Research Question: What is the activation energy (kJmol^{-1}) of the hydrolysis reaction of 1.4 × 10^{-3} M acetylsalicylic acid ($C_9H_8O_4$) at pH 7, determined by analysing the spectrophotometric absorbance of ultraviolet radiation of wavelength 300nm by the salicylic acid ($C_7H_6O_3$) produced in the reaction carried out at different temperatures (K)?

1. Introduction

Like millions of people around the world, I take aspirin tablets to cure my headaches and fevers from time to time. On the box of many medicinal tablets including aspirin, an instruction reads "store in a cool and dry place". I always wondered why Aspirin needed to be stored in that certain environment, so I decided to dig deep into the chemical composition of an Aspirin tablet and its mechanism. Aspirin or acetylsalicylic acid or 2-ethanoyloxybenzoic is an ester prepared by the synthesis of salicylic acid (2-hydroxybenzoic acid) with excess acetic anhydride. The way Aspirin cures pain, reduces swelling and reduces a raised body temperature is by inhibiting the production of prostaglandins which are produced by the enzymes called cyclooxygenase i.e., COX-1 and COX-2 enzymes[1]. However, salicylic acid produces many adverse effects in the body like upper gastrointestinal bleeding, injury to gastric mucosa, irritation in stomach and formation of gastric ulcers[2]. Aspirin undergoes hydrolysis to break down into salicylic acid and acetic acid according to the following reaction[1]:

$$C_9H_8O_4 + H_2O \longrightarrow C_7H_6O_3 + CH_3COOH$$
(acetylsalicylic acid) (salicylic acid)(acetic acid)

To gain a better understanding of Aspirin's effects on the stomach and intestines, I discussed this with my father, who is a gastroenterologist. He explained to me that most doctors do not prescribe aspirin for pain relief, specially to elderly patients and instead prescribe alternatives that will not dissociate into salicylic acid. Apart from its detrimental gastric effects, hydrolysis of aspirin can also cause asthmatic attacks and flares of urticaria in some patients[3]. It is axiomatic that the reason why aspirin tablets must be stored in a cool and dry place is because in the presence of moisture, the acetylsalicylic acid would hydrolyse and presumably, at a higher temperature, this reaction might be faster. This reaction remained at the back of my mind while we studied chemical kinetics in class. Considering the importance of this hydrolysis reaction, I questioned the value of its Activation Energy (E_A) which led me to my research question; **"*What is the activation energy (kJmol^{-1}) of the hydrolysis reaction of 1.4 × 10^{-3} M acetylsalicylic acid ($C_9H_8O_4$) at pH 7, determined by analysing the spectrophotometric absorbance of ultraviolet radiation of wavelength 300nm by the salicylic acid ($C_7H_6O_3$) produced in the reaction carried out at different temperatures (K)?*"**

2. Investigation

2.1: Background Information

Rate of reaction: The rate of any chemical reaction depends on the order of the reaction. Previous studies have shown that the hydrolysis of aspirin is a second order reaction since its rate depends on the concentration of acetylsalicylic acid and the pH. However, if the pH is kept constant using a buffer solution, the reaction is presumed to be a pseudo-first order reaction[4] whose rate can thus be determined by the equation:

$$rate = -\frac{d[A]}{dt} = k[A]$$

Here, [A] is the concentration of acetylsalicylic acid (mol dm^{-3}), t is the time passed (s) and k is the rate constant (s^{-1}). The integrated form of this equation can be written as:

$$\ln [A_t] = -kt + \ln [A_0]$$

Here, $[A_t]$ is the remaining concentration of acetylsalicylic acid (mol dm^{-3}) at time "t" (s), $[A_0]$ is the concentration of acetylsalicylic acid (mol l^{-3}) at time t = 0 s and *k* is again the rate constant (s^{-1}).

Beer-Lambert Law: An instrument called spectrophotometer is used to determine the absorption of ultraviolet radiations by a solution. When the solution is of low to moderate concentration, Beer-Lambert law states that, "The absorbance is directly proportional to the concentration of the solution" [5] according to the equation:

$$a = \varepsilon l c$$

Here 'a' represents the absorbance of radiation, 'ε' represents the molar absorptivity of the solution (L mol^{-1} cm^{-1}), 'l' represents the path length (cm) and 'c' represents the concentration of the solution (mol dm^{-3}).

Activation Energy: The Activation Energy (E_A) of a reaction is the minimum amount of energy required for the reaction to occur. The value of the activation energy (kJmol^{-1}) is independent of the temperature but does depend on the pH of the reaction mixture. The activation energy is determined by the Arrhenius equation which is given by

$$\ln k = \ln A - \frac{E_A}{RT}$$

Here, A is the frequency factor or the pre-exponential factor (s^{-1}) which takes into account the frequency of reactions and the likelihood of correct molecular orientation, R is the gas constant whose value is 8.314 J K^{-1} mol^{-1}, T is the temperature (K) and E_A is the activation energy (J mol^{-1}).

2.2: Calculations

In this investigation, after obtaining a graphical relationship between absorbance of radiation of 300 nm wavelength and concentration of salicylic acid using a calibration curve, an equation of the form $a = m[S]+b$ was obtained, where a is the absorbance and [S] is the concentration of salicylic acid. Through several repetitions of the hydrolysis experiment, we obtained the mean absorbance of radiation of wavelength 300 nm at different time intervals. Using the equation, $a = m[S]+b$, we determined the value of concentration of salicylic acid produced in the reaction at different time intervals. The moles of salicylic acid produced was calculated from the concentration by the formula:

moles= n = (concentration×volume)/(molar mass)

Since the hydrolysis reaction has 1:1 molar ratio between salicylic acid produced and acetylsalicylic acid consumed, the concentration of acetylsalicylic acid remaining was calculated by:

concentration = $[A_t]$ = (initial concentration) - (moles)/(volume)

This value is used to calculate the ln $[A_t]$ at time t (seconds). The graph of ln $[A_t]$ vs t gives the negative of rate constant "-k" as the gradient in $seconds^{-1}$. The (ln k) values at different temperatures are plotted against the 1/T values of temperature according to the equation; $\ln k = \ln A - \frac{E_A}{RT}$.

