Research Question - How does the temperature (°C/± 0.05 °C) of acetylsalicylic acid (25 °C, 35 °C, 45 °C, 55 °C, 65 °C & 75 °C; ± 0.05 °C) affect the concentration (mol dm^{-3}) of salicylic acid determined through the absorbance using a spectrophotometer (± 0.050 arbitrary units)?

Introduction - Working part-time in my uncle's pharmacy during the summer holidays I managed the distribution of medicine and online orders after school. During one of the hottest summers in Pakistan, I encountered 30 complaints from customers that bought Aspirin and Chloramphenicol (used for bacterial infection), who reported that the medicine was ineffective and caused a lot of irritation. After numerous complaints, my uncle advised me to discard all the leftover stocks saying that the drugs have decomposed after being subject to high temperatures. Upon further research (in an article in ABC news), I found a similar statement suggesting that Aspirin should not be kept in medicine cabinets due to high temperatures. Witnessing the incident in my uncle's pharmacy and the research present in the article, I became more curious about the chemical stability of aspirin. Interested in a career of biochemistry and pharmaceuticals myself, I seized the internal assessment as an opportunity and I decided to investigate the impact of thermal degradation on the hydrolysis of aspirin. This medicine in particular was chosen for the IA for its vast applications and because it is a common commodity in every household. This allowed me to pose the research question above to examine the effect of temperature on the concentration of salicylic acid using the technique of UV spectrophotometry which will help me understand how true the article's opinion is about not storing aspirin in bathroom medicine cabinets and at what temperatures does the concentration exceed the acceptable threshold that causes it to be ineffective.

Background Information – Aspirin, with an IUPAC name of 2-Acetoxybenzoic acid (commonly referred with the name of acetylsalicylic acid in the report), is an analgesic drug that has many versatile uses from pain relief from headaches to an effective blood-thinner. However, despite its medical benefits, its effectiveness is vulnerable to its chemical stability as it is ineffective after it has degraded. Aspirin is an ester bond of salicylic acid (classified as a salicylate) which hydrolyses into salicylic acid, and acetic acid (see figure 1.1).

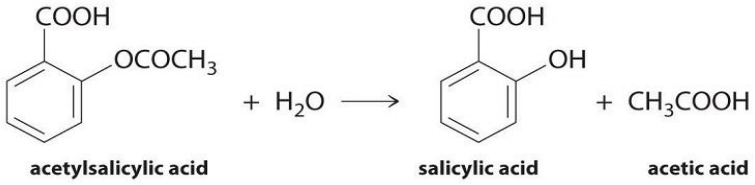


Figure 1 *Hydrolysis of Acetylsalicylic Acid (Libretexts. "Chapter 13.2: Reaction Rates")*

$$C_9H_8O_{4(s)} + H_2O_{(l)} \rightarrow C_7H_6O_{3(aq)} + C_2H_4O_{2(aq)} \qquad (Arias)$$

Since salicylic acid itself cannot be ingested directly because it causes severe tissue damage, it undergoes a synthesis reaction with acetic acid to form acetylsalicylic acid which is safe to consume (Miller). Once it is orally ingested, it undergoes hydrolysis where the ester bond between the acetic acid and salicylic acid breaks. Other factors like temperature and pH levels and the age of acetylsalicylic acid also affect the rate of hydrolysis. In this particular investigation, the correlation of the temperature of acetylsalicylic acid vs. the concentration of salicylic acid will be examined using a spectrophotometer. Alternatively, a back titration can also be performed to determine the concentration, however, spectrophotometry is used because it is more accurate and less sensitive than a titration. Whilst manufacturing aspirin commercial additives are added to allow the binding, therefore the

acetylsalicylic acid within aspirin tablets is not pure. To obtain pure acetylsalicylic acid, aspirin tablets will be dissolved in propan-2-ol as it is more soluble in alcohol than water, followed by recrystallization to extract pure acetylsalicylic acid. An aspirin molecule is composed of an ester, a benzene ring and a carboxyl-group containing both non-polar and polar components. However, the affinity of water (a polar molecule) for aspirin is not as large as the affinity of water for itself (Alkhafaji). As oppose to propan-2-ol (which is slightly polar) has an affinity for aspirin molecules similar to that of propan-2-ol for itself, therefore making propan-2-ol a better solvent than water.

The impact of varying temperatures of acetylsalicylic acid on the concentration of salicylic acid can be analyzed using UV-spectrophotometry, a technique that is based on the absorption of ultraviolet and visible light by coloured chemical compounds at particular wavelengths producing a distinct absorption spectrum. Acetylsalicylic acid itself does not absorb light in the visible spectrum, however, when it is hydrolyzed, the salicylic acid can react with iron(III) Nitrate (a chromophore reagent) to produce a chromophore *(Miller)*, which is a coloured compound that absorbs light at a particular wavelength. This reaction produces a salicylate dianion which forms a violet (tetraaquosalicylatroiron (III)) complex that can absorb green light in the visible spectrum.

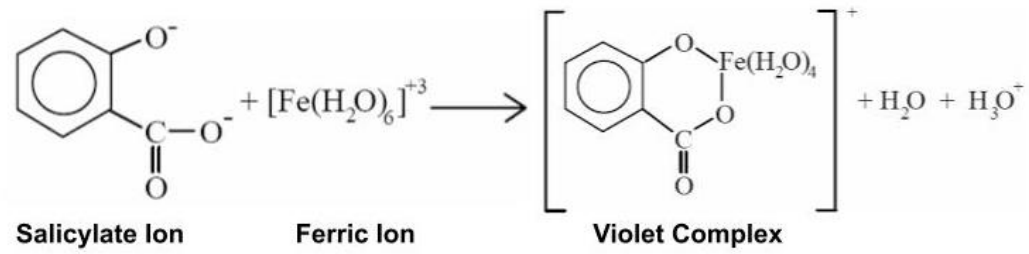


Figure 2 *Forming a chromophore (Violet Complex) of the Salicylate Dianion (Ali)*

The peak absorbance (λ_{max}) of this tetraaquosalicylatroiron (III) complex for salicylic acid can be typically measured at the wavelength of 530 nm. According to Beer Lambert's Law, the intensity of the colour produced by the complex will depend upon the concentration of salicylic acid in the sample. Beer Lambert's Law states that:

$\mathbf{A = \varepsilon\, l\, c}$	Where A = absorbance (expressed in arbitrary units) ε = Molar Absorptivity, extinction coefficient (mol $dm^{-3})^{-1}$ cm^{-1} l = optical path length, length of the cuvette (cm) – equivalent to 1 cm ± 0.01 cm c = concentration (mol dm^{-3})

Since the length of the cuvette is 1 cm, it can be rewritten as A = ε c, representing a linear relationship of the form y = mx: where y is the absorbance, x expresses the concentration and ε is the gradient of the graph.

