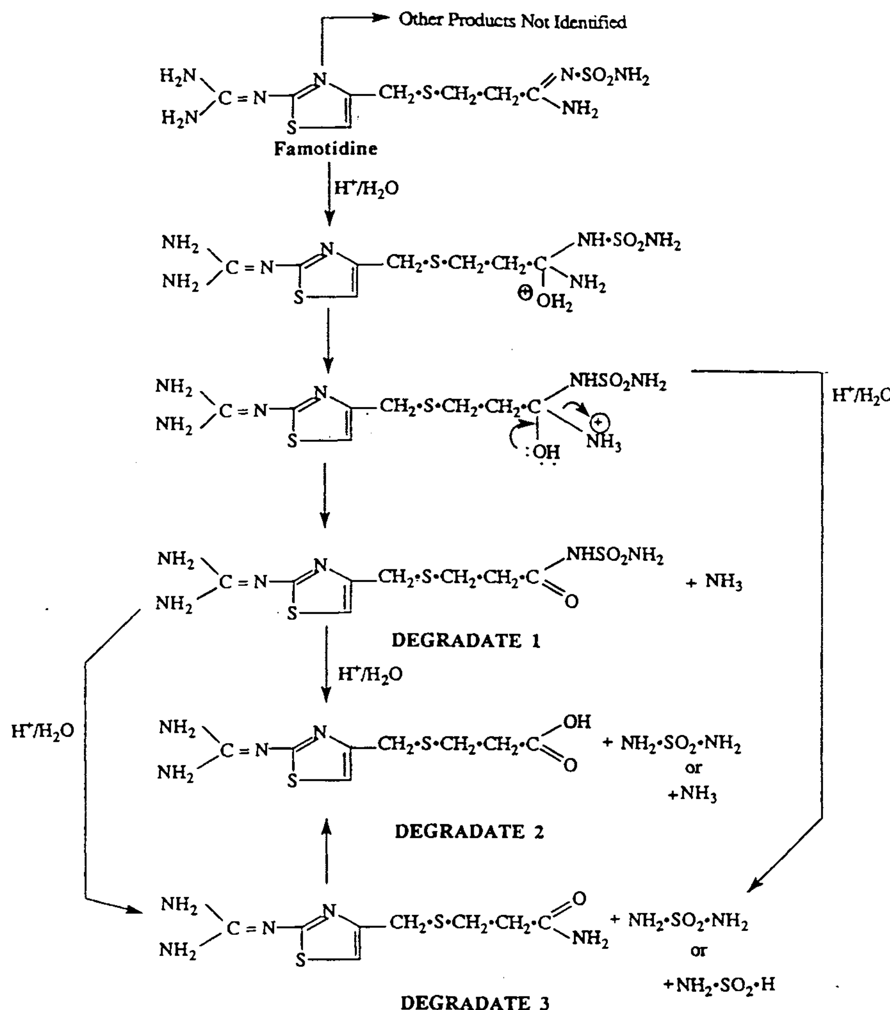
## Logk - pH Profile

The logk - pH profile for the degradation of famotidine at 85, 70 and 55°C is shown in Figure 2. In the acidic region, the slope of the lines is close to $-1$ indicating that famotidine undergoes specific acid catalyzed hydrolysis in the acidic range. The logk decreases with increasing pH in the acidic range. In the alkaline region the slope of the lines is close to $+0.72$ indicating that famotidine may be undergoing general base catalyzed hydrolysis in the alkaline region. The logk increases with increasing pH in the alkaline region. The overall rate of loss of famotidine can be described by equation (2)

$$dF_T/dt = k_1[H^+][FH^+] + k_5[F] + k_6[OH^+][F] \quad (2)$$

Equation (2) can be rewritten in terms of the fraction of famotidine that exists in solution as the protonated form ($FH^+$) and/or the unionized form (F). This leads to equation (3)

$$(dF_T/dt)(1/F_T) = k_{obs} = k_1[H^+]f_{FH^+} + k_5 f_F + k_6[OH^+]f_F \quad (3)$$




Scheme I. Proposed scheme for acid catalyzed hydrolysis of famotidine.

