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The Effect of pH on the Hydrolysis of Acetylsalicylic Acid (C9H8O4) to Salicylic Acid (C7H6O3) and Acetic Acid (C2H4O2) at 333.15 K (60 °C): A Spectrophotometric Analysis †

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Abstract: This study investigates the effect of pH on the hydrolysis of acetylsalicylic acid (C9H8O4) to salicylic acid (C7H6O3) and acetic acid (C2H4O2) at a temperature of 333.15 K (60 °C). The hydrolysis reaction was analyzed by measuring the concentration of salicylic acid using spectrophotometry at different pH levels (2.0, 4.0, 6.0, 8.0, and 10.0). The findings indicate that as the pH increases, the concentration of salicylic acid also increases, suggesting that acetylsalicylic acid is less stable in more basic conditions. This supports the hypothesis that the hydrolysis rate of acetylsalicylic acid is pH-dependent, with higher pH levels accelerating the reaction. The study's results are consistent with previous research and provide a deeper understanding of the chemical behavior of aspirin in varying pH environments. These findings have significant health implications, as they support the understanding that aspirin is more stable in the acidic environment of the stomach and is primarily hydrolyzed in the more basic environment of the small intestine. This knowledge is crucial for optimizing the therapeutic efficacy of aspirin and minimizing potential gastrointestinal side effects by considering the pH-dependent stability and absorption of the drug in the body.

Keywords: acetylsalicylic acid; hydrolysis; pH dependence; spectrophotometry; aspirin stability

1. Introduction

Various nonsteroidal anti-inflammatory (NSAID) drugs, particularly enzyme inhibitors, play a significant role in medicine. The common drug aspirin stands out due to its unique mechanism as an irreversible enzyme inhibitor. Aspirin irreversibly inactivates the cyclooxygenase (COX) enzyme, providing relief from inflammation and pain. It is generally used for headaches, fevers, colds, toothaches, and muscle pains. The chemical name for aspirin is acetylsalicylic acid (ASA), with the chemical formula C9H8O4. Aspirin tablets primarily contain ASA as the active ingredient, along with several inactive ingredients [1] for preservation, coating, or flavoring, such as corn starch, croscarmellose sodium, hypromellose, microcrystalline cellulose, mineral oil, and titanium dioxide [2].

Aspirin functions as an acetylating agent by attaching an acetyl group to a serine residue in the active site of the COX enzyme [3]. One of the products formed after the hydrolysis of ASA is salicylic acid (C7H6O3). Considering that aspirin tablets initially reach the acidic environment of the stomach, the pH dependence of the aspirin or ASA hydrolysis reaction becomes a topic of interest. Previous studies indicate that aspirin remains unchanged in the highly acidic stomach and only undergoes hydrolysis upon reaching the somewhat basic small intestine. The pH of an environment is crucial to ester hydrolysis, and aspirin is an ester. Therefore, the research question arises: "How does pH affect acetylsalicylic acid (C9H8O4) hydrolysis to salicylic acid (C7H6O3) and acetic acid (C2H4O2) at temperature 333.15 K (60 °C) by measuring the concentration of salicylic acid through spectrophotometry?"

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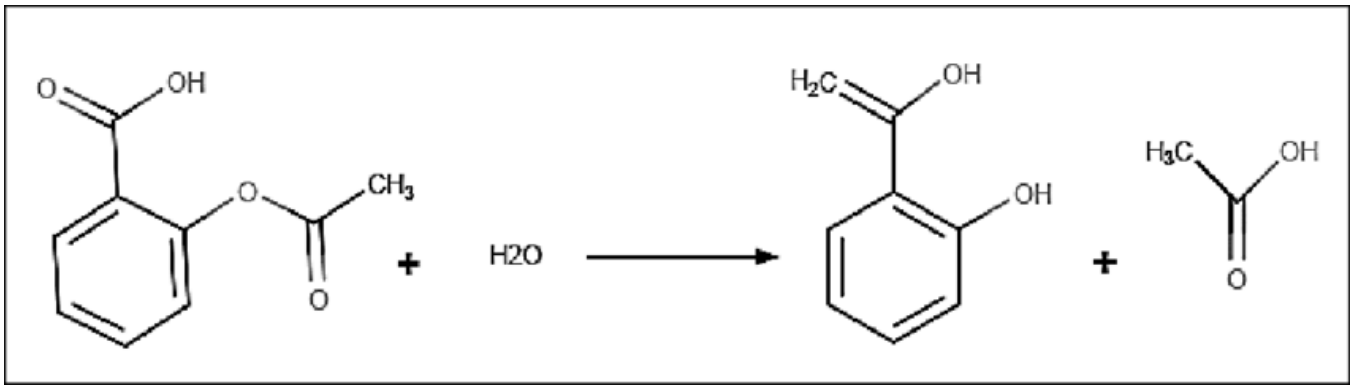
2. Main Text