Since the molar extinction coefficient, ε, for Salicylic Acid is unknown, it will be determined using a calorimetric analysis of standard solutions of salicylic acid, which will produce a calibration curve (Beer Lambert's Plot of Concentration Vs. Absorbance) and its gradient will represent the molar extinction coefficient. This molar extinction coefficient will be used to calculate the unknown concentration of salicylic acid at varying temperatures of acetylsalicylic acid by dividing its absorbance with the extinction coefficient.

Hypothesis – If acetylsalicylic acid is dissolved at a greater temperature (at 75°C ± 1°C) then the concentration (mol dm^{-3}) of salicylic acid will be the greatest. There will be an exponential relationship between temperature and concentration because, according to Maxwell-Boltzmann's distribution curve, a higher temperature will speed up the rate of hydrolysis and more reactant molecules will have the minimum activation energy to overcome the ester bond between acetic acid and salicylic producing a greater concentration of salicylic acid.

Variables – The variables acknowledged in this investigation are the following:

Independent Variable (IV)	Dependent Variable (DV)
The temperature (°C) of Acetylsalicylic acid (25 °C, 35 °C, 45 °C, 55 °C, 65 °C & 75 °C) - This will be manipulated by using a water bath to change the temperature of the pH 7 buffer solution and the ethanol to the given range. These **particular increments** are chosen because the temperature in a typical home can range from ambient (room) temperature to extremely high temperatures in the kitchen and therefore this range will reflect the real-life situation. The water bath will evenly distribute the heat to the mixture, and the temperature will be checked using a thermometer (± 1°C).	**Raw DV – Absorbance (arbitrary units) at 530 nm (of wavelength)** measured using a UV-VIS spectrophotometer with a photometric uncertainty of ± 0.050 arbitrary units. The absorbance will be recorded for both the standard solution and the solution with varying temperatures of acetylsalicylic acid.
	Processed DV – Average Concentration (mol dm^{-3}) of Salicylic acid - Using the average absorbance of the unknown concentrations of salicylic acid at varying temperatures, the average concentration will be determined by dividing the average absorbance with the molar extinction coefficient determined in the calorimetric analysis of standard solutions of salicylic acid. The uncertainty in the concentration of salicylic acid will be propagated using equipment uncertainties.

Controlled Variables – The following variables are controlled to ensure reliable data is obtained:

Variable	Why it needs to be controlled? (Potential for Error)	How is it controlled?
The pH of the Acetylsalicylic acid	The rate of reaction of hydrolysis is pH-dependent. As the pH of the medium becomes more basic/alkali, the rate of hydrolysis of aspirin becomes faster and therefore the formation of salicylic acid increases within the sample causing a discrepancy in data and hence reducing the validity and reliability.	The pH of acetylsalicylic acid will be controlled by using a pH 7.0 phosphate buffer solution (0.2 mol dm^{-3} in each trial).
The initial concentration of acetylsalicylic acid	The formation of salicylic acid is dependent upon the original concentration of acetylsalicylic acid. Hence, if a greater concentration of acetylsalicylic acid is used, then the formation of salicylic acid will be the greatest too due to a greater collision frequency and faster reaction rate which leads to imprecise data.	The concentration of acetylsalicylic acid is controlled by adding only 0.4 g (using a balance) for each trial.
Volume & concentration of Iron (III) Nitrate added	If a greater volume of Iron (III) Nitrate and/or greater concentration is added to the hydrolysis product then more salicylate dianions will react with $[Fe(H_2O)_6]^{3+}$ due to a greater concentration of ferric ions and hence a darker colour will be produced causing the absorbance to fluctuate.	This can be controlled by adding 5 cm^3 of iron (III) nitrate with a concentration of 0.125 mol dm^{-3}.
Time allowed for aspirin to undergo hydrolysis	If the time allowed for the acetylsalicylic acid to hydrolyze varied, it would have caused the concentration of salicylic acid to vary as well, because greater time would allow the formation of more salicylic acid and vice versa.	The hydrolysis reaction time will be controlled by using a stopwatch (for a 30 minutes timer).
Time allowed for solutions to react with Iron (III) Nitrate	Reaction time to allow standard and unknown solutions of salicylic acid to react with Iron (III) Nitrate solution affect the intensity of the violet complex formed and hence can cause the final absorbance to fluctuate. For instance, a greater time would allow a darker-coloured complex and vice versa.	This will be controlled by allowing only 5 minutes (measured using a stopwatch) for this complex formation to occur.

Safety concerns: The following hazards (taken from *CLEAPSS Student Safety Sheets)* will be addressed:

Risk	Hazard	Precaution
Propan-2-ol **Ethanol**	Highly flammable & irritant, can cause serious damage to the eye, dizziness, and drowsiness	- Use small concentration and volume possible, wear goggles for eye protection and ensure the laboratory is well-ventilated, keep away from naked flames, use a hot plate or water bath and avoid reaching the boiling point whilst heating.
Acetylsalicylic & Salicylic Acid	Causes irritation to the skin and eyes.	- Handle with care and wear gloves and protective gear.
Hot Plate	Hot- can burn skin	- Handle with care, and avoid touching the metal plate
Iron (III) Nitrate	Irritant - can cause skin and eye irritation and can also cause respiratory and digestive tract irritation.	-Wear gloves, goggles and protective gear, if in contact flush eyes and skin with plenty of water and remove any and all contaminated clothing. Avoid consuming any chemicals.
pH 7 Buffer	Causes irritation to skin & eye	
Glassware	Fragile, glassware can shatter into pieces and can injure and cause damage to skin tissues	- Handle all glassware apparatus with care and avoid placing it at the edge of a table. If broken, avoid touching it with bare hands.

Ethical & Environmental Consideration – Use small concentrations and volumes of chemicals to reduce waste and to minimize the disposal of toxic chemicals into the environment and dispose all chemical waste into the waste bottle. The concentration and volumes of chemicals have been chosen to conduct a small-scale experiment that is scaled sufficiently to minimize the impact on uncertainty, producing decently large data values to keep the percentage uncertainty significantly small and increasing the reliability of the results obtained. Otherwise, there are no significant ethical considerations.