where $F_T$ is the total concentration of famotidine and $f_{(x)}$ are the respective fractions of the different species in solution. Finally, $k_1$, $k_5$ and $k_6$ are the catalytic rate constants. The fraction of protonated famotidine ($FH^+$) is given by equation (4)

$$f_{FH^+} = K_b/([OH^-] + K_b) \text{ or } 1/(1 + 10^{pH-pK_a}) \text{ or } H^+/(H^+ + K_a) \quad (4)$$

and the fraction of famotidine in the unionized form can be estimated using equation (5)

$$f_F = [OH^-]/[OH^-] + K_b) \text{ or } 1/(1 + 10^{pK_a-pH}) \text{ or } K_a/(H^+ + K_a) \quad (5)$$

In the acidic region, most of the famotidine exits in the protonated form ($FH^+ > 99\%$), and it undergoes specific acid catalysis. So, for the acidic region equation (3) can be reduced to equation (6)

$$k_{obs} = k_1[H^+]^n f_{FH^+} \quad (6)$$

where $n$ is the order of reaction with respect of hydrogen ion. Similarly, in the alkaline region most of the famotidine exists in the undissociated form (F) and the concentration of $[H^+]$ can be considered to be negligible. Thus the observed rate constant can be expressed as equation (7)

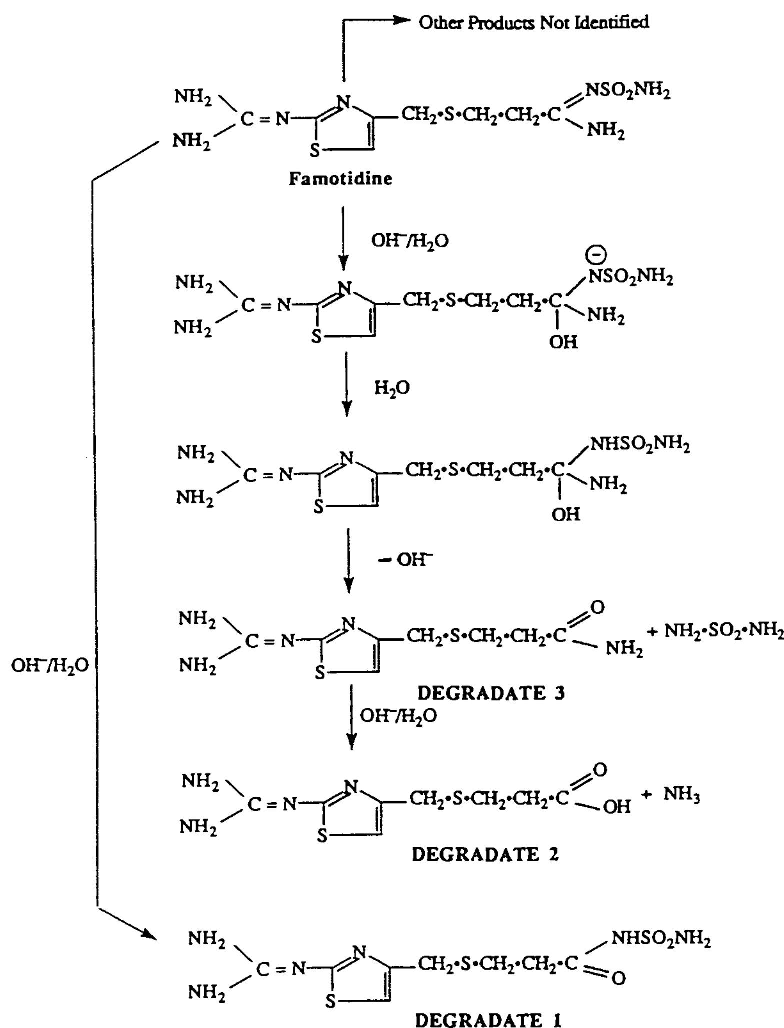
$$k_{obs} = k_5 f_F + k_6[OH^+]^m f_F \quad (7)$$

where $m$ is the order of reaction with respect of hydroxide ion.

The catalytic rate constants at each of the temperatures were obtained by fitting the data for $k_{obs}$ and pH to equations (6) and (7) using NONLIN, a nonlinear digital computer program (PCNONLIN Ver. 3.0, SCI Software). The catalytic rate constants $K_1(^1/_{M\text{-}min})$, $K_5(^1/_{min})$, and $K_6(^1/_{M\text{-}min})$ were found to be at 85°C:2.09, 5.08E$^{-04}$, 18.72; at 70°C:0.76, 1.07E$^{-04}$, 4.46; and at 55°C:0.22, 2.32E$^{-05}$, and 0.304, respectively.

