Aspirin Stability

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Objectives

- To describe how accelerated stability testing at elevated temperatures can be used to explain the stability of the product at room temperature.
- How to classify reactions as zero, first, and second order reactions and their corresponding reactions rates.
- To determine the activation energy necessary by constructing an Arrhenius plot.
- To predict the rate constant at a given temperature by using reaction rate data at elevated temperatures
- To determine the shelf life of the product using an integrated rate law.

Introduction

The chemical stability of a 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.

Hydrolysis of the drug entity can be a major factor in the instability of solutions. Aspirin, for example, undergoes hydrolysis with the resultant degradation products being salicylic acid and acetic acid.

$$\text{Aspirin} \rightarrow \text{Salysilic Acid} + \text{Acetic Acid}$$

The rate of this reaction is said to be second order, since it is dependent not only upon the aspirin concentration, but upon solution pH. At pH = 7.5, the rate expression for the hydrolysis of aspirin may be written:

$$\frac{-d[A]}{dt} = K[A][OH^-] \tag{1}$$

where,

[A] = the concentration of aspirin (acetyl salysilic acid) (mol/L)
[OH^-] = hydroxyl ion concentration (mol/L)
K = second order rate constant (L/min·mol)
t = time (min)

The rate expression above is called *second order* because it is proportional to two concentrations, [A] and [OH^-].

If the solution is buffered so that the hydroxyl ion concentration remains essentially constant, the rate expression may be rewritten in a form that combines the constant values of K and [OH^-] into one constant:

$$\frac{-dA}{dt} = K_{app}A \tag{2}$$

where,

A = mass of aspirin (mg)
K_{app} = a constant equal to $K[OH^-]$, with units of min^{-1}.

From the above equation, it can be seen that the degradation of aspirin in a solution buffered at pH = 7.5 will follow first order kinetics; that is, the reaction will appear to be a first order reaction, dependent only on the concentration of one reactant; i.e. aspirin (ASA or simply A).

The integrated form of a first order rate expression above is:

$$\boxed{\ln A_t = \ln A_0 - K_{app} t} \qquad (3)$$

where,

A_t = mass of aspirin remaining at time t (mg)
A_0 = mass of aspirin initially present (mg)
K_{app} = apparent first order rate constant (min^{-1})
t = time of sampling (min). For example, 0 min, 15 min, 30 min etc.

This equation is of the form:

$$y = mx + b$$

where,

m = the slope of the line
b = the y intercept

For the hydrolysis of aspirin in buffered solution (pH = 7.5), a semi-log plot of the aspirin concentration remaining versus time should yield a straight line with a negative slope equal to $-K_{app}$.

Since the experimentally determined first order rate constant (K_{app}) is related to the true second order rate constant (K) by the expression:

$$K_{app} = K[OH^-]$$

Kapp is called a "pseudo-first order rate constant" and can be determined experimentally by studying the change in concentration of aspirin with time.

It is easier to measure the increasing concentration of the product, salicylic acid (SA) than it is to measure the decreasing aspirin concentration, A. Therefore the experiment follows the appearance of SA, and relates the SA concentration to the concentration of A.

One mole of salicylic acid is produced when one mole of aspirin degrades; so, using the ratio of the molecular weights of aspirin to salicylic acid, we can determine the weight of aspirin degraded for each mg of salicylic acid produced.

$$\frac{180.15\frac{\text{g aspirin}}{\text{mol}}}{138.12\frac{\text{g SA}}{\text{mol}}} = \frac{1.304\,\text{mg ASA}}{1\,\text{mg SA}}$$

Therefore, each milligram of salicylic acid present represents the degradation of 1.3 milligrams of aspirin. Since the amount of aspirin initially present is known and since the amount of aspirin, which has degraded, can be determined, the amount of aspirin remaining can be calculated.

It is desirable to determine the stability of the active ingredient in the drug so that a shelf life or expiration date may be assigned to the product. The shelf life is the length of time required for the product potency to be reduced to some percentage of its original value. For most products, this is the t_{90} or time at which the product retains 90% of its original potency. Although the drug's stability at room temperature is of interest, a stability study at room temperature would take too long. Therefore, studies are conducted at elevated temperatures in accordance with the Arrhenius equation:

$$K_{app} = \alpha e^{\frac{-E_a}{RT}} \tag{4}$$

where,

K_{app} = apparent rate constant (min^{-1})
α = frequency constant (min^{-1})
E_a = activation energy (cal/mol)
R = gas constant (1.987 cal./K mol)
T = absolute temperature (K)

The Arrhenius equation can be rewritten as:

$$\boxed{\ln K_{app} = \ln \alpha - \frac{E_a}{R} \cdot \frac{1}{T}} \tag{5}$$

Again, an equation of the form y = mx + b is generated, indicating that a semi-log plot of K_{app} vs. the reciprocal of the absolute temperature (1/T) should yield a straight line with a negative slope equal to $-E_a/R$. This line can be extrapolated to the value of 1/T that corresponds to room temperature and the predicted rate constant for the reaction at room temperature can be taken from the y- axis.