2.1. Background Information

Acetylsalicylic acid (or 2-ethanoyloxybenzoic acid), or ASA, when reacted with water, produces salicylic acid (or 2-hydroxybenzoic acid) and acetic acid (or ethanoic acid) [4]. As seen from the diagrams, ASA has an

aromatic ring with two functional groups: a carboxylic acid and an ester group. It is therefore an ester.

$$C_8H_7O_2COOH + H_2O = C_7H_6O_3 + CH_3COOH$$



During hydrolysis, the water molecule acts as a nucleophile and attacks aspirin's ester group, forming a tetrahedral intermediate. The intermediate collapses, releasing the alcohol group (-OH) as a leaving group, resulting in the formation of acetic acid. The remaining portion of the aspirin molecule after acetic acid is formed is now salicylic acid.

pH can affect ASA hydrolysis because in basic conditions, hydroxide ions (OH-) act as a nucleophile, attacking the carbonyl carbon of the ester group like water does, promoting the hydrolysis reaction. However, at more acidic conditions, ASA is present in a protonated form, which increases its susceptibility to nucleophilic attacks. Moreover, one of the intermediate reactions in the hydrolysis is an acid-base reaction, so it will be interesting to see how pH affects the hydrolysis reaction in detail.

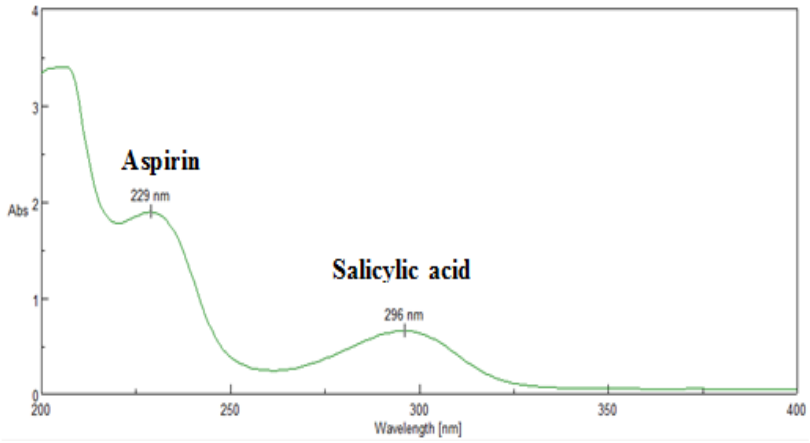
This will be done by measuring the concentration of salicylic acid at different reaction pH values. The presence of salicylic acid cannot be detected by the human eye as it does not involve a significant color change. It was found that Iron(III) chloride is often used in aspirin purity tests: it turns into a dark purple complex in the presence of any molecule with a phenol group [5]. Therefore, it does not turn purple in a solution of "pure" aspirin (that is, without any hydrolysis). It turns purple in the presence of salicylic acid (which does have a phenol group), so adding this molecule can help determine the concentration of salicylic acid.

The human eye cannot detect differences between how concentrated a color is, unless at two extremes (very dark purple versus very light purple). Therefore, it is necessary to use a spectrophotometer to resolve any minute concentration differences. Beer's law, [6] noted below, is used in spectrophotometry. Since concentration is directly proportional to absorbance, the spectrophotometer can effectively measure the concentration of salicylic acid.

$$A = \varepsilon c l$$

A	Absorbance	
ε	Molar absorption coefficient	$M^{-1}cm^{-1}$
c	Molar concentration	M
l	optical path length	cm

After looking at an absorbance versus wavelength graph, [7] maximum absorbance occurs at around 300 nm (exactly 296 nm) for salicylic acid. Therefore, a wavelength of 300 nm will be used for the spectrophotometer.