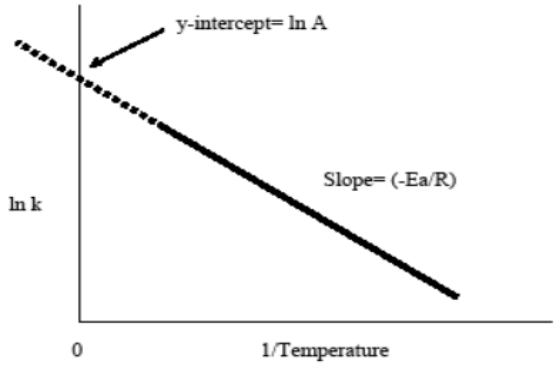


Figure 1: The graph of ln k vs 1/ T according to the Arrhenius equation[8]

3. Variables

3.1 Independent Variable

Temperature (K): The temperature was chosen as the independent variable because it can be easily varied, and different increments can provide us different rate equations as rate of reaction is dependent on the temperature of the reaction mixture. The temperatures that were chosen are 308 K, 323 K, 338 K and 353 K because previous studies have shown that the hydrolysis reaction of aspirin occurs much faster at elevated temperatures[4] and it wouldn't have been possible to record substantial data at room temperature (293 K) since the reaction would be extremely slow.

3.2 Dependent Variable

Pseudo first order rate constant, "k" (s^{-1}): The rate constant was chosen as the dependent variable in order to determine the activation energy of the reaction. Different temperatures would yield different values for the rate constant and these can be plotted on a graph using the equation $\ln k = \ln A - \frac{E_a}{RT}$. The rate constant was determined by UV-spectrometry method i.e., the concentration of salicylic acid produced was determined by the observing the absorbance of radiation by the solution at different time intervals for different temperatures.

3.3 Controlled Variables

Variable	How has it been controlled	Why has it been controlled	Controlled Value
pH	The pH is kept constant with the use of a sodium dihydrogen phosphate and disodium hydrogen phosphate buffer solution. A buffer solution resists a change in its pH when small amounts of acids or bases are added, so in this experiment, it allowed the pH to remain constant.	The rate equation of a first order reaction would only be valid if the pH of the solution remains constant. Keeping the pH constant ensures that the pH change is not a factor influencing the rate of reaction. The value of pH 7 was chosen because the pH in small intestine is about pH 7 (ranges from 6 to 7.4)[6] and that's where majority of the aspirin is absorbed in the patient's body. Thus, calculating the activation energy of the reaction at this pH will help us understand the reaction as it occurs in the small intestine.	pH 7 ± 0.2
Initial concentration of acetylsalicylic acid	Stock solution of aspirin is prepared with a fixed concentration and the amount of aspirin stock solution added is kept constant at 1 ml which ensures that the initial concentration of acetylsalicylic acid is constant.	The concentration of acetylsalicylic acid influences the rate of reaction according to the relation "rate=k[A]" where A is the concentration. Controlling the initial concentration and keeping it at a constant value ensures that the concentration of the salicylic acid produced is only affected by the varying temperature. The low concentration is chosen as the spectrophotometer's range for absorbance readings is 0.1- 1. Hence, if a higher concentration of salicylic acid is produced, it would be impossible to measure the absorbance reading which could go beyond 1.	1.4×10^{-3} M

Wavelength of electromagnetic radiation from the spectro-photometer	The spectrophotometer's radiation's wavelength is set at the specific value using the control panel and appropriate settings are applied.	Research shows that salicylic acid's maximum absorbance occurs at 300 nm and at this wavelength, acetylsalicylic shows no absorbance[7]. Hence, the absorbance reading of the spectrophotometer would be directly related to the concentration of salicylic acid produced according to the Beer's law equation only at this wavelength.	300 nm
Volume of the reaction mixture	For every iteration of the experiment, the volume of the reaction mixture is kept constant by adding 19 ml of the pH 7 buffer solution to 1 ml of aspirin stock solution. A graduated pipette was used to add and measure the solutions.	The volume of the mixture can affect the concentration of salicylic acid produced as concentration is given by mass per unit volume and a varying volume of the reaction mixture would result in varying concentration which would affect the calculated rate constant.	20 ml or 0.020 l

4. Methodology

4.1 Materials and Apparatus Required

- Systronics AIM Spectrophotometer 104
- (3) 3 ml UV cuvettes
- (5) Test tubes and stoppers
- (2) 1 ml (±0.01 ml) graduated pipettes
- (2) Volumetric flasks- 1000ml, 100 ml
- (7) Measuring cylinders- 50 ml, 100 ml (±0.5 ml)
- pH meter
- China dish
- Magnetic stirrer
- Water Bath (±0.1 K)
- Funnel
- Filter paper
- 10 g Salicylic Acid
- Sodium dihydrogen phosphate 1.20 g
- Disodium hydrogen phosphate 0.885 g
- 2 M HCl and NaOH
- 500 mg Aspirin Tablet
- 200 ml Spectrophotometer grade methanol
- 1000 ml deionized water

4.2 Experimental Procedure

Preparing pH 7 ±0.02 Buffer Solution

1. Take 1.20 g of sodium dihydrogen phosphate and 0.885 g of disodium hydrogen phosphate in a 1000 ml volumetric flask and fill it to the mark with desionized water. Seal the flask with a cork and shake the solution well to ensure the contents dissolve.
2. Extract approximately 10 ml of this solution in a measuring cylinder and using a pH meter, check its pH. If the pH is not around pH 7, add a small amount of 2M HCL (if pH higher than 7) or 2M NaOH (if pH lower than 7) in the flask to attain the required pH.