### pH of Maximum Stability

The pH of maximum stability can be approximated using equation (8). This equation can be approximated as follows:




Scheme II. Proposed scheme for base catalyzed hydrolysis of Famotidine.

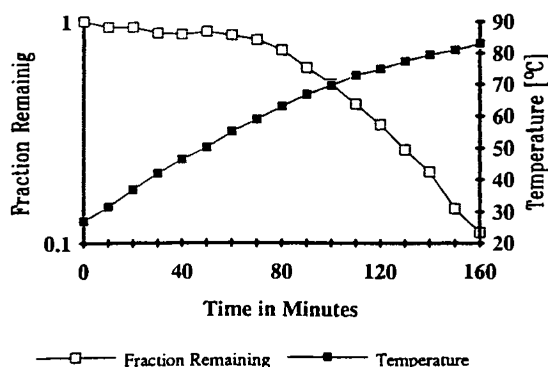



Fig. 3. Semilogarithmic plot of nonisothermal decomposition of famotidine at pH 1.71.

$$k_{obs} = k_1[H^+] + k_5 + k_6[OH^-] \quad (8)$$

The pH of maximum stability can be approximated by differentiating $k_{obs}$ with respect to $[H^+]$ in equation (8) and equating to zero. Thus,

$$k_1 - (K_w * k_6)/[H^+]^2 = 0 \quad (9)$$

or

$$[H^+]^2 = (K_W * k_6)/k_1 \quad (10)$$

Using equation (10), the maximum pH for 85, 70 and 55°C was estimated to be 6.52, 6.61 and 6.92, respectively. The shelf-life of famotidine in solution at 25°C is predicted to be 43 days at pH 6.92.

### Rate Dependence on Temperature

The dependence of catalytic rate constants on temperature is given by the Arrhenius equation

$$k = Ae^{-E_a/RT} \quad (11)$$

where $E_a$ is the activation energy and $A$ the frequency factor. The Arrhenius parameters calculated in accordance with equation (11) are given in Table I. The activation energy in the alkaline region is higher than in the acidic region indicating that famotidine is more stable in the alkaline region than in the acidic region.

### Mechanism of Degradation

In the TLC studies, a sample obtained at 3 hours during the isothermal studies at pH 2, 85°C, showed absence of an spot corresponding to famotidine, but showed presence of spots corresponding to degradates, referred to in the text as degradate 1, degradate 2 and degradate 3. It also showed a spot corresponding to $R_f$ value of 0.3 which has not been characterized. This indicates the formation of three known degradates and one unknown degradate due to acidic hydrolysis of famotidine. A sample obtained at 2.5 hours during the isothermal studies at pH 10, 85°C, 2.5 hours showed absence of a spot corresponding to famotidine, but showed an intense spot corresponding to degradate 3. The spots corresponding to degradate 1 and degradate 2 had very low intensity. This sample also showed presence of two more spots which did not correspond to any of the known degradates. It could be concluded that the major decomposition product of alkaline hydrolysis of famotidine is degradate 3. Degradate 1 and degradate 2 are minor decomposition products. Two more degradates are also formed during alkaline hydrolysis which were not characterized.

The mechanism of degradation of famotidine in acidic and alkaline media is proposed in Schemes I and II.

### Comparison of Nonisothermal and Isothermal Kinetics

Analysis of the nonisothermal kinetic data was done using a computer program NISO7,[15] which uses the integral method of data analysis. The data (time, temperature and fraction of drug remaining) were fitted to the integral form of the first order rate equation:

$$C = C_o\exp\{-A \int \exp(-E_a/RT(t))dt\} \quad (12)$$

It was found from isothermal experiments that the degradation follows first order kinetics. The program NISO7 uses the iterative simplex algorithm of Nelder and Mead to perform a least squares fit to time temperature and concentration data to appropriate kinetic model. The optimal values of activation energy and the preexponential factor are obtained using the program.

