STABILITY OF SORBIC ACID IN SOLUTION

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THESIS

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I certify that this thesis was prepared under my supervision at the University of Baghdad as a partial requirement for the degree of Master of Science in Pharmacy.

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In view of the available recommendation I forward this thesis for debate by the Examining Committee.

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We, The Examining Committee, after reading this Thesis and examining the student in its content, find it adequate with standing as a Thesis for the degree of Master of Science in Pharmacy.

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SUMMARY

The pH-profile of the rate of disappearance of Sorbic Acid from aqueous solution was determined at room temperature. It was also determined at 65° and sealed conditions. The degradation rate of Sorbic Acid was obtained by determining the concentration of unoxidized Sorbic Acid versus time using a spectrophotometer (U.V. method - λ max 262 mu). An apparent first order rate of degradation was observed. The maximum stability for Sorbic Acid was at pH (4.8-6) at room temperature and at pH 8 at 65° and sealed conditions.

The effect of ionic strength and that of light were studied and showed no effect on the oxidation rate of Sorbic Acid.

The degradation rate was also studied in the presence of Sulfuric Acid at different concentration of Sorbic Acid, Sulfuric Acid and different temperature. Any increase in initial concentration resulted in an increase in the reaction rate. The increase in concentration of Sulfuric Acid also increased the reaction rate and decreased the lag time for the decomposition. The increase in temperature caused a decrease in reaction rate.

CHAPTER I

I N T R O D U C T I O N

1.1 PHYSICAL PROPERTIES AND REACTIONS

Sorbic Acid (trans, trans - 2.4 - hexadienoic acid) CH_3-CH = CH-CH = CH-COOH is a white crystalline solid. It has been used as a preservative for foods, cosmetics and pharmaceuticals for over 25 years because of its antimicrobial activity and low toxicity. It discolors somewhat on storage and is only slightly soluble in water (about 0.16% at room temperature).

Sorbic Acid is found in nature. It was first obtained from rowanberry oil in 1859 (1).

Sorbic Acid forms salts readily with alkalies. The esters, amides and acid chlorides of the acid are formed in the conventional manner.

Sorbic Acid undergoes addition reactions and Diels-Alder reaction with many dienophiles (3).

Oxidizing agents attack the olefinic bonds of Sorbic Acid and autoxidation results in the formation of peroxides followed by degradation and polymerization. Ozone is the only oxidizing agent which attacks both double bonds.

The physical properties of Sorbic Acid are shown in table 1. (2)

Table 1

Chemical Formula	$CH_3CH = CH-CH = CH-COOH$
Molecular weight	112.12
Melting Point	135 - 137°
Solubility: H_2O at 20°	0.16 g/100 ml
Ethanol at 25°	14.8 g/100 ml
Fat & Oil at 20°	0.5 - 1%
Heat of combustion- E	-11.927 btu/lb
Ionization constant K at 25°	1.73×10^{-5}
Odor	Odorless
Taste	Acidic

1.2 METHODS OF PREPARATION

1. Sorbic Acid can be prepared from malonic acid and crotonaldehyde in the presence of pyridine (4)

$$CH_3CH = CH-CHO + H_2C(CO_2H)_2 \xrightarrow[H^+]{\text{pyridine}} CH_3CH = CHCH = CHCOOH + CO_2 + H_2O$$

Sorbic Acid

2. Sorbic Acid prepared commercially from the oxidation of 2,4 - Hexadienal (1) which is obtained by the condensation of three molecules of acetaldehyde as shown below:

$$CH_3-CH=CH-CH=CH-CHO \xrightarrow[air]{O_2} \underset{\text{Sorbic Acid}}{CH_3-CH=CH-CH=CH-COOH}$$

3. Sorbic Acid is also prepared commercially by the reaction of ketene and crotonaldehyde (1) as shown:

$$CH_3-CH=CH-CHO + CH_2=C=O \xrightarrow[\text{Boron trifluride}]{\text{(Acid Catalyst)}}$$

$$\underset{\text{Sorbic Acid}}{CH_3-CH=CH-CH=CH-C(=O)-OH} \xleftarrow[\text{aq.HCl}]{H^+} \underset{\text{polymeric product poly(hexeno-B-lactone)}}{\left(\begin{array}{l}CH=CHCH_3\\ |\\ -CH-CH_2-C(=O)-O-\end{array}\right)_x}$$

1.3 ANTIMICROBIAL ACTIVITY AND MECHANISM OF ACTION

Sorbic Acid falls into a group of short chain organic acids which together with their salts have been shown to exhibit antimicrobial properties.