Making the Calibration Curve for concentration of salicylic acid vs absorbance

1. Weigh 0.12 mg, 0.22 mg, 0.32 mg, 0.42 mg and 0.52 mg of salicylic acid and put the samples in different 50 ml measuring cylinders (±0.5 ml) and dissolve them each in 1 ml spectrophotometer grade methanol, transferred using a graduated pipette (±0.01 ml). While taking measurements from a pipette or measuring cylinder, take the reading at the meniscus for accuracy.
2. Slowly add 19 ml of the buffer solution to each measuring cylinder using a funnel to fill each cylinder to the 20 ml mark. Stir the solution with the magnetic stirrer.

3. One by one, transfer 1 ml of each solution into a UV cuvette using a graduated pipette (±0.01 ml). Set the wavelength of the spectrophotometer radiation at 300 nm and place the cuvette in it. Note down the different readings of absorbance at different concentrations of salicylic acid.

Hydrolysis of Aspirin

1. Set the water bath to 308 K (±0.1 K).
2. Take a tablet of Aspirin rated 500 mg and crush it in a china dish to obtain it in a powdered form. Transfer the powder into a 100 ml (±0.5 ml) volumetric flask and fill it to the 100 ml mark with spectrophotometer grade methanol. Using a magnetic stirrer, dissolve the powder in methanol and after most of the powder is dissolved, filter out the impurities using a filter paper while transferring the solution into another identical volumetric flask. These impurities were the coating of the tablet and after removing them we obtain a stock solution of acetylsalicylic acid in methanol of concentration 5 mg/ml. Immediately seal the flask.
3. Add 19 ml of the pH 7 buffer solution into a test tube using a graduated pipette (±0.01 ml) and place it into the water bath in a test tube stand. Minimal volume of the buffer solution is taken so that the concentration of the salicylic acid produced is high enough to be detected in the spectrophotometer.
4. Using a graduated pipette (±0.01 ml), transfer 1 ml of Aspirin stock solution into the test tube in the water bath, place a stopper on the test tube and start the stopwatch.
5. After 10 minutes, remove the stopper on the test tube and transfer 1 ml of the reaction mixture into a UV cuvette using a graduated pipette (±0.01 ml). Set the wavelength of the spectrophotometer radiation at 300 nm and place the cuvette in it and take the reading of absorbance.
6. Transfer the 1 ml reaction mixture back to the test tube and place the stopper again. Do not remove the test tube at any point to ensure that the temperature remains stable.
7. Repeat step 5 and 6 after 20, 30, 40, 50 and 60 minutes to obtain the increase in absorbance with time.
8. Repeat the steps 3-8 for 4 repetitions to minimize the impact of random errors.
9. Repeat the steps 3-9 by setting the water bath at the following temperatures: 323 K, 338 K and 353 K (±0.1 K)

4.3 Risk Assessment

Safety Precautions: Salicylic acid has several health hazards[9]. If spilled on human body, it can cause chemical burns and inhaling its fumes can cause respiratory problems. Although it is used in low concentrations in this experiment, precautions were taken to be safe because other chemicals like HCl were also used in the experiment which are highly corrosive. Thus, a mask, a lab coat, latex gloves, and goggles, were worn throughout the duration of the experiment.
Environmental concerns- The chemical wastes produced after the experiment could harm the environment if carelessly dumped or disposed in the sink. Thus, all the chemical wastes were stored in plastic bottles to be handed over to ETP (Effluent Treatment Plant) for further disposal.

5. Data Collection

5.1 Qualitative Data:

1. The colour of the reaction mixture remained constant while the reaction occurred, a very faded white colour.
2. While transferring the 1 ml solution into the cuvette and back from the cuvette to the reaction mixture, a very small volume of solution was left behind in the dropper. Since we are dealing with only 20 ml total volume, a small volume loss can make a minor difference in the concentration of the reactant and the product.

5.2 Raw Data

Table 2: Variation of absorbance of radiation (a_1, a_2, a_3, a_4, a_5) with time (min) when the experiment was conducted at different temperatures (K)

Time (min) ± 0.5 min		**10**	**20**	**30**	**40**	**50**	**60**
Temp= 308 K (±0.1K)	a_1	0.351	0.381	0.433	0.480	0.571	0.661
	a_2	0.362	0.395	0.454	0.517	0.609	0.693
	a_3	0.318	0.362	0.419	0.443	0.552	0.649
	a_4	0.343	0.389	0.403	0.464	0.561	0.651
	a_5	0.328	0.352	0.423	0.451	0.544	0.635
Temp= 323 K (±0.1K)	a_1	0.372	0.442	0.496	0.591	0.719	0.863
	a_2	0.393	0.472	0.521	0.598	0.704	0.841
	a_3	0.360	0.419	0.473	0.562	0.673	0.823
	a_4	0.382	0.459	0.486	0.591	0.723	0.838
	a_5	0.388	0.443	0.464	0.583	0.705	0.819
Temp= 338 K (±0.1K)	a_1	0.470	0.523	0.631	0.743	0.883	0.931
	a_2	0.431	0.501	0.629	0.721	0.862	0.923
	a_3	0.423	0.485	0.604	0.738	0.836	0.908
	a_4	0.454	0.499	0.614	0.733	0.841	0.911
	a_5	0.407	0.458	0.583	0.709	0.833	0.884
Temp= 353 K (±0.1K)	a_1	0.489	0.543	0.687	0.825	0.932	0.962
	a_2	0.499	0.561	0.708	0.819	0.921	0.973
	a_3	0.496	0.584	0.693	0.839	0.918	0.988
	a_4	0.475	0.591	0.713	0.852	0.923	0.994
	a_5	0.471	0.572	0.694	0.844	0.942	0.985