The spectrophotometer will output absorbance values, which can be converted to concentration values by using a calibration curve (a graph of absorbance versus concentration). A previous research study was found, wherein the researchers created a calibration curve for salicylic acid at 300 nm. Since molar absorption coefficient stays constant between spectrophotometers (it is a constant value for each chemical species), and the study used the same width of cuvette (therefore, the same optical path length), a new calibration curve for salicylic acid is not necessary. This previously done curve can also be used in our study.

2.2. Hypothesis

Previously, it was mentioned that when consumed, aspirin tablets pass through the stomach's acidic environment unchanged, and only hydrolyze into salicylic acid when reaching the somewhat alkaline environment of the small intestine. Therefore, it is hypothesized that as pH increases, the salicylic acid concentration will also increase. In other words, aspirin is less stable at higher pH values.

2.3. Variables

Independent Variable: pH of the aspirin hydrolysis solution. This variable will be varied through the usage of different buffer solutions of pH 2.0, 4.0, 6.0, 8.0, and 10.0.
Dependent Variable: Concentration of salicylic acid from aspirin hydrolysis reaction. This variable will vary as different pH buffers are used. The concentration of salicylic acid is directly proportional to the absorbance (Beer's law), and absorbance will be directly measured from the spectrophotometer. The calibration curve will be used in order to find the corresponding concentration of salicylic acid.

2.4. Controlled Variables

Controlled Variable	Reason to Control the Variable	How to Control the Variable
Temperature of Reaction	Temperature affects the rate of hydrolysis to a very large extent- in fact, it will be kept constant at 333.15 K or 60 °C because lab manuals show that this temperature is ideal for the reaction (it is fastest at this temperature condition) [8].	Heating plates will be used that can maintain and bring a solution to this temperature.
Amount and concentration of Iron (III) chloride used	Iron (III) chloride reacts with salicylic acid to produce a dark purple compound. As the concentration and volume of Iron (III) chloride used increases, the amount of purple compound also increases, meaning that the absorbance results from the spectrophotometer will also be skewed.	0.5 mL of 1 M Iron (III) chloride will be pipetted into all cuvettes, for all pH values. It will not change for different pH values.
Time of Reaction (10 min)	The more time the ASA solution and buffer are left to react, the more salicylic acid is produced. To ensure that this was not a factor for any differences in salicylic acid concentration, time will be kept constant for all trials, at all pH values.	The buffer and ASA solution will be left to react for 10 min (for all pH values), and no trial will be left for a longer or shorter time than that.
Amount of buffer solution used	pH is a factor which can affect the rate of ASA hydrolysis, so the volume of buffer solution used must be kept constant between trials.	15 mL of the buffer solution will be used for every trial, to ensure that only the change in the pH of the buffer would affect the concentration of salicylic acid.
Amount of ASA used	If more ASA (which is a reactant) is present, then the concentration of salicylic acid produced will also be higher. Keeping the reactant concentration constant will ensure that the final results are not affected by any factor other than pH.	0.1 g of ASA will be used for every pH and every trial. It will be measured out using a scale and the amount will remain constant between trials.
Cuvette Width and Volume	Beer's law involves the optical path length variable, which is essentially the width of the cuvette used. Changing the cuvette width/ volume/ brand would render the calibration curve ineffective, and the entire method would need to be changed.	The same type and brand of cuvette will be used for every single trial.
Amount of Water Used to React with ASA	Water is a reactant in the ASA hydrolysis. As the amount of water used increases, the number of moles	15 mL of water will be used for all trials, regardless of the pH value varied.

	of water would also increase, affecting the reaction kinetics and salicylic acid concentration.	
Room Conditions- Temperature, pressure, and humidity	The room temperature may change the temperature of the reactant solutions, affecting the rate of the reaction (temperature must be kept constant for all trials), so it must be kept constant, or as close to constant as possible. It is the same with pressure and humidity, which can change water concentration in a reaction and number of reactant collisions.	The experiment will be done in a room with all windows closed to prevent any pressure changes, and all trials will be done in succession to prevent any temperature and humidity changes from affecting the experiment.