Thomas et.al (5) studied the influence of Sorbic Acid on the growth of certain species of bacteria, yeasts, filamentous fungi and found that the pH of the culture medium is the principal factor controlling the activity of Sorbic Acid as an inhibitor for microbial growth.

The efficient use of Sorbic Acid as an antimicrobial agent would be at pH value which results in higher concentration of undissociated acid.

All of the organisms studied grew in media containing 0.1% Sorbic Acid at pH 7. The yeasts and filamentous fungi were inhibited in media containing 0.1% Sorbic Acid at pH 4.5, the lactic acid bacteria were inhibited at this concentration of the chemical at pH 3.5.

Leo et.at (6) described the use of Sorbic Acid in media to selectivily favor the growth of catalase negative lactic acid bacteria. The effectiveness of Sorbic Acid was found to depend on its concentration, the type and pH of the medium.

It was also found that 0.12% Sorbic Acid in liver media permitted the growth of lactic acid bacteria and clostridia but inhibited the catalase positive actinomycets, bacteria, molds, yeasts when the pH was in the range of 5-5.5. The use of Sorbic Acid in liver infusion or some equally efficient basal medium was suggested for enrichment and presumptive isolation of catalase (-) bacteria specially Lactobacillus.

F.J. Bandelin (7) studied the effect of pH on the efficiency of various mold inhibiting compounds. He has used twelve compounds at pH 3, 5, 7, 9. The molds used were Chaetomium globosum, Altemaria solani, Pencillium citrinum and Aspergillus niger. The organic acids used such as benzoic, propionic and Sorbic Acids showed loss of activity with the increase of pH. The activity of Sorbic Acid at pH 3 was quite high, at pH 5 was fair, at pH 7 had reduced activity, and the activity failed at pH 9.

Orville Wyss (8) studied the microbial inhibition by food preservatives and suggested that the short chain fatty acids act by competitive inhibition of enzymes.

The mechanisms in the inhibition of microorganisms by Sorbic Acid was studied by George K. et.al (9). They found that the oxidative assimilation of glucose, acetate, succinate and fumurate by washed cells of Escherichia Coli, Pseudomonas aeruginosa and Saccharomyeas cerevisia was inhibited by concentration of Sorbic Acid ranging from 15 to 105 mg/100 ml.

Oxidative phosphorylation by submicroscopic particles of E. Coli was reduced by about 30% by 37 mg/100 ml of Sorbic Acid. The sulfhydryl enzymes fumarase, asparatase and succinic dehydrogenase were inhibit by Sorbic Acid. The loss of activity of Sorbic Acid after reacting with cysteine suggested that a thiol addition occurred which is believed to be the mechanism of action against sulfhydryl enzymes or cofactors which contain SH-group in their molecules. As a result of this work the inhibition of various enzymetic reactions by Sorbic Acid has been suggested as the mechanism by which microbial growth is inhibited.

John A. Troller (10) found the catalase inhibition as a possible mechanism of the fungistatic action of Sorbic Acid and this inhibition is related to Sorbic Acid concentration, pH of the system and length of time that the catalase was exposed to the acid.

1.4 TOXICITY AND METABOLISM

The toxicity of Sorbic Acid is extremely low, it is one third of that of benzoic acid. The U.S. Food & Drug Administration recognized Sorbic Acid as generally safe.

Gunnel Westoo and Others (11,1) have found that Sorbic Acid is metabolized into CO_2, H_2O in the same manner as other unsaturated fatty acids normally found in food (β - oxidation).

1.5 RELATED STABILITY STUDIES

Before discussing kinetics of Sorbic Acid in solution it may be of value to review some experimental conditions and results for related stability studies.

The autoxidation of ascorbic acid in sealed containers was studied by J.C. Bauernfeind (12). The results indicated that the destruction of ascorbic acid is directly proportional to the amount of available oxygen (O_2) in the container.

The decomposition rate of ascorbic acid in the presence and absence of O_2 at different pH and fixed ionic strength was studied by Krowezynski et.al (13). They found that the oxidation of ascorbic acid occured rapidly at pH 7.05, whereas in the absence of oxygen it takes place more rapidly at pH 3.3. On the basis of these results the workers concluded that higher stability of ascorbic acid solution is obtained by excluding O_2 and adjusting the pH from 6-7.

A. R. Rogers et.al (14) studied the effect of pH on aerobic degradation of ascorbic acid, and they found that the degradation rate shows maximum near pH 4 and minimum near pH 5.6 (temperature 25°).

Other reports on the anaerobic degradation of ascorbic acid indicate that the maximum degradation rate is at pH 4.

This was believed to be due to the formation of a I:I Complex between an ascorbic acid molecules and a hydrogen ascorbate ion which would be present in higher concentration at pH 4. The