Table 1: Data for calibration curve of Salicylic acid concentration vs Absorbance of radiation

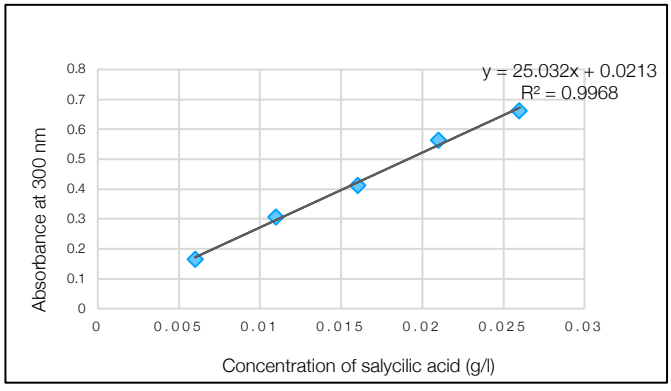
	Concentration of Salicylic Acid (g/l)	Absorbance at 300 nm
1.	0.006	0.165
2.	0.011	0.305
3.	0.016	0.414
4.	0.021	0.562
5.	0.026	0.662

Table 1 shows the variation of absorbance of radiation of wavelength 300 nm with the increasing concentration of salicylic acid (g/l) which will be used to form the calibration curve for absorbance vs concentration. **Table 2** shows the gradual increase in absorbance of the radiation with time as aspirin hydrolyses and forms salicylic acid. The table shows 5 repetitions of the experiment used to obtain different absorbance values (a_1, a_2, a_3, a_4, a_5) at different temperatures; 308 K, 323 K, 338 K, and 353 K.

5.3 Processed Data

The data from **Table 1** was used to plot a graph of concentration of salicylic acid vs absorbance of radiation. **Graph 1** depicts the linear relationship between the two quantities and the equation of the line of best fit is used to establish the mathematical relationship.

Graph 1: Calibration curve for absorbance vs concentration of salicylic acid



Line of best fit: y = 25.032x+ 0.0213

The y-axis represents the absorbance (a) and the x-axis represents the concentration of salicylic acid ([S]).
Thus,

a= 25.032[S] + 0.021

Table 3: A table showing the variation of average absorbance of radiation (a_{avg}) with time (min), the uncertainty in the average absorbance (Δ a) and the standard deviation (σ) from the different trials when the experiment was conducted at different temperatures (K)

Temp	**308 K (±0.1 K)**			**323 K (±0.1 K)**			**338 K (±0.1 K)**			**353 K (±0.1 K)**		
Time (min) ±0.5	a_{avg}	Δ a	σ	a_{avg}	Δ a	σ	a_{avg}	Δ a	σ	a_{avg}	Δ a	σ
10	0.340	0.022	0.017	0.379	0.016	0.013	0.437	0.032	0.025	0.486	0.014	0.012
20	0.376	0.021	0.018	0.447	0.026	0.020	0.493	0.032	0.024	0.570	0.024	0.019
30	0.426	0.025	0.019	0.488	0.028	0.022	0.612	0.024	0.020	0.699	0.013	0.010
40	0.471	0.037	0.029	0.585	0.018	0.014	0.729	0.017	0.014	0.835	0.016	0.013
50	0.567	0.032	0.025	0.705	0.025	0.020	0.851	0.025	0.021	0.927	0.012	0.009
60	0.658	0.029	0.021	0.837	0.022	0.017	0.911	0.024	0.018	0.980	0.016	0.012

The data from **Table 2** was used to determine the average absorbance for each time interval (a_{avg}) by finding the mean of the values, uncertainty of the average absorbance (Δ a) by dividing the range of the data by 2, and the standard deviation of the values (σ) by using the formula for standard deviation. A sample calculation for the data in **Table 3** is shown in the box on the right.

Sample calculation for **Table 3**:

At temperature = 308K and time = 20 mins,

$$a_{avg} = \frac{a_1 + a_2 + a_3 + a_4 + a_5}{5}$$

$$= \frac{0.381 + 0.395 + 0.362 + 0.389 + 0.352}{5} \approx 0.376$$

$$\Delta a = \frac{a_{max} - a_{min}}{2} = \frac{0.395 - 0.352}{2} \approx 0.021$$

$$\sigma = \sqrt{\frac{\sum_5^{i=1}(a_i - a_{avg})^2}{5}} \approx 0.018$$

Using the formula, a = 25.032[S] + 0.0213, we can determine the concentration of salicylic acid produced (g/l) at different time intervals by using the values of absorbance (a_{avg}) from **Table 3**. We can then find the number of moles of salicylic acid produced by using the formula, *moles= n =(concentration×volume)/(molar mass)*, and finally find the concentration of acetylsalicylic acid remaining by using the formula; *concentration = $[A_t]$ = (initial concentration) - (moles)/(volume)*, since we know the initial concentration of acetylsalicylic acid which is the same for each sample. **Table 4** shows the calculated values for the remaining concentrations of acetylsalicylic acid ($[A_t]$) for each corresponding time interval (t) and the natural log for each value (ln $[A_t]$). A sample calculation for **Table 4** is also shown on the next page.