3. Materials

- 50 325 mg Aspirin Tablets
- Mortar and Pestle
- Glass rod for stirring
- 150 mL Acetone
- Spectrophotometer Cuvettes of 10 mm path size, 5x
- Spectrophotometer (at 300 nm wavelength setting)
- Electric Heating Plate, 2x
- pH Buffer Solutions at 2.0, 4.0, 6.0, 8.0, and 10.0 pH
- 1 M Iron (III) chloride, 50 mL
- Distilled water
- Cuvette wipes/ tissues
- Safety wear: Gloves and Goggles
- pH strips

Materials	**Quantity Measured**	**Absolute Uncertainty**	**Percentage Uncertainty**
15 mL Volumetric Flasks, 5×	15 mL	±0.02 mL	0.001%
Spectrophotometer absorbance		±0.001	
150 mL beaker	100 mL	±5 mL	0.05%
Scale	0.1g	±0.0005	0.005%
10 mL pipette, 2×	0.5 mL	±0.05 mL	0.01%

4. Procedure

4.1. Preliminary Extraction of ASA from Aspirin Tablets

Since commercially sold aspirin tablets contain extraneous materials (listed in the Introduction) other than ASA, ASA will firstly be extracted from the tablets.

1. 50,325-milligram aspirin tablets were taken and crushed to a fine powder with a mortar and pestle.
2. Previous lab manuals [9] dictate that 1 Liter of acetone, ethanol, or methanol be used for 500,325 mg tablets. Therefore, 100 mL of acetone was poured into a beaker (first by measuring it out through a 150 mL beaker), and the crushed tablets were slowly added to the acetone.
3. A glass rod was used to stir the aspirin and acetone solution for a few minutes.

4. The beaker was left in the fume hood overnight to let the acetone evaporate and allow pure ASA to be extracted.
5. The ASA, which formed crystalline structures on the beaker, was scraped and removed. It was crushed with a mortar and pestle again to reduce it to a powder.

4.2. ASA Hydrolysis Procedure

1. 15 mL of water was poured out in a volumetric flask, and then transferred to a 150 mL beaker. It was continually heated for 20 min at a temperature of 60 °C on a heating plate.
2. Simultaneously, 15 mL of 2.0 pH buffer solution was heated for 20 min at a temperature of 60 °C on a different heating plate.
3. 0.1 g of crushed ASA was measured out and placed separately.
4. Both beakers of the buffer solution and water were removed; the ASA was added to the water, and immediately after, the buffer was added to the water beaker. It was stirred a few times, and left to react for 10 min.
5. In the meantime, using a 10 mL pipette, measure out 0.5 mL of Iron (III) chloride into a cuvette.
6. After the ten minutes are complete, using a separate pipette to avoid contamination, fill the cuvette with the buffer, ASA, and water solution until its maximum mark is filled.
7. Zero the spectrophotometer using a cuvette with distilled water. Then add the previously prepared cuvette and note down the absorbance value of the solution. While handling the cuvettes, wipe them down with tissues (without moisture) to ensure that fingerprints and other outside contamination affects the results.
8. The above steps are repeated for the other pH values of 4.0, 6.0, 8.0, and 10.0.

4.3. Safety and Risk Assessment

Safety considerations: Eye protection and goggles are necessary, especially because ferric chloride is a corrosive chemical that can cause serious damage [10]. Gloves were used when handling all chemicals, including acetone because they can cause skin irritation. Moreover, because acetone is highly flammable, it was necessary to be vigilant [11].

Ethical considerations: There were no ethical concerns to take into account.

Environmental considerations: Iron (III) chloride pollutes the environment and can be quite toxic to aquatic organisms [12]. Therefore, it was not disposed of through the sink; instead it was neutralized before disposal. Ferric chloride is a strong acid with a pH of 1.8, so sodium bicarbonate was continually added to it until the pH of the solution measured around 7.0 to 7.5 (pH strips were continuously used to see if the solution reached the value) [13].

5. Data and Analysis

5.1. Qualitative Observations

1. After leaving the mixture of acetone and crushed aspirin tablets to evaporate overnight, a block of white crystals formed on the beaker. It seems that the ASA dries and turns into several sheets with small crystals protruding.
2. As expected, the water, buffer, and ASA solution was clear. However, when iron chloride and solution were reacted together in the cuvette, a dark purple color was seen, a result of the salicylic acid forming in the cuvette.
3. Although one could not tell the difference between the "amount" of purple compounds forming in the cuvette between close pH values like 2.0 and 4.0, after several trials, it was noticed that at a pH of 10.0, there was much more color than at 2.0.