Finally, the shelf life of the drug at room temperature can be determined. We will consider the shelf life to be the time for which at least 90% of the drug remains active, and this will be denoted t_{90}:

$$A_{t_{90}} = 0.9A_0 \tag{6}$$

where,

t_{90} = The length of time for which at least 90% of the original drug is present (min)
A_{t90} = The amount of drug present at time t_{90} (i.e., 90% of the original amount of drug)
A0 = The amount of drug initially present (mg)

In order to find the value of t_{90}, we will use Equation 3. This equation relates the amount of aspirin remaining to the time lapsed:

$$\ln A_{t90} = \ln A_0 - K_{app} t_{90}$$

This equation can be simplified. First, equation 6 is substituted for A_{t90}, and then the equation is rearranged:

$$\ln(0.9A_0) = \ln(A_0) - K_{app} t_{90}$$

$$\ln\left(\frac{0.9A_0}{A_0}\right) = -K_{app} t_{90}$$

$$\ln(0.9) = -K_{app} t_{90}$$

$$\boxed{\frac{0.1054}{K_{app}} = t_{90}} \tag{7}$$

In equation 7, the value of K_{app} is taken at room temperature (T=298K).

Procedure

In this experiment, three people will work in a group; each group member will conduct the experiment at a different temperature (50°C, 70°C, and 80°C) and then collaborate the data to hand in one lab report per group.

Equipment and Materials

- UV spectrometer
- (1) 1000 mL beaker
- (1) graduated pipette, to measure 1.0 ml sample
- (6) 15 mL sample bottles containing 14 mL water
- (1) 100 mL volumetric flask
- (1) 200 mL Erlenmeyer flask
- (1) weighing boat
- Parafilm
- Labeling tape
- Salicylic Acid Standard Solutions (0.00-0.133 mg/mL), labeled
- Beakers, labeled, for small quantities of standard solutions (one for each standard)
- One 3-ml plastic dropper for each beaker of standard solution
- Phosphate Buffer Solution, 750 mL for 5 teams

- Acetylsalicylic acid
- Methanol (Spectrophotometric Grade) in a beaker, with 3-ml plastic dropper
- (1) Hot plates
- (1) Thermometer 0-100 °C, preferably an alcohol thermometer
- (1) Cuvette for spectrophotometer at 310 nm
- Hothands
- Funnel

UV Spectrophotometer

The UV spectrophotometer is a helpful and simple piece of equipment to understand and use in the lab. However, one should be extremely cautious when using it, because it is very expensive. Set the wavelength to 310nm. Only UV grade cuvets should be used to test samples. These UV grade cuvets can hold approximately 3 mL of solution. Use DI water as the standard; carefully place the cuvet in the proper holding device.

Standard Curve

1. You will be provided with prepared standard solutions of salysalic acid (SA) between 0-0.133 mg/ml.

2. Using the UV spectrophotometer, measure the absorbance of each of concentrations of salicylic acid. Record the results in Table 1.

Conc. of SA (mg/mL)	Absorbance

Table 1 **Absorbance of SA in given concentrations**

3. Make a Beer's Law plot of concentration vs. absorbance. Determine the slope, intercept, and R^2 value. A sample plot is provided below.

Hydrolysis of Aspirin

Each team will perform the experiment at one temperature, and data for different temperature runs will be shared among teams. A constant temperature bath is made using a large beaker of water on a hotplate. The flask containing the aspirin is placed in the beaker of water.

*****Note: Read the procedure, especially steps 1-5 before you begin!! As soon as aspirin is added to water, it begins to degrade. It is therefore important to take your initial sample quickly after water is added to the aspirin in step 2. Likewise, it is important to read the***

absorbance of samples very quickly after the sample is taken, since the aspirin in the sample continues to degrade.

1. Add *approximately* 120 mL of the phosphate buffer solution (pH=7.5) into a 200 ml Erelenmeyer flask; cover the flask with parafilm and place into the constant temperature bath. Allow the contents to come to the specified temperature.
2. Weigh 200 mg of aspirin and add the powder to a 100 mL volumetric flask. Add *approximately* 2.0 mL of methanol to dissolve the aspirin. (There are two types of methanol in the lab, make sure that the spectrometric grade methanol is used, the other type of methanol is only good for UV readings at wavelengths lower then 210). Add the hot-buffered solution to the flask until a total volume *exactly* of 100 mL is reached. Quickly cover with parafilm and invert the volumetric flask many times to ensure a good mixture.
3. Obtain the "**Eppendorf pipette**" which will allow you to pipette *exactly* 1.0 ml of sample from your flask. Obtain a plastic sample container which already contains 14.0 ml distilled water. Immediately pipet a 1.0 mL sample and place in a sample container. This sample will correspond to time zero. Then, place the volumetric flask containing the aspirin solution in the water bath. (note that the sample has been diluted by a factor of 15 – 1 ml of aspirin diluted to a total of 15.0 ml).
4. Use a dropper to fill the UV cuvet about ¾ full (approximately 3 mL) and read the absorbance at 310 nm using the spectrophotometer. Record the absorbance value in Table 2
5. Continue to pipet and analyze 1 mL samples from the buffer solution every 10-15 minutes for 60 minutes. Do not remove the flask when sampling so the temperature can remain stable. Record the results in Table 2.