The values of ln $[A_t]$ have been plotted in **Graph 2** against the values of (t) for each of the four temperature values. The line of best fit shows the linear relationship predicted by the equation $\ln [A_t] = -kt + \ln [A_0]$.

Table 4: A table showing the remaining concentration of acetylsalicylic acid **$[A_t]$** at different time intervals for each temperature and the natural log for every concentration value **ln $[A_t]$**

Temp	**308 K (±0.1 K)**		**323 K (±0.1 K)**		**338 K (±0.1 K)**		**353 K (±0.1 K)**	
Time (s) ± 30 s	**$[A_t]$ (mol dm^{-3})**	**ln $[A_t]$**	**$[A_t]$ (mol dm^{-3})**	**ln $[A_t]$**	**$[A_t]$ (mol dm^{-3})**	**ln $[A_t]$**	**$[A_t]$ (mol dm^{-3})**	**ln $[A_t]$**
600	0.00131	-6.64	0.00130	-6.65	0.00128	-6.66	0.00126	-6.67
1200	0.00130	-6.65	0.00128	-6.66	0.00126	-6.67	0.00124	-6.69
1800	0.00128	-6.66	0.00126	-6.67	0.00123	-6.70	0.00120	-6.72
2400	0.00127	-6.67	0.00124	-6.69	0.00120	-6.73	0.00116	-6.76
3000	0.00124	-6.69	0.00120	-6.72	0.00116	-6.76	0.00114	-6.78
3600	0.00122	-6.71	0.00116	-6.75	0.00114	-6.77	0.00112	-6.79

Sample calculation for **Table 4**:

At temperature = 308K and time = 1200 s; $a_{avg} = 0.376$

We know, $a = 25.032[S] + 0.021$

Concentration of salicylic acid produced ([S]) $= \frac{(0.376 - 0.021)}{25.032} \approx 0.0142$ g/l

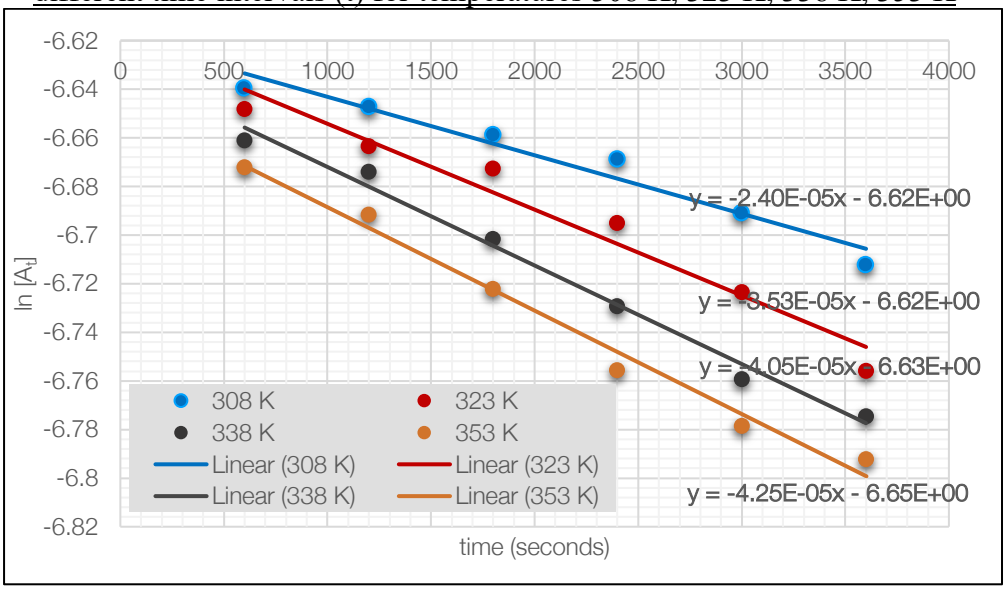
Moles of salicylic acid produced (n) $= \frac{(concentration \times volume)}{(molar\ mass)} = \frac{(0.0142 \times 0.020)}{(138.121)} \approx 2.06 \times 10^{-6}$ mol

Remaining concentration of acetylsalicylic acid ($[A_t]$) $= initial\ concentration - \frac{(moles)}{volume}$

$= 1.4 \times 10^{-3} - \frac{2.06 \times 10^{-6}}{0.020} \approx 0.00130$ mol dm^{-3}

ln($[A_t]$) = ln (0.00130) ≈ - 6.65

Graph 2: A graph of natural log of remaining concentration of acetylsalicylic acid (ln $[A_t]$) at different time intervals (t) for temperatures 308 K, 323 K, 338 K, 353 K



Calculating the rate constant (k): According to the rate equation, $\ln [A_t] = -kt + \ln [A_0]$. Therefore, the gradients of the line of best fits in **Graph 2** where x-axis represents (t) and the y-axis represents (ln $[A_t]$) should give us (-k) or the negative rate constant. For each temperature, different values of the rate constant can be determined and are given below in **Table 5.**

Calculating the uncertainty in the rate constant: Due to the random and systematic errors in the experiment i.e. uncertainty in the absorbance and time readings, the rate constant calculated for each temperature will have some uncertainity. The uncertainity for each rate constant is given in **Table 5** and a sample calculation is also shown in the box below.