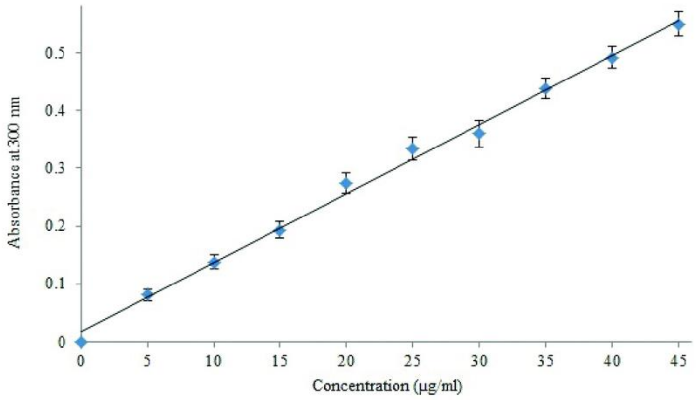
5.2. Quantitative Observations

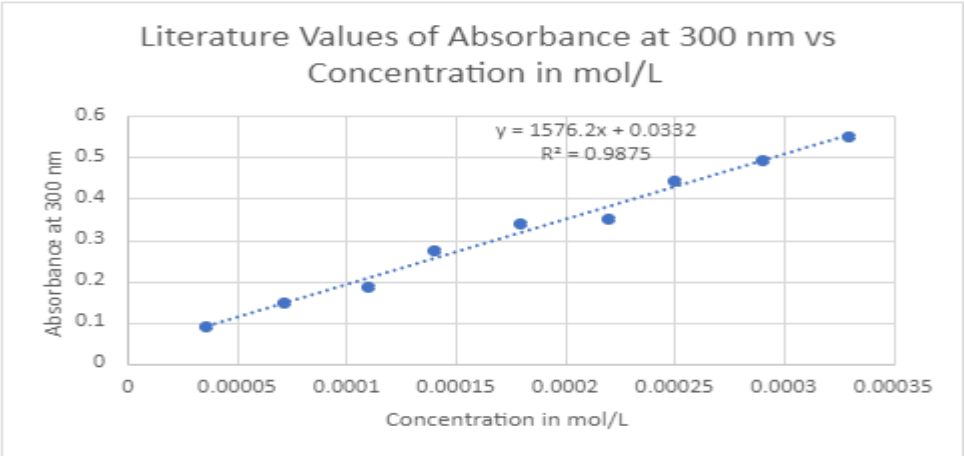
5.2.1. Constructing the Calibration Curve

The first graph is a Calibration Curve for salicylic acid, from a previously done research study [14].Since its units of concentration are in microgram/ mL, and because this experiment uses mol/ L, the first graph's data was noted down, and then converted to the units of mol/ L by using the molar mass of salicylic acid as 138.121 g/ mol. This data was again graphed to find the linear equation for future use in the investigation. It is also noted below:

$$y = 1576.2\,x + 0.0332$$

where y= absorbance at 300 nm and x= concentration of salicylic acid in mol/ L





5.2.2. Spectrophotometer Absorbance Data at Different pH Values

pH	**2.0**	**4.0**	**6.0**	**8.0**	**10.0**
Trial	Absorbance Measured from Spectrophotometer ± 0.001	Absorbance Measured from Spectrophotometer ± 0.001	Absorbance Measured from Spectrophotometer ± 0.001	Absorbance Measured from Spectrophotometer ± 0.001	Absorbance Measured from Spectrophotometer ± 0.001
1	0.380	0.381	0.478	0.572	0.740
2	0.364	0.375	0.489	0.585	0.761
3	0.370	0.371	0.501	0.573	0.745
4	0.391	0.387	0.475	0.598	0.780
5	0.375	0.386	0.479	0.575	0.798

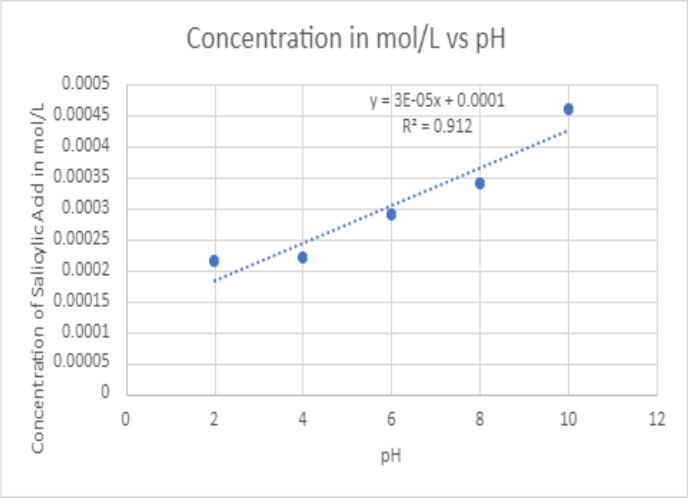
Using the calibration curve linear equation (y= 1576.2 x + 0.0332) and above absorbance values, the corresponding concentration values were found and recorded in a table below.