Materials – The following equipment will be required to conduct the practical:

Apparatus		Chemicals & Consumables		
Equipment Name	**Qty.**	**Chemical**	**Concentration**	**Volume**
Mortar & Pestle	x1	Distilled Water	2 Bottles	
Electric Water Bath (± 1°C)	x1	Propan-2-ol	1.0	25 cm^3
Glass Stirring Rod	x1	Ethanol	0.1 mol dm^{-3}	100 cm^3
Spatula	x1	pH 7 Phosphate Buffer Solution	0.2 mol dm^{-3}	500 cm^3
Weighing Dish	x5	Powdered Salicylic Acid	NA	50 g
Stopwatch (± 0.01 s)	x1	Iron (III) Nitrate	0.025 mol dm^{-3}	100 cm^3
UV Spectrophotometer (± 0.050)	x1	**Glassware**		
Hot Plate	x1	**Equipment**	**Uncertainty**	**Qty.**
Weighing Balance (± 0.01g)	x1	10 cm^3 Volumetric Pipette	± 0.04 cm^3	x1
Pipette Filler	x1	100 cm^3 Beaker	± 1.0 cm^3	x1
Filter Paper	x5	10 cm^3 Measuring cylinder	± 0.2 cm^3	x1
Test Tube Rack	x1	Test Tubes	NA	x5
Cuvettes	x15	150 cm^3 Conical Flask	NA	x5
Thermometer (± 0.5 °C)	x1	100 cm^3 Volumetric Flask	± 0.2 cm^3	x2
Filter Funnel	x2	100 cm^3 measuring cylinder	± 1.0 cm^3	x1

Experimental Set-up:

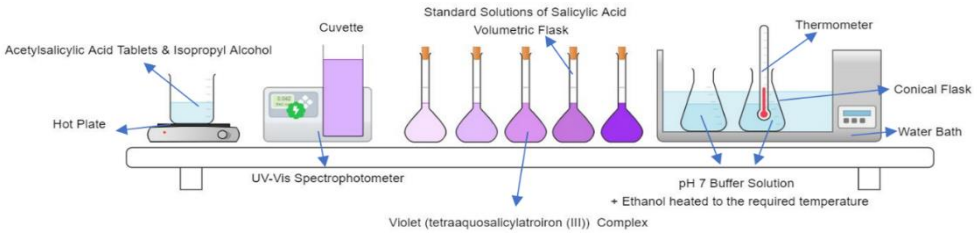


***Figure 3** Experimental Set-up for determining the Absorbance of Salicylic Acid at varying temperatures (Self-Made by the Candidate)*

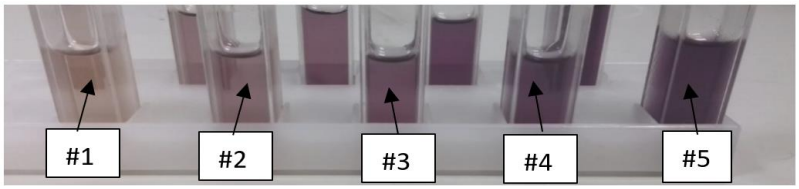


Figure 4 *Standard Solutions of Salicylic Acid with different concentrations forming a violet complex with Iron (III) Nitrate (Self-taken by the candidate on 16 April)*

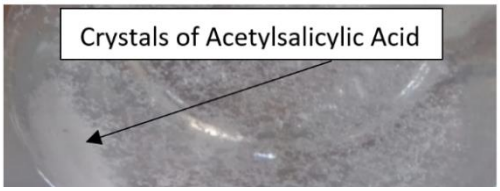


Figure 5 *Crystallization of Acetylsalicylic Acid (Self-Taken by the candidate on 20 April 2021)*

Preliminary Data Collection: I initially chose the range of IVs between 10°C to 35°C with a 5-degree increment between each, however, the trend obtained wasn't significant with small shifts, therefore the method was adapted with a new range from 25 °C to 75 °C with 10-degree increment for a visible and reliable trend.

Pre-Lab Calculations – The following calculations are made before conducting the practical:

CALCULATION 1: Calculating the concentration of Salicylic ($C_7H_6O_3$) acid in the stock solution

m ($C_7H_6O_3$)=0.09 g **M_r ($C_7H_6O_3$)**=138.121 g mol^{-1} **n ($C_7H_6O_3$)=** $\frac{m}{M_r} = \frac{0.09\ g}{138.121\ g\ mol^{-1}}$ = 6.52 x 10^{-4} mol

Volume of buffer solution & ethanol = 100 cm^3 = 0.1 dm^3

c ($C_7H_6O_3$) = $\frac{n}{V} = \frac{6.52 \times 10^{-4}\ mol}{0.1\ dm^3} = 6.52 \times 10^{-3}$ mol dm^{-3}

Propagating Uncertainty for the concentration of Salicylic Acid

Uncertainty in moles of ($C_7H_6O_3$) = equal to uncertainty of mass → ± 11.11 %

Total Uncertainty in Volume = % uncertainty of ethanol + % uncertainty of pH 7 Buffer = 1.11% +0.4% = 1.15%

Total % uncertainty in c = the sum of uncertainties in n and v = 11.11% + 1.15% = 12.26%

Absolute uncertainty in concentration = $\frac{Percentage\ Uncertainty}{100} \times Concentration = \frac{12.26}{100} \times 0.00652 = 0.00080$

Therefore, the concentration of salicylic acid in the stock solution = $6.52 \times 10^{-3}\ mol\ dm^{-3}$ ± 0.00080

Methodology/Procedure: The method has been adapted from ChemLibretexts (Libretexts. "Experiment _613_Spectrophotometric Determination of Aspirin") in which a calibration curve (using colorimetric analysis) is produced from standard solutions of salicylic acid to determine the molar absorptivity, extinction coefficient and then the concentration of salicylic acid is determined within aspirin tablets. The volumes and concentration of the chromophore (Iron (III) Nitrate Solution) and ethanol have been modified to minimize the disposal of chemical waste and to reduce the impact on uncertainty by yielding large readings with small percentage uncertainties.

Further Adaptation: Since pure acetylsalicylic acid was not available in the laboratory, it was extracted from aspirin tablets (500 mg) using propan-2-ol .

Part 1: Preparing Salicylic acid stock solution for the colorimetric analysis

1. Pour 90 cm^3 of phosphate pH 7 buffer measured using a 100 cm^3 graduated cylinder into the beaker.
2. Add 10 cm^3 of ethanol solution using a 10 cm^3 volumetric pipette and filler.
3. Using an electric weighing balance and weighing dish, measure 0.09 g of Salicylic acid and transfer it into the beaker of Ethanol and the pH 7 buffer solution.
4. Place the mixture in the beaker into the water bath and heat the solution until all the salicylic acid has dissolved. Stir the solution with a stirring rod whilst heating.