Arrhenius equation: According to the equation, $\ln k = \ln A - \frac{E_A}{RT}$, we need to plot (ln k) against (1/T) on a graph. So, **Table 5** shows these values along with their uncertainities. Sample calculation is shown in the box.

Table 5: A table showing the values of calculated rate constant (k) at different temperatures, natural log of these values (ln k) along with corresponding temperatures (T), (1/T) values and the uncertainity of all values (Δk), (Δ ln k) and (Δ 1/T)

T (K)	1/T (K^{-1})	Δ 1/T (K^{-1})	k (s^{-1})	Δk(s^{-1})	ln k	Δ ln k
308	3.25×10^{-3}	1.06×10^{-6}	2.40×10^{-5}	8.87×10^{-7}	-10.64	0.037
323	3.09×10^{-3}	9.57×10^{-7}	3.53×10^{-5}	1.21×10^{-6}	-10.25	0.034
338	2.96×10^{-3}	8.76×10^{-7}	4.05×10^{-5}	1.38×10^{-6}	-10.11	0.034
353	2.83×10^{-3}	8.01×10^{-7}	4.25×10^{-5}	1.33×10^{-6}	-10.07	0.031

Sample calculation for uncertainities:

For T = 308 K, t = 600 seconds,

Relative uncertainity in the calculated moles of acetylsalicylic acid:

$$\frac{\Delta n}{n} = \frac{\Delta[S]}{[S]} + \frac{\Delta Volume}{Volume} = \frac{\Delta a}{a_{avg}} + \frac{\Delta Volume}{Volume} = \frac{0.022}{0.340} + \frac{0.0005}{0.020} = 0.09$$

Relative uncertainity in the calculated concentration of acetylsalicylic acid:

$$(\Delta \ln[A_t]) = \frac{\Delta[A_t]}{[A_t]} = \frac{\Delta n}{n} + \frac{\Delta Volume}{Volume} = 0.09 + \frac{0.0005}{0.020} = 0.115$$

Uncertainty in the rate constant for each time interval

$$\frac{\Delta k}{k} = \frac{\Delta \ln[A_t]}{\ln[A_t]} + \frac{\Delta t}{t} = \frac{0.115}{6.64} + \frac{30}{600} = 0.067$$

By finding the average of uncertainties in rate constant for all time intervals, we can find the uncertainty for in the rate constant for a specific temperature (308 K in this case)

$$\Delta \ln k = \frac{\Delta k}{k} = \frac{0.067 + 0.041 + 0.033 + 0.032 + 0.026 + 0.022}{6} = 0.037$$

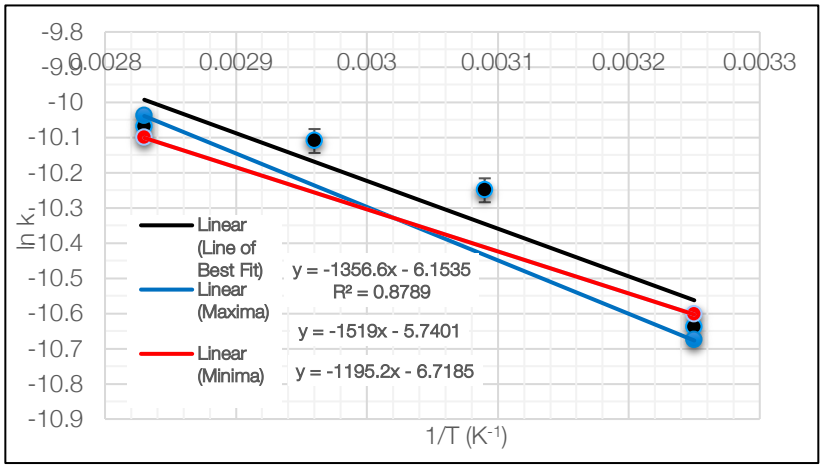
$\Delta k = k \times 0.037 = 2.40 \times 10^{-5} \times 0.037 \approx 8.87 \times 10^{-7}\ s^{-1}$

Uncertainty in 1/T,

$$\frac{\Delta 1/T}{1/T} = \frac{\Delta T}{T} = \frac{0.1}{308} = 3.25 \times 10^{-4}$$

$\Delta 1/T = 3.25 \times 10^{-4} \times 3.25 \times 10^{-3} = 1.06 \times 10^{-6}\ K^{-1}$

Graph 3: A graph of 1/T vs ln k showing the data points, line of best fit, maxima and minima



According to the equation $\ln k = \ln A - \frac{E_A}{RT}$, the gradient of the graph of 1/T vs ln k gives us $(-\frac{E_A}{R})$ and the y-intercept gives us ln A where A is the frequency factor or the pre-exponential factor.

Activation Energy (E_A): The line of best fit, the maxima and the minima are all straight lines, and their gradients are -1356.6, -1519, -1195.2 J mol^{-1} respectively. Multiplying the gradient of the line of best fit by -R, we get the activation energy. Thus,

$$E_A = -1356.6 \times -8.31 = 11273.35\ J\ mol^{-1} = 11.27\ kJ\ mol^{-1}$$

The uncertainty in the activation energy can be calculated by considering the gradients of the maxima and minima lines such that,

$$\text{Uncertainty} = \frac{E_{A(max)} - E_{A(min)}}{2} = \frac{(-1519 \times -8.31) - (-1195.2 \times -8.31)}{2} = 1345.4\ J\ mol^{-1} = 1.34\ kJ\ mol^{-1}$$