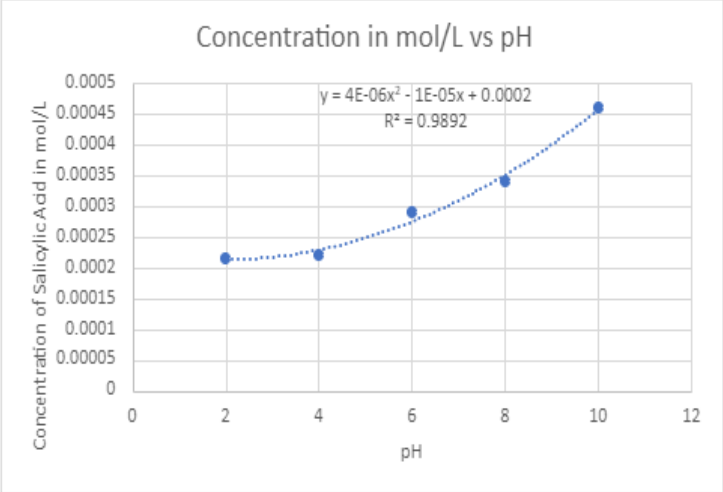
pH	**2.0**	**4.0**	**6.0**	**8.0**	**10.0**
Trial	Concentration in mol/ L ±0.001	Concentration in mol/ L ±0.001	Concentration in mol/ L ±0.001	Concentration in mol/ L ±0.001	Concentration in mol/ L ±0.001
1	0.00022	0.00022	0.00028	0.00034	0.00045
2	0.00021	0.00022	0.00029	0.00035	0.00046
3	0.00021	0.00021	0.00030	0.00034	0.00045
4	0.00023	0.00022	0.00028	0.00036	0.00047
5	0.00022	0.00022	0.00028	0.00034	0.00049
Average Concentration	0.00022	0.00022	0.00029	0.00034	0.00046
Uncertainty of Average Concentration	±0.00051	±0.00051	±0.00051	±0.00051	±0.00052

We find the concentration values from the absorbance values, which have an uncertainty of ±0.001. By the principle of propagation of errors, since absorbance is subtracted and then divided by constants without any uncertainty values (absorbance − 0.0332/1576.2), the uncertainty of the concentration values is also ±0.001. \

The uncertainty of the average concentrations, however, was found by the commonly used (maximum − minimum)/2 formula. Note that the uncertainty of the concentrations (0.001) was added to the maximum concentration, and then the formula was applied.

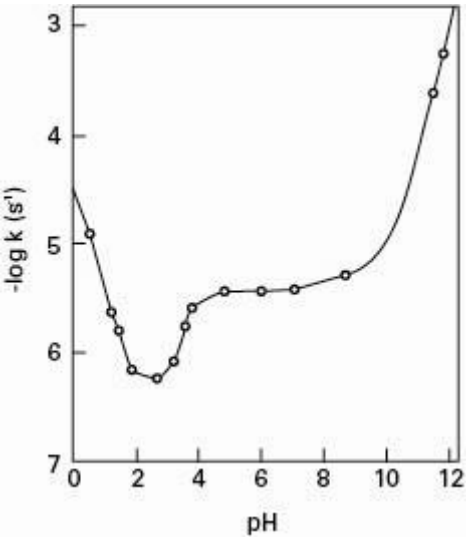
The average concentrations for each of the pH values were then graphed. After trying both a linear regression and a quadratic one, it was seen that the quadratic regression was the best fit with the data.





6. Conclusions

The research question has been answered: as pH increases, acetylsalicylic acid ($C_9H_8O_4$) hydrolysis to salicylic acid ($C_7H_6O_3$) and acetic acid ($C_2H_4O_2$) at temperature 333.15 K (60 °C) also increases, as measured the concentration of salicylic acid through spectrophotometry. The initial hypothesis has been confirmed, but it is important to consider the mechanisms and investigation in more detail.