Before running the program, an ASCII file containing the data in specific format was created. Also, a polynomial order (3rd to 20th) to fit the temperature data to time was specified. The first order rate model was chosen and the number of polynomial terms chosen was varied to produce the best fit to the data. The initial estimates of activation energy and the pre-exponential factor were also given. These estimates were obtained from the isothermal experiments at the specific pH.

The results of nonisothermal run at pH 1.71 are shown in Figure 3. This shows that with increase in temperature rate of disappearance of the drug increases. Table II gives a comparison of the Arrhenius parameters obtained using the

Table II. Comparison of Activation Energies Obtained from Isothermal and Nonisothermal Methods

| | Activation Energy (cal/mole) | | | Arrhenius Factor (1/min) | | |
|---|---|---|---|---|---|---|
| pH | Isothermal | Nonisothermal | %Difference | Isothermal | Nonisothermal | %Difference |
| 1.71 | 16815 | 16745 | 0.42 | 9.77E+08 | 8.14E+08 | 16.68 |
| 2.24 | 17563 | 17038 | 2.99 | 7.99E+08 | 3.58E+08 | 55.19 |
| 2.66 | 17259 | 17494 | −1.36 | 1.78E+08 | 2.94E+08 | −65.17 |
| 10.00 | 30810 | 29173 | 5.31 | 1.74E+17 | 2.06E+16 | 88.14 |

%Difference = (Isothermal − Nonisothermal)*100/Isothermal.

Table III. Comparison of Rate Constants and Half-Lives Obtained Using Arrhenius Parameters From Isothermal and Nonisothermal (Noniso.) Studies

| pH | Temperature (°C) | $k_{obs}$(1/min) | | Half-life(h) | |
|---|---|---|---|---|---|
| | | Isothermal | Noniso. | Isothermal | Noniso. |
| 1.71 | 85 | 0.05290 | 0.04860 | 0.22 | 0.24 |
| | 70 | 0.01880 | 0.01730 | 0.61 | 0.67 |
| | 55 | 0.00610 | 0.00570 | 1.89 | 2.03 |
| | 25 | 0.00045 | 0.00043 | 25.67 | 26.86 |
| 2.24 | 85 | 0.01510 | 0.01410 | 0.76 | 0.82 |
| | 70 | 0.00510 | 0.00500 | 2.26 | 2.31 |
| | 55 | 0.00160 | 0.00160 | 7.22 | 7.22 |
| | 25 | 0.00010 | 0.00011 | 115.50 | 105.00 |
| 2.66 | 85 | 0.00520 | 0.00610 | 2.22 | 1.89 |
| | 70 | 0.00180 | 0.00210 | 6.42 | 5.50 |
| | 55 | 0.00056 | 0.00065 | 20.63 | 17.77 |
| | 25 | $3.90E^{-05}$ | $4.30E^{-05}$ | 296.15 | 268.60 |
| 10.00 | 85 | 0.02690 | 0.03190 | 0.43 | 0.36 |
| | 70 | 0.00410 | 0.00530 | 2.82 | 2.18 |
| | 55 | 0.00051 | 0.00075 | 22.65 | 15.40 |
| | 25 | $4.00E^{-06}$ | $8.00E^{-06}$ | 2887.50 | 1443.75 |

isothermal and the nonisothermal studies. This indicates that there is close agreement in the values of activation energy obtained from isothermal and nonisothermal studies. The percentage difference between the value of activation energy obtained by isothermal and nonisothermal is between −1.36 to 5.31. The data indicates that nonisothermal studies appear to yield an accurate estimation of the activation energy. The percentage difference for the Arrhenius factor is high because this is estimated from the intercept of Arrhenius plot in the isothermal studies. Thus, a slight variation in the slope of the line will affect the intercept considerably. A comparison of the values of rate constants and corresponding half-lives calculated at 85, 70, 55 and 25°C, using the Arrhenius parameters obtained by isothermal and nonisothermal studies is given in Table III. Considerable variation in the predicted half-life at pH 10, 25°C is observed between the calculated half-lives using the isothermal and nonisothermal method. This variability may be due to the variation in the Arrhenius factor at pH 10.

These results indicate that nonisothermal methods will enable estimation of the shelf-life of a pharmaceutical preparation without resorting to much time-consuming kinetic analysis at several different temperatures, as is done in isothermal approach.

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