Part 2: Producing a calibration curve: using the colorimetric analysis of salicylic acid

1. To prepare the first standard solution, measure 2 cm^3 of the salicylic acid solution prepared in part 2 using a 10 cm^3 graduated cylinder and filler and pour it into a 100 cm^3 graduated cylinder.
2. Fill up the remaining volume of the graduated cylinder with iron (III) nitrate solution (of concentration 0.025 mol dm^{-3}). This will form a violet iron complex. Allow it to react for at least 3 minutes.
3. Fill a cuvette about 2/3 with the 2-hydroxybenzoic acid- iron (III) complex.
4. Blank the spectrophotometer with a cuvette filled 2/3 with distilled water.
5. Place the 2-hydroxybenzoic acid- iron (III) complex cuvette inside the spectrophotometer ensuring that the transparent side is aligned with the light source. Record results in an appropriate table.
6. Repeat steps 1-6 to make different concentrations of salicylic acid with a volume of the standard solution of 4 cm^3, 6 cm^3, 8 cm^3 & 10 cm^3.

Part 3: Extracting pure acetylsalicylic acid powder from Aspirin tablets

1. Roughly grind 10 aspirin tablets using a mortar and pestle and transfer it to an Erlenmeyer flask.
2. Measure 25 cm^3 of propan-2-ol using a 10 cm^3 graduated cylinder and add it to the flask.
3. Place the flask with a thermometer on a hot plate and heat the mixture until it is nearly boiling.
4. Ensure all tablets have dissolved within the propan-2-ol , if not keep heating the mixture.
5. Filter the mixture using the filter funnel and paper and transfer the filtrate into a beaker.
6. Add 100 cm^3 of cold water within the beaker and wait for roughly 24 hours to crystallize.
7. Collect the recrystallized acetylsalicylic acid using the filter funnel and paper and allow it to dry.

Part 4: Determining the concentrations of Salicylic acid at different temps. of Acetylsalicylic Acid

1. Measure 25 cm^3 of 0.1 mol dm^{-3} ethanol and 25 cm^3 of pH 7 phosphate buffer solution and pour it into a 150 cm^3 conical flask. Place a thermometer into the mixture and ensure the temperature is 25 °C.
2. Using a weighing dish and measuring balance, measure 0.05 g of Acetylsalicylic acid powder (extracted in Part 3) and transfer it into the conical flask of the pH 7 Buffer and ethanol.
3. Allow the acetylsalicylic acid to completely dissolve, then start the stopwatch for 30 minutes to react.
4. Using a 10 cm^3 measuring cylinder, measure 5 cm^3 of Iron (III) Nitrate and add to the first test tube.
5. Measure 2 cm^3 of the product of hydrolysis of acetylsalicylic acid using a cleaned 10 cm^3 measuring cylinder and pour it into the test tube and allow it to react for 5 minutes (using a stopwatch).
6. Blank the spectrophotometer using a distilled water cuvette.
7. Fill a cuvette 2/3 with the solution of the violet complex and place it in a spectrophotometer.
8. Repeat 5 trials for ethanol solution at room temperature (25°C).
9. Repeat Steps 1-8 for ethanol solution at other temperatures (35 °C, 45 °C, 55 °C & 65 °C & 75 °C).

Propagation of Uncertainties – Calculated using the formula % uncertainty = $\frac{Absolute\ Uncertainty}{Reading} \times 100$

Apparatus	Purpose	Percentage Uncertainty
Weighing Balance (± 0.01 g)	Measuring 0.09 g of Salicylic Acid	11.11 %
Volumetric Pipette (± 0.04 cm^3)	Measuring 10 cm^3 ethanol	0.4%
Measuring Cylinder (± 1.0 cm^3)	Measuring 90 cm^3 of pH 7 buffer solution	1.11%

DATA COLLECTION (I) → COLORIMETRIC ANALYSIS for the Calibration Curve

Qualitative Data – Qualitative observations made when standard solutions reacted with Iron (III) Nitrate

Sol.	Colour Change observed	Other observations
1	Transparent, Light yellow/purple	*When the stock solution for salicylic acid was prepared by putting salicylic acid powder within pH7 buffer & ethanol, some powder didn't dissolve completely which could have affected absorbance readings and produced a higher random error in the concentration. The intensity of the colour progressively increased as the concentration increased.
2	Translucent Violet	
3	Violet/purple colour	
4	Opaque Purple	
5	Extremely Dark Opaque Violet	

Raw Table 1.1– Concentration (mol dm^{-3}) of standard solutions of salicylic acid vs. Absorbance at 530 nm measured using a spectrophotometer (± 0.050)

Standard Solution No.	Volume (cm^3) of Salicylic Acid Stock Solution added (± 0.02 cm^3)	Volume (cm^3) of Iron (III) Nitrate Solution added (± 1.0 cm^3)	Concentration of Salicylic Acid (mol dm^{-3}) *	Absolute Uncertainty* in the concentration	Absorbance at 530 nm (± 0.050)
1	2.00	98.0	1.30×10^{-4}	$\pm 1.99 \times 10^{-5}$	0.336
2	4.00	96.0	2.61×10^{-4}	$\pm 3.73 \times 10^{-5}$	0.602
3	6.00	94.0	3.92×10^{-4}	$\pm 5.48 \times 10^{-5}$	0.890
4	8.00	92.0	5.22×10^{-4}	$\pm 7.23 \times 10^{-5}$	1.463
5	10.00	90.0	6.52×10^{-4}	$\pm 8.98 \times 10^{-5}$	1.902
Average Percentage Uncertainty in concentration				9.24 %	-

*__Note__ the concentration of salicylic acid and its absolute uncertainties are recognized as **processed values** but are merged into the raw data table for clarity and concision. The concentration and absorbance will be graphed on the x and y-axis respectively to determine the molar absorptivity coefficient.

Sample Calculations for the colorimetric analysis – The following calculations are made to process the concentrations for standard solutions of salicylic acid with their relative uncertainties.

Calculating the concentration of standard solutions of salicylic acid *(shown for standard solution 1 only)*
**The same calculations (for processing concentration and uncertainties) were repeated for all other standard solutions as well with different volumes of stock solution.*

Concentration of the stock solution = 6.52×10^{-3} mol dm^{-3} (See pre-lab calculations)
Volume of stock solution added to make standard solution 1 = 2.00 cm^3 = 0.002 dm^3
n(standard solution) = c x v = $6.52 \times 10^{-3} \times 0.002 = 1.304 \times 10^{-5}\ mol$
After adding Iron (III) Nitrate, the total volume (salicylic acid stock + Iron (III) Nitrate) = 100 cm^3 = 0.1 dm^3
New concentration (standard solution) = $\frac{n}{V} = \frac{1.304 \times 10^{-5}}{0.1} = 1.304 \times 10^{-4}$ mol dm^{-3}

Propagating Uncertainties in the concentration of standard solutions *(shown for standard solution 1 only)*