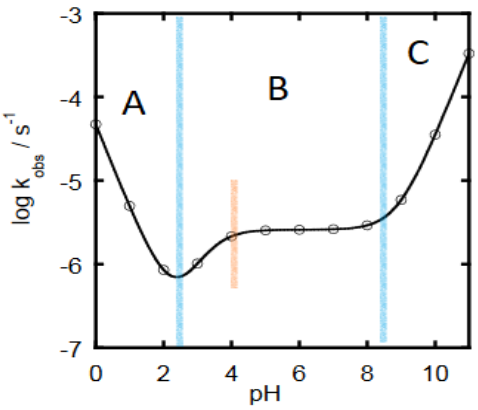


Firstly, this previous research study [15] and pH profile from Dr. Loyd Allen corroborates with the results of this investigation. A solution of higher pH will result in more rapid hydrolysis, increasing the rate of the reaction and producing more salicylic acid product. Since the y axis is a negative log of ASA hydrolysis' rate constant k, the minimum points (such as the one near pH 2), correspond to a smaller reaction rate k. The trend seen in this previous study is almost quadratic, just as in this investigation, adding to the credibility of the results found here.

No prior data with the exact same criteria (300 nm wavelength used, varying pH concentrations) exists, so it will not be possible to do an experimental error calculation. However, aside from the matching of graphical trends corroborating with previous studies to show accuracy in the investigation, one can also internally look at the obtained data and compare it within itself. The standard deviation and standard error were calculated for each set of absorbance values for each pH, and they showed that the data was very precise. The data was not that spread out, adding to the trend's credibility.

pH	2.0	4.0	6.0	8.0	10.0
Standard Deviation of Absorbance Values	0.010	0.007	0.011	0.011	0.024
Standard Error of the Mean of Absorbance Values	0.005	0.003	0.005	0.005	0.010

Finally, the detailed chemical reasoning behind the trend was understood to identify any errors (systematic and random), explained in the following Evaluation section. Chemist SR Maiti writes that there are 3 different possible mechanisms seen through Dr. Allen's pH profile for aspirin hydrolysis, separated by the blue lines in the figure below [16]. Maiti considers a sharp turn or change in slope of the graph as a sign to whether the mechanism of the reaction changes.



At segment A, the usual acid ester hydrolysis mechanism is followed (so as pH increases, hydrolysis decreases). The main components in the reaction are the protonated form of aspirin (since its pK_a is 3.5) and H_3O^+, meaning the rate law is rate = k [ASA] [H_3O^+], but the observed rate law is rate = k_{obs} [ASA] since aspirin hydrolysis a pseudo first order reaction (it looks like a second order reaction but behaves as a first order reaction).

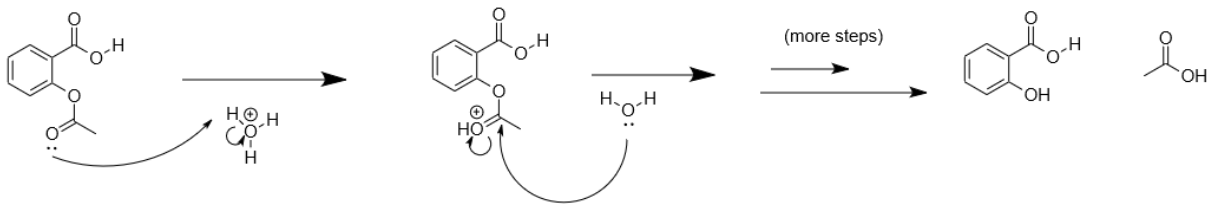
Maiti derives:

$k_{obs}=k\,[H_3O^+]$

$\log(k_{obs}) = \log(k) + \log([H_3O^+])$

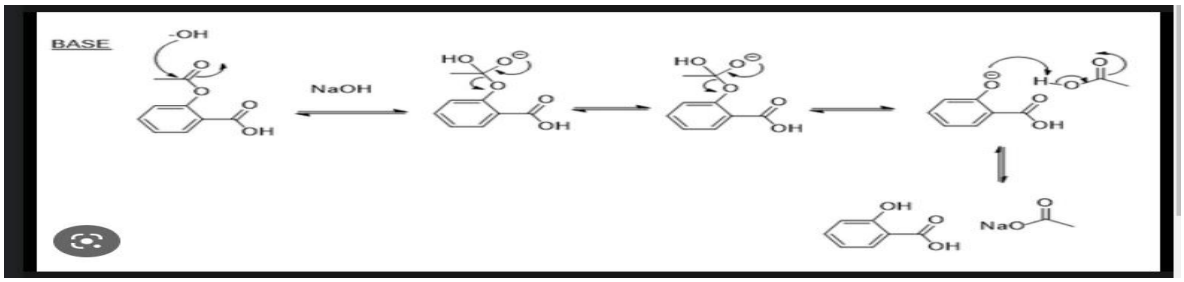
$\log(k_{obs}) = \log(k) - pH$, which is why a negative trend is seen in the graph.