Data Analysis

Please note: Please show sample calculations for each equation that you use. You may reproduce the tables below using Excel, and perform your calculations on the spreadsheet instead of performing them on a hand calculator.

1. Using the equation you obtained in the Beer's Law plot (Refer to Step 3 in the procedure for making a Standard Curve), determine the concentration of salicylic acid (SA) for each absorbance reading. Remember, the equation of the line is of the form y=mx+b, where concentration is the "y" value, the absorbance is the "x value", and m and b are the slope and intercept of the line. Record the concentration in Table 2.

2. Determine the amount of acetylsalicylic acid degraded A_{drgd} for each sample time and record results in Table 2. Use the following equation.

$$A_{dgrd} = [SA] \cdot V(\mathrm{DF}) \cdot R$$

where,
A_{drgd} = the mass of Aspirin degraded (mg)
[SA] = concentration of SA (mg/ml).
DF = Dilution Factor, 15 in this case.
V = total volume of SA solution (ml), 100 ml in this case.

R = ratio of A degraded to SA formed; (1.304 mg A)/(mg SA)

3. Determine the amount of acetylsalicylic acid remaining for each sample time and record results in Table 2.

$$A_{rem} = A_0 - A_{dgrd}$$

Where,
A_{rem} = the mass of Aspirin remaining (not yet degraded), (mg).
A_0 = the initial mass of Aspirin in the experiment, (mg), about 200 mg for this experiment.

Temperature =				
Time (min)	**Absorbance**	**Concentration of Salicylic Acid (mg/mL)**	**Acetylsalicylic acid degraded (mg)**	**Acetylsalicylic acid remaining (mg)**
0				

Table 2. Experimental data and calculated values for Aspirin degradation at T = _____

4. Obtain the results for two ***different*** temperatures from other teams and record the results in Table 3 and Table 4. *(You may obtain the entire data table from the team, but you are responsible for making sure their calculations are correct! You are also responsible for obtaining data from another team if you determine that the data you have is not sufficient.)*

Temperature =				
Time (min)	**Absorbance**	**Concentration of Salicylic Acid (mg/mL)**	**Acetylsalicylic acid degraded (mg)**	**Acetylsalicylic acid remaining (mg)**
0				

Table 3. Shared experimental data and calculated values for Aspirin degradation at T = _____

Temperature =				
Time (min)	**Absorbance**	**Concentration of Salicylic Acid (mg/mL)**	**Acetylsalicylic acid degraded (mg)**	**Acetylsalicylic acid remaining (mg)**
0				

Table 4. Shared experimental data and calculated values for Aspirin degradation at T = _____

Data Analysis (Record the following data in Table 5)

1. Plot ln (A_{rem}) vs. time for each temperature and determine the slope of the line at each temperature. A sample plot is shown below for a single temperature. You may plot the data for the other temperatures on the same graph, or on separate graphs, as you wish.

2. Calculate K_{app} for each temperature: $-K_{app}$ = slope of the above plot. In the example above, K_{app} = -slope = 0.0025 min^{-1}. Record the results in Table 5.

3. For each temperature, calculate the value of 1/T, where T is the temperature in Kelvin. Record the values in Table 5. Also calculate and record the values of ln(K_{app}) for each Temperature.

4. Construct an Arrhenius Plot by plotting ln K_{app} vs. 1/T. Find the slope and intercept. The slope will be equal to –E/R and the intercept will be equal to ln(α). A sample plot is provided below. Please note that there will be only one line on this plot. Each data point represents the K value from a different temperature, so you will have three data points.

5. Use Equation 5 Determine the K_{app} at room temperature (25 °C = 298K). Remember, (–E/R) is the slope of the plot above, and ln(α) is the intercept.

$$\ln K_{app} = \ln \alpha - \frac{E_a}{R} \cdot \frac{1}{T}$$

   Remember from algebra that $K_{app} = \exp\{\ln K_{app}\} = e^{\{\ln K_{app}\}}$.

6. Determine the shelf life of aspirin in a buffered solution at t_{90} using Equation 7.

$$\frac{0.1054}{K_{app}} = t_{90}$$

Temp (°C)	Temp (°K)	1/T (°K)	K_{app} (min^{-1})	ln(K_{app})

Table 5. Data analysis for the Aspirin degradation experiment.

Assignment

One assignment per team is due at the beginning of the period next week.
Write the Results and Conclusion sections of a laboratory report (only these sections – you do not need to write the introduction/background/theory/procedure for this assignment). You may refer to this laboratory handout for theory/equations that are used in your results section. The results and conclusions should include:

- All calculations requested in the laboratory handout
- All data analysis requested (including tables and graphs) in the laboratory handout
- A discussion of the significance of the results
- Meaningful conclusions that are drawn *from your results*

Don't forget, tables and graphs must be explained/discussed in the text!

In addition, you should include your yellow laboratory notebook page as an appendix. Also in an appendix should be sample calculations for all the calculations performed, showing numbers and units. .