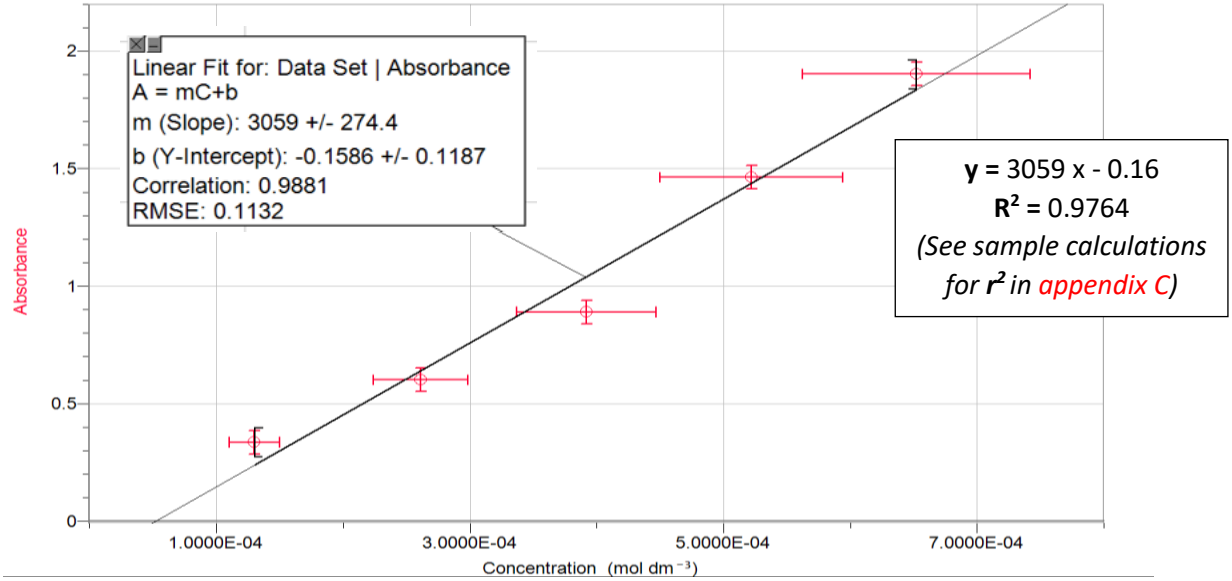
Apparatus	Purpose	Percentage Uncertainty
10 cm^3 measuring cylinder (± 0.02 cm^3)	To measure 2 cm^3 volume of salicylic stock solution added to standard solution 1.	1.00 %
100 cm^3 measuring cylinder (± 1.0 cm^3)	To measure 98 cm^3 volume of Iron (III) Nitrate added to standard solution 1.	1.02 %

Propagation of uncertainty
% uncertainty in moles = % uncertainty in concentration (of stock solution)+ % uncertainty in volume of salicylic acid stock solution = 12.26 % (pre-lab calculation) + 1.00 % = 13.26 %
% uncertainty in total volume = % uncertainty in $C_7H_6O_3$ + % uncertainty in $Fe(NO_3)_3$ = 2.02 %
% uncertainty in final concentration = % uncertainty in moles + % uncertainty in total volume =
= 13.26 % + 2.02 % = 15.28 % *[See Appendix A for Excel Calculations]*
Absolute Uncertainty = $\frac{Percentage\ Uncertainty}{100} \times$ concentration = $\frac{15.28}{100} \times 1.304 \times 10^{-4} = 1.99 \times 10^{-5}$

Justification of Uncertainties – Whilst propagating the uncertainties in stock solution and standards solutions, percentage uncertainties were added for all readings (of concentration, volume and moles) to account for the impact of small measurements and their equipment on their overall uncertainties.

Calibration Curve (Colorimetric analysis of Salicylic Acid) - Concentration (mol dm^{-3}) of standard solutions of salicylic acid vs. Absorbance at 530 nm measured using a spectrophotometer (± 0.050). Vertical error bars reflect the photometric uncertainty of the UV-Vis spectrophotometer whilst the horizontal error bars reflect the uncertainty in concentration propagated using the percentage uncertainties of volumes and masses used to prepare the standard solutions of salicylic acid.

Figure 6 *Calibration Curve of Salicylic Acid - Concentration (mol dm^{-3}) Vs. Absorbance (Self-Made by the Candidate on LoggerPro)*



Analysis of the Calibration Curve - By examining the graph above of concentration of standard solutions vs. its absorbance at the wavelength of 530 nm, there is a strong positive correlation between the two variables as the coefficient of determination (**r^2,** see *Appendix C* for sample calculations) is extremely close to 1 (the value being 0.9764) which suggests that the linear association between the variables is strong (as predicted in the background information) which increases the reliability of this curve. However, despite the strong correlation, there is a high systematic error as there is a deviation in the y-intercept of this line. The theoretical y-intercept of this graph is supposed to be zero because there is a direct proportionality between absorbance with concentration predicted by **$A = \varepsilon c$**, since the length of the cuvette is 1 cm. This systematic error can be attributed to the fact that not all salicylic acid was dissolved completely in the stock solution which could have caused the true concentration of salicylic acid in the stock solution to be much lower than the calculations as a result leading to a lower absorbance than expected and causing the graph to shift to the right.

Using the graph **molar absorptivity coefficient** is determined to be $\varepsilon = \frac{A}{c} = \mathbf{3059}$ (mol dm^{-3})$^{-1}$ cm^{-1}

Propagating uncertainty in $\varepsilon = \sum \%age\ uncertainty\ in\ spectrophotometer + average\ \%age\ uncertainty\ in\ concen.$

Percentage Uncertainty = 14.23 % + 5 % = ± **19.23 %**

DATA COLLECTION (II) → TEMPERATURE VS. SALICYLIC ACID CONCENTRATION

Qualitative Data – Observations were made whilst reacting acetylsalicylic acid at different temperatures:

Temp. (°C)	Color Change observed	Other observations
25	Light Yellow (almost original colour of Iron (III) Nitrate solution)	*While extracting acetylsalicylic, when aspirin tablets were dissolved in propan-2-ol some of the white suspended substance was insoluble which were presumably the binding commercial additives. *After acetylsalicylic acid was filtered from propan-2-ol it formed shiny crystals which were suspended in the bottom of the container.
35	Darker more pale yellow	
45	Translucent/light purple	
55	Darker shade of purple	
65	Opaque purple	
75	Extremely dark violet Complex	

Raw Table 1.2 - How does the temperature (°C) of acetylsalicylic acid (25 °C, 35 °C, 45 °C, 55 °C, 65 °C & 75 °C) affect the absorbance of light by salicylic acid when measured using a spectrophotometer (± 0.050)?

Temperature (°C) of Acetylsalicylic Acid (± 0.5 °C)	Absorbance (± 0.050) of light at a wavelength of 530 nm after 30 mins				
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
25	0.634	0.641	0.637	0.642	0.630
35	0.768	0.776	0.773	0.764	0.772
45	0.934	0.924	0.927	0.932	0.921
55	1.122	1.128	1.120	1.130	1.130
65	1.486	1.494	1.497	1.483	1.494
75	1.946	1.952	1.958	1.956	1.958

Sample Calculations – *All calculations have been shown for acetylsalicylic acid at 25 °C [See Appendix B]*

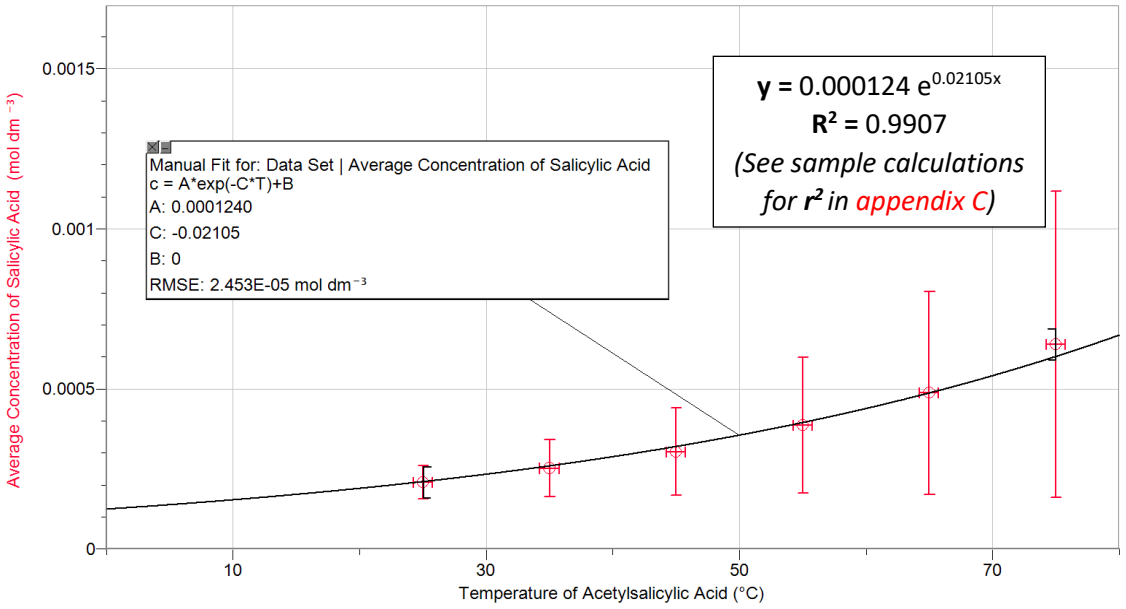
Calculation	Sample Calculation
Average Absorbance	$\text{Average} = \frac{\sum Trials}{No.\ of\ Trials} = \frac{0.634+0.641+0.637+0.642+0.630}{5} = 0.637$
Average Absolute Uncertainty in absorbance	$\text{Absolute Uncertainty} = \frac{\sum Absolute\ Uncertainty\ in\ each\ trial}{5} = 0.050$
Propagating % uncertainty in average absorbance	$\%\ \text{uncertainty} = \frac{Average\ Absolute\ Uncertainty\ in\ Absorbance}{Actual\ Average\ Absorbance} = \frac{0.050}{0.637} = 7.85\ \%$
Average Concentration of Salicylic Acid	$\text{Concentration} = \frac{Average\ Absorbance}{Molar\ Extinction\ Coefficient} = \frac{0.637}{3059} = 2.083 \times 10^{-4}\ mol\ dm^{-3}$
Propagating % uncertainty in average concentration	**% uncertainty** = $\sum$ % *Uncertainty in Average Absorbance* + % *uncertainty in molar extinction coefficient* **E.g.,** 7.85 % + 19.23 % = ± 27.08 % (for 25 °C)

Processed Data – How does temperature (°C) of acetylsalicylic acid (25 °C, 35 °C, 45 °C, 55 °C, 65 °C & 75 °C) affect the average concentration (mol dm^{-3}) of salicylic acid determined using the absorbance (± 0.050)?

Temperature (°C) of Acetylsalicylic Acid (± 0.5 °C)	Average Absorbance (± 0.050)	% Uncertainty in average Absorbance	Average Concentration (mol dm^{-3}) of Salicylic Acid	% Uncertainty in the Average Concentration
25	0.637	± 7.85 %	2.083×10^{-4}	± 27.08 %
35	0.771	± 6.48 %	2.521×10^{-4}	± 25.71 %
45	0.928	± 5.39 %	3.034×10^{-4}	± 24.62 %
55	1.126	± 4.44 %	3.863×10^{-4}	± 23.67 %
65	1.491	± 3.35 %	4.875×10^{-4}	± 22.58 %
75	1.954	± 2.56 %	6.389×10^{-4}	± 21.79 %
Average Percentage Uncertainty in Concentration				± 24.24 %

Graph – A scatter graph (with an exponential relationship) has been used to plot the temperature (°C) of acetylsalicylic acid vs. the concentration (mol dm^{-3}) of salicylic acid in the sample because the data obtained is numerical and continuous and hence is best represented by a scatter diagram. The horizontal error bars reflect the equipment uncertainty of the thermometer and the vertical error bars reflect the uncertainty in the average concentration. Whilst propagating the uncertainty in the final average concentration of salicylic acid, the percentage uncertainties were averaged to account for the impact of small readings on the overall uncertainty of the measurement.

Figure 7 *Exponential Curve of temperature of acetylsalicylic acid (25 °C, 35 °C, 45 °C, 55 °C, 65 °C & 75 °C) vs. the average concentration of salicylic acid in the sample (Self-Made by the Candidate on LoggerPro)*



Data Analysis– The percentage error is calculated for the concentration at different temperatures using the theoretical value processed from a report published by Andrew Chen and then is averaged to obtain an overall percentage error. The formula used to calculate the percentage error is $\left|\frac{Theoretical\ Value - Experimental\ Value}{Theoretical\ Value}\right| \times 100$.

Temperature (°C)	Experimental value of average concentration (mol dm^{-3})	Theoretical Value (mol dm^{-3}) *(Chen)*	Percentage Error
25	2.083×10^{-4}	1.936×10^{-4}	14.52 %
35	2.521×10^{-4}	2.153×10^{-4}	17.21 %
45	3.034×10^{-4}	2.623×10^{-4}	15.66 %
55	3.863×10^{-4}	3.430×10^{-4}	12.62 %
65	4.875×10^{-4}	4.122×10^{-4}	18.24 %
75	6.389×10^{-4}	5.529×10^{-4}	15.56 %
Average Percentage Error in Concentration			**15.64 %**

**Comparison between the percentage error and uncertainty is made later in Conclusion*

Conclusion – The research question about investigating "the thermal stability of aspirin" was answered using relevant data collected from the absorbance readings on the spectrophotometer. The results clearly support the hypothesis & present an exponential relationship between the temperature and the concentration of salicylic acid modelled by the line of best fit **y = 0.000124 e$^{0.02105x}$** with a coefficient of determination **(r^2)** of 0.9907 (refer to *appendix C* for calculation) which is extremely close to the value of 1 suggesting that the correlation between temperature and concentration is very strong. This exponential trend is supported by the data values of the average concentration of salicylic acid from 2.083×10^{-4} mol dm^{-3} progressively to 6.389×10^{-4} mol dm^{-3} at 25°C and

75°C respectively. This is explained by Maxwell-Boltzmann's distribution curve that as temperature increases more particles possess the minimum activation energy required to break the ester bond between salicylic acid & acetic acid, causing the formation of salicylic acid to increase exponentially and allowing for more effective collision and increasing the rate of hydrolysis.