Essentially, the carbonyl oxygen of ASA is protonated, making it more electrophilic. A water molecule attacks the carbonyl carbon, and an intermediate is formed, which is then deprotonated, breaking the carbon-oxygen bond and resulting in the formation of salicylic acid and acetic acid.



Meanwhile, at segment C, the main components of the solution are ASA and OH^-, so the base catalyzed mechanism starts to dominate more. As pH increases, the graph also rises because OH^- is the "perfect" nucleophile, [17] as Dr. Robert DiGiovanni describes, for ester hydrolysis of ASA. Hydroxide ions (OH-) act as a nucleophile, attacking the carbonyl

carbon of the ester group like water does, promoting the hydrolysis reaction, and increasing rate as pH increases. This mechanism is drawn out in the figure below [18].



At segment B, several mechanisms take place because ASA is both protonated and deprotonated, and there is a lower concentration of H_3O^+, so Maiti writes that there are two "parts": since rate increases till pH is 4, and then stays constant. The lengthy derivations and details are not fully necessary to explain the results of this investigation, and specifics of the mechanism are still being argued by the scientific community, but the mechanisms in section B are catalyzed by neither acid nor base (instead due to a complex interaction between deprotonated ASA and protonated ASA with the other reactants).

Error and Explanation	**Type of Error**	**Effect of the Error on the Final Result**	**Preventing the Error in Further Experimentation**
The concentration values were calculated from the literature values' calibration curve, which used a spectrophotometer cuvette of the same optical path length as was used in this investigation. There is, however, a small systematic error, because for a concentration of zero, there is still a non zero y intercept (0.0332), which is chemically not possible.	Systematic	An increase in salicylic acid concentration calculations from the calibration plot	Instead of relying on literature value calibration data (which was done because of lack of access to salicylic acid) and the tools to measure such small concentrations necessary to obtain the calibration plot, a future experiment could involve the construction of such a plot from scratch. Multiple data points could be considered as well (just 9 were done in the calibration plot used here).
Much care was taken to prevent contamination and fingerprints on the cuvettes (including glove usage and frequent wiping) which would blur it and affect the absorbance reading on the spectrophotometer. However, it was not possible to have perfectly clean, new looking, cuvettes every trial because of "human error" and the inevitability of dust particles and contamination.	Random	Inaccurate absorbance values obtained from spectrophotometer	This error is not fully preventable, but if possible, a new cuvette can be used for every trial. Cleaning could be done more frequently; the researcher could employ more researchers to help with this task, to finish and ready the cuvettes efficiently.
In the experiment, ASA was added first to the water, and then the buffer solution was added to the same beaker. While these steps were done in quick succession, the results could still be affected because some molecules of ASA and water would have already started reacting before the buffer was added and the pH was established in the solution.	Systematic	A slight increase in salicylic acid concentrations, and therefore absorbances	In future experiments, the buffer can first be added to the water, and then the ASA. Otherwise, both the ASA and water can be simultaneously poured into the buffer solution.

pH and temperature are two crucial aspects of every reaction, so it will be interesting to consider if changing the temperature affects the concentration of salicylic acid. Generally, it is accepted that as temperature increases, so too does the rate of the reaction. However, as mentioned previously in this investigation, previous research studies have found that 60° C is the ideal temperature for this reaction to produce maximum salicylic acid output. How will increasing the temperature beyond this point impact the reaction? Will the trend be quadratic, like the pH graphs generated in this investigation? It will be an interesting further exploration, especially considering the relevance of aspirin in the human body and medical field.

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Institutional Review Board Statement:.

Informed Consent Statement:.

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Conflicts of Interest: The author declares that there are no competing interests.

Ethics Approval and Consent to Participate: Not applicable.

Consent for Publication: Not applicable.

List of Abbreviations

ASA	Acetylsalicylic Acid
COX	Cyclooxygenase
NSAID	Nonsteroidal Anti-Inflammatory Drug
pH	Potential of Hydrogen

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