Therefore, $E_A = 11.27\ kJ\ mol^{-1} \pm 1.34\ kJ\ mol^{-1}$

Frequency Factor (A): Similarly, y-intercepts of the LOBF, maxima and minima are -6.15, -5.74, -6.72

$$A = e^{-6.15} = 2.13 \times 10^{-3}\ s^{-1}$$

$$\text{Uncertainty} = \frac{A_{(max)} - A_{(min)}}{2} = \frac{e^{-5.74} - e^{-6.72}}{2} = 1.00 \times 10^{-3}\ s^{-1}$$

Therefore, $A = 2.13 \times 10^{-3}\ s^{-1} \pm 1.00 \times 10^{-3}\ s^{-1}$

6. Conclusion and Evaluation

6.1 Conclusion

The activation energy (E_A) of the hydrolysis of $C_9H_8O_4$ (aspirin or acetylsalicylic acid) of 1.4×10^{-3} M concentration at pH 7 ± 0.2 was successfully determined in the course of this investigation to be 11.27 kJ mol^{-1} ± 1.34 kJ mol^{-1} and the frequency factor of the reaction was determined to be 2.13×10^{-3} s^{-1}± 1.00×10^{-3} s^{-1}. While the percentage error in the calculated activation energy is about 11.89 %, the error in the calculated frequency factor is about 46.95% which is extremely high. This is because ln k (natural log of rate constant) is only linear over a narrow range of temperature[14] but this investigation deals with a broader range of temperature. While the graph obtained in this investigation produces a linear line of best fit for ln k over the range of temperature, the low R^2 value (0.879) proves this point as R^2 is the correlation coefficient and a lower value depicts a weak correlation between the dependant (ln k) and independent variable (1/T) due to the broad range of temperature.

Despite the uncertainties in the calculated values, I still have high confidence in the results of this investigation because of their precision (which is evident by the low standard deviation amongst the replicates of absorbance). Moreover, the results are in agreement with the current scientific consensus, such as the increase in the concentration of product with time, which is substantiated by the observed increase in absorbance of radiation, and also the increase in the rate constant as temperature increases, which is substantiated by the increase in the calculated rate constant with temperature. Thus, the results of this investigation provide us crucial information about the hydrolysis of aspirin at pH 7 ± 0.2 which help us understand the reaction as it occurs in the small intestine of the human body. At this pH, the reaction is fairly slow at low temperatures which was observed in the experiment as the successive absorbance readings of the reaction mixture did not show a drastic increase at lower temperatures. However, at elevated temperatures, the rate of hydrolysis increases, and the reaction can produce more salicylic acid than the amount required by the body.

6.2 Strengths

The equipment and apparatus (measuring cylinders, weighing scale, thermometer, spectrophotometer) used in the experiment had very low uncertainty which increases the certainty of the conclusion drawn above. Moreover, for every temperature, 5 repetitions were taken for the hydrolysis experiment, which minimized the random error and yielded a very low standard deviation (σ) in the absorbance of radiation by the reaction mixture. This proves the fact that the results were immensely precise. In addition to that, the high R^2 value (0.997) indicated in **Graph 1** and the y-intercept (0.021) lying close to the expected value (0) shows the accuracy of the calibration curve that is used to establish the mathematical relationship between absorbance and concentration and used to calculate the increasing concentrations of salicylic acid which further proves the accuracy of the processed data which is why it is in agreement with the established scientific theories. The choice of equipment is also a strength of this investigation. For example, the use of a water bath ensured that the thermal energy was uniformly distributed in the solution which allowed the independent variable, temperature, to be effectively controlled and the use of a magnetic stirrer ensured that the chemicals were uniformly dissolved, and the concentrations were accurately measured.

6.3 Weaknesses

However, there are a number of weaknesses in this experiment that must be addressed.
The scope of data for the independent variable, temperature, is limited since the experiment was conducted at only 308K, 323K, 338K and 353K. Therefore, only four data points were obtained on the Arrhenius graph (**Graph 3**) due to which the impact of random error on the investigation was increased, as depicted by the relatively high uncertainty in the calculated activation energy (11.89%). This also affected the gradient value calculated by Microsoft Excel. Since there were only 4 coordinates on the graph, it is possible that the gradient is not an accurate representation of the true gradient, thus impeding the reliability of the calculated value of E_A.

The broad range of temperature was another weakness as highlighted in the conclusion; however, the temperature increase of 15 K in each subsequent experiment was necessary to observe a substantial increase in the rate constant as a preliminary experiment proved that varying the temperature by 5 K or 10 K had minimal impact on the absorbance readings. Ultimately, the investigation is limited by the use of only 4 data points for the Arrhenius graph, and the broad range of temperature affects the accuracy of the results. Both these factors reduce the certainty of the conclusion drawn. These weaknesses can be resolved by conducting the experiment at even higher temperatures like 368 K and 383 K but not at lower temperatures, since the reaction would be extremely slow at temperatures closer to or below room temperature.

The volumetric flask in which the 100 ml aspirin solution was stored was not free of moisture. Therefore, it is possible that even before the solution was transferred into the test tubes, a small amount of acetylsalicylic acid ($C_9H_8O_4$) had hydrolysed and the concentration of $C_9H_8O_4$ was lesser than 1.4×10^{-3} M before the experiment started. This change was not accounted for in the calculations, thereby reducing the reliability of the calculated rate constant and ultimately the value of E_A. This could have been avoided by preparing the aspirin solution right before each trial of the experiment which would have been time-consuming but would have provided more accurate results.