Considering the reliability of the extinction coefficient used to process the absorbance at varying temperatures, it is apparent that the percentage uncertainty of the molar extinction coefficient is really high (**19.23 %)** which indicates that there is a high random error due to the fact that the salicylic acid didn't completely dissolve when preparing the stock solution causing the actual concentration to be much lower than expected. The data obtained for the average concentration of salicylic acid is relatively accurate with an average percentage error of **±15.64 %**, suggesting that the results are accurate & valid (close to the theoretical value) due to the accuracy of the method. This is also supported by the fact that the percentage error **±15.64 %** was relatively smaller than the average percentage uncertainty of **24.24 %** which verifies the validity. Despite, the greater validity there is a high random error which could have been contributed by the fact that only a single trial was conducted for the calibration curve, the absorbance readings on the spectrophotometer kept fluctuating & there were impurities present in the cuvette which could have interfered with the absorbance of light. Examining the graph of temperature vs. concentration of salicylic acid, there is evidence of two anomalies, at 45°C and 75°C, (i.e. two points where the error bars do not intersect with the line of best fit) which is due to the fact that although the water bath distributed the heat evenly, there was heat lost to the surroundings, possibly causing the average concentration to be lower than expected.

Placing this in a scientific context, the acceptable threshold of concentration of free salicylic acid in aspirin is **0.15 %** according to LAHC education. However, the concentration of salicylic acid exceeds this threshold very quickly at temperatures after 35°C suggesting that the stability of aspirin is very vulnerable to temperatures higher than ambient temperature. As mentioned earlier in the introduction, this investigation was conducted to investigate the statement in ABCnews about why medicine cabinets are not a suitable place to store medications, the results obtained clearly supports this statement showing that even a small increase in temperature 10-15°C causes a decently large increase in the concentration of salicylic acid reducing the effectiveness of the drug and making patients' tissues vulnerable to damages of salicylic acid. Linking back to the incident in my Uncle's pharmacy, temperatures beyond 25°C cause a significant increase in the concentration of salicylic acid that is certainly capable of minimizing the efficacy of the tablets, which explains the irritation reported by numerous customers.

Strengths & Justifications -

Using a UV-VIS Spectrophotometer - Since a UV spectrophotometer has a greater precision (relative to a calorimeter and a Specto-VIS Plus) and more versatility, it makes the data obtained more precise and reliable. In addition, absorbance on a calorimeter can be measured for only a restricted range of wavelengths, but because a UV spectrophotometer was utilized the absorption spectrum was visible which allowed the peak absorbance to be interpolated from Beer Lambert's plot increasing the validity of the results.
Collection of Relevant Data - The collection of relevant data is evident, since the absorbance of salicylic acid at different temperatures of the solution clearly allowed the concentration of salicylic acid to be determined from the extinction coefficient displaying a strong exponential relationship between the two variables & producing sufficient data.

Evaluation – The following limitations caused the percentage uncertainty to be greater than percentage error:

Limitation		How does it affect the data?	Improvements
Random Error – These factors caused the percentage error to be very high (24.24 %), which is greater than the percentage error, this justifies the numerous limitations in this section.	Impurities present in the plastic cuvette	Although the cuvettes were cleaned with distilled water, some of the cuvettes utilized were used and had scratches on them which increased the scattering of light and hence affected the precision of the absorbance readings on the spectrophotometer.	This can be improved by utilizing new cuvettes and cleaning them with a microfiber cloth.
	Number of Trials conducted	The number of trials conducted within this experiment was 5 which were sufficient enough to calculate an average; identify and eliminate anomalies; and draw a trend, however, a greater number of trials will reduce the random error and reduce the percentage uncertainty. Also for the calibration curve, only one trial was conducted; a greater number of trials for each standard solution will increase the precision of the extinction coefficient.	This can be improved by increasing the number of trials conducted from 5 to 10-12 trials for each temperature and for the calibration curve allowing 3 repeats for precision.
	Fluctuating readings on the UV spectrophotometer	The UV-Vis spectrophotometer displayed varying readings of absorbance of both the standard solutions and the unknown solutions, and measured absorbance kept fluctuating with time randomly which could have increased the random error within the values obtained. And allowing the readings to become stagnant will result in the time allowed for the reaction to occur between the hydrolysis product with Iron (III) Nitrate solution to vary which could affect the intensity of the violet complex.	This can be improved by collecting the first set of data values on the graph obtained on Logger Pro and click collect as soon as a reasonable graph of absorbance vs. wavelength is produced.
Systematic Error – Evidence of small systematic error (15.64%).	Using a water bath to heat the solution	It was assumed that the water bath evenly distributed heat (uniformly) and that no heat & temperature loss occurred between the time span of taking the pH 7 buffer & ethanol mixture out and adding acetylsalicylic acid. However, this assumption was limited by the hole in the lid (to check the temperature) which could yield some heat loss. The time span between adding the acetylsalicylic acid suggests that there was some heat loss and thus a temperature decrease that must have happened which would lead the concentration to much lower than what is theoretically expected.	This can be improved by sealing the lid with tape and styrofoam and adding acetylsalicylic acid powder when the solution is inside the water bath to ensure that there is minimal heat loss.
Data Limitation	The range of IVs used	There were 6 increments of IVs from 25 °C to 75 °C, which were sufficient enough to produce a visible trend however a greater range of IVs would allow for better conclusions to be drawn. In addition, the range of standard solutions used to produce the calibration curve was sufficient to draw a line of best fit and determine the extinction coefficient; however, a greater range of standard solutions would increase the accuracy of this coefficient.	This can be improved by looking at a range of IVs from 15 °C (typically in the fridge) to 90 °C and increasing the range of standard solutions in the calibration curve.

Extensions – The following are plausible extensions to this investigation:

(a)	Investigating the effect of the **concentration of hydroxyl ions** on the chemical stability of aspirin – Since the rate of hydrolysis is dependent upon the pH of the medium, the impact of varying pH on the concentration of salicylic acid could be investigated, by utilizing different buffer solutions. This can have real-life applications when considering the type of container to store aspirin tablets. Other factors like the age of the tablets, the duration of heat applied to aspirin, the percentage yield of aspirin at various temperatures can be examined.
(b)	The chemical kinetics of aspirin tablets at different temperatures can be examined by calculating the rate of degradation of acetylsalicylic acid at various temperatures using the formula **rate = k[A]**: where k is the rate constant of acetylsalicylic acid and A is the concentration. This can also be used to predict the mass of aspirin left within the samples at different temperatures using $\mathbf{\ln A_t = \ln A_0 - K_{app}\, t}$ where A_t the mass of the aspirin, A_0 is the initial mass of aspirin and K_{app} is the apparent first-order rate constant and t is sampling time. And lastly, the half-life of acetylsalicylic acid can be determined using the formula $\mathbf{t_{1/2} = \frac{ln2}{k}}$ where k is the rate constant and $t_{1/2}$ is the time for the amount of aspirin to half, which has potential applications in determining the shelf-life.

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Appendix A – Propagating Percentage Uncertainties for the Calibration Curve

Volume of Salicylic Acid Stock Solution	% Uncertainty in volume (of Salicylic Acid)	% Uncertainty in moles	Volume of Iron Nitrate	% uncertainty in Iron Nitrate	% uncertainty in volume	Total % Uncertainty in concentration	Concentration of Salicylic Acid	Absolute Uncertainty
2.0	1.000000	13.260000	98.000000	1.020408	2.020408	15.280408	0.000130	0.000020
4.0	0.500000	12.760000	96.000000	1.041667	1.541667	14.301667	0.000261	0.000037
6.0	0.333333	12.593333	94.000000	1.063830	1.397163	13.990496	0.000392	0.000055
8.0	0.250000	12.510000	92.000000	1.086957	1.336957	13.846957	0.000522	0.000072
10.0	0.200000	12.460000	90.000000	1.111111	1.311111	13.771111	0.000652	0.000090
			Average % Uncertainty in Concentration			14.23812778		

Figure 8 *Calculations for propagating uncertainties in the concentration of salicylic acid in the samples used for the calibration curve (Conducted on Microsoft Excel Spreadsheet by the Candidate)*

Appendix B – Processing the Average Concentration of Salicylic Acid at Different Temperatures

The average concentration of salicylic acid at different temperatures is determined by dividing the average absorbance with the molar extinction coefficient determined from the calibration curve.

Temperature (°C) of Acetylsalicylic Acid (± 0.5 °C)	Absorbance (± 0.050) of light at a wavelength of 530 nm after 30 mins					Average Absorbance	Average Concentration of Salicylic Acid
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5		
25	0.634	0.641	0.637	0.642	0.630	0.63680	0.000208173
35	0.768	0.776	0.773	0.764	0.772	0.77060	0.000251912
45	0.934	0.924	0.927	0.932	0.921	0.92760	0.000303236
55	1.122	1.128	1.120	1.130	1.130	1.12600	0.000368094
65	1.486	1.494	1.497	1.483	1.494	1.49080	0.000487349
75	1.946	1.952	1.958	1.956	1.958	1.95400	0.000638771

Figure 9 *Calculations for processing the average concentration of salicylic acid at different temperatures (Conducted on Microsoft Excel Spreadsheet by the Candidate)*

Temperature (°C) of Acetylsalicylic Acid (± 0.5 °C)	Absolute Uncertainty in Absorbance	Average Absorbance (± 0.050 arbitrary units)	% Uncertainty in the Average Absorbance	% Uncertainty in the Average Concentration of Salicylic Acid
25	0.05	0.6372	7.8468	27.08
35	0.05	0.7713	6.4826	25.71
45	0.05	0.9281	5.3874	24.62
55	0.05	1.1266	4.4381	23.67
65	0.05	1.4912	3.3530	22.58
75	0.05	1.9543	2.5585	21.79
		Average % Uncertainty		24.24

Figure 10 *Calculations for processing the percentage uncertainties in the average concentrations of salicylic acid (Conducted on Microsoft Excel Spreadsheet by the Candidate)*

Appendix C – Calculations for r^2 (The Coefficient of Determination)

The coefficient of determination (r^2) will be determined using the formula:

$R^2 = \frac{MSS}{TSS} = \frac{TSS-RSS}{TSS}$	Where MSS = Modal Sum of Squares TSS = Total Sum of Squares associated with the outcome variable RSS = Residual Sum of Squares

Finding the linear regression for the **calibration curve** on excel:

	A	B	C	D	E	F
1						
2	X-Values	Y-Values				
3	**Concentration of Salicylic Acid (mol dm-3)**	**Absorbance at at 530 nm (± 0.050)**			Calculations	
4				**Parameter**	**Formula**	**Result**
5	**0.000130**	0.336		r	[=CORREL(A5:A9, B5:B9)]	0.9881426
6	**0.000261**	0.602		R Squared	[=F5*F5]	0.9764257
7	**0.000392**	0.89		R Squared using RSQ	[=RSQ(B5:B9, A5:A9)]	0.9764257
8	**0.000522**	1.463				
9	**0.000652**	1.902				

Figure 11 *Calculations for determining the coefficient of determination for the calibration curve, concentration vs. absorbance of salicylic acid (Conducted on Microsoft Excel Spreadsheet by the Candidate)*

Finding the coefficient of determination for the **exponential graph** ($y = 0.000124\ e^{0.02105x}$) of temperature of acetylsalicylic acid vs. salicylic acid concentration in the sample:

To find the coefficient of determination for the exponential graph, the linear regression between the x values and the logarithm of Y values [$Log_e^{0.02105}(Y)$] was used to determine the strength of correlation.

	A	B	C	D	E	F	G
1							
2	X-Values	Y-Values					
3	**Temperature (°C) of Acetylsalicylic Acid (± 0.5 °C)**	**Average Concentration of Salicylic Acid**	**Log e^0.02105 (Y)**		Calculations		
4					**Parameter**	**Formula**	**Result**
5	**25**	0.000208173	-402.7145412		r	[=CORREL(A5:A10, C5:C10)]	0.9953150
6	**35**	0.000251912	-393.6546669		R Squared	[=G5*G5]	0.9906519
7	**45**	0.000303236	-384.8455666		R Squared using RSQ	[=RSQ(C5:C10, A5:A10)]	0.9906519
8	**55**	0.000368094	-375.637635				
9	**65**	0.000487349	-362.305466				
10	**75**	0.000638771	-349.4519972				

Figure 12 *Calculations for determining the coefficient of determination for the exponential graph of temperature of acetylsalicylic acid and concentration of salicylic acid (Conducted on Microsoft Excel Spreadsheet by the Candidate)*