The time intervals noted for the absorbance readings show the time when the sample was removed from the mixture, not when it was placed in the spectrophotometer to note down the absorbance of radiation. As it took about 30 seconds to transfer the sample into the spectrophotometer, the uncertainty of ±0.5 minutes was assumed in the calculations. However, this estimate was not an accurate representation of the uncertainty in the time. This impedes the reliability of the rate constants *k* calculated for different temperatures which likely do not reflect actual values. While it is possible to note down the exact time when the absorbance readings were taken, it would lead to different time intervals for each iteration each instead of the constant 10 min intervals and average time would have to be calculated which would ultimately increase the random error in the calculation of rate constants.

Furthermore, when the 1 ml sample was removed from the test tube in the water bath to be placed into the spectrophotometer, the stopwatch wasn't stopped, and the sample was placed back into the water bath after checking the absorbance. During the few seconds of this transfer, the 1 ml sample and the remaining mixture hydrolyse at different rates since the sample's temperature gradually decreases when it is removed from the mixture as it tries to attain equilibrium with the room temperature and then gradually increases when placed back into the test tube in the water bath. This means that not only is the temperature used in the calculation of rate constant not an accurate representation of the solution's true temperature (as the temperature decreases during transfer to the spectrophotometer but it was assumed to be the same as that of the water bath), but also the calculated rate of reaction *k* is in fact an approximation of the actual rate of reaction which is affected by the constant removal and addition of the 1 ml sample. The former of these problems can be solved by checking the temperature of the sample right before measuring the absorbance of radiation and then taking the average value of this temperature from each trial as the actual temperature of the sample.

Another weakness lies in the usage of the spectrophotometric method to calculate the concentration of salicylic acid produced. During the experiment, small amounts of stray light can leak into the spectrophotometer which can affect the readings of absorbance produced by the instrument[15]. Thus, these readings might not be an accurate representation of the actual absorbance by the solution and these inaccurate readings were used to calculate the concentration of salicylic acid produced at different time intervals, and subsequently the rates of reaction and activation energy (E_A). A better spectrophotometer that minimizes the amount of stray light/radiation that can leak into it could have been used for the experiment but due to limited resources, we had to settle for a less efficient instrument.

6.4 Extensions-

The experiment can be carried out at different pH to understand the correlation between the pH and the rate of reaction and subsequently the activation energy. An acidic pH and a basic pH would have different effects on the rate. Moreover, the experiment can be conducted for different initial concentrations of aspirin to prove that the initial concentration has a minimal effect on the calculated activation energy. The reaction can be studied under room temperature, to gain a better understanding of the stability of aspirin at room temperature and understand why aspirin tablets cannot be stored in moist environments. However, observing the reaction at room temperature would require a long duration of time intervals since the reaction would be very slow. Different catalysts like sodium carbonate can also be used in the aspirin hydrolysis reaction and their effect on the Activation energy can be evaluated.

Bibliography

[1] Aspirin Foundation – Chemistry. Retrieved from "https://www.aspirin-foundation.com/history/chemistry/" date of access 21/01/2021, 11:15 pm

[2] H.T. Sørensen, L. Mellemkjaer & W.J. Blot, *Risk of upper gastrointestinal bleeding associated with use of low-dose aspirin*, Am. J. Gastroenterol, 2000

[3] Berges-Gimeno, M. P., & Stevenson, D. D. *Nonsteroidal Anti-inflammatory Drug-Induced Reactions and Desensitization*. Journal of Asthma, 41(4), 2004

[4] E. Barry & L.L. Borer, *Experiments with aspirin*, J. Chem. Educ, p. 354, 2000

[5] D. W. Ball. *Field Guide to Spectroscopy*. SPIE Press, Bellingham, WA, 2006

[6] Fallingborg J. *Intraluminal pH of the human gastrointestinal tract.* Dan Med Bull, 1999 Jun

[7] Iwunze, M. O. *Absorptiometric Determination of Acetylsalicylic Acid in Aqueous Ethanolic Solution*. Analytical Letters, 41(16), 2944–2953, 2008

[8] John, D., 2013. The Arrhenius law: Arrhenius Plots. Chemwiki. Chemwiki.ucdavis.edu. Retrieved from http://chemwiki.ucdavis.edu/Core/Physical_Chemistry/Kinetics/Modeling_Reaction_Kinetics/TemperatureDependence_of_Reaction_Rates/The_Arrhenius_Law/The_Arrhenius_Law%3A_Arrhenius_Plots, date of access 19/12/2020, 9:10 p.m.

[9] MSD Online, Salicylic acid safety tips, retrieved from https://www.msdsonline.com/2015/07/08/salicylic-acid-safety-tips-hazards/", date of access: 17/12/2020, 10:04 pm

[10] U.S. Pharmacopeia USP 42, NF37 vol 4. Revision: 1 / 2019

[11] Delloyd's Lab Tech resources reagent and solution: Preparation of pH buffer solutions

[12] Lapage S., Shelton J. & Mitchell, T. *Methods in Microbiology*. Norris J. and Ribbons D., (Eds.), Vol. 3A, Academic Press, London, 1970

[13] MacFaddin, J. F. *Biochemical Tests for Identification of Medical Bacteria*. 3rd Ed., Lippincott, Williams and Wilkins, Baltimore, 2000

[14] Arrhenius Equation. (2020, September 11). Retrieved from https://chem.libretexts.org/@go/page/1443, date of access 13/11/2020, 4:00 pm

[15] Reule A. G. Errors in Spectrophotometry and Calibration Procedures to Avoid Them. Journal of research of the National Bureau of Standards. Section A, Physics and chemistry, 80A(4), 609–624. https://doi.org/10.6028/jres.080A.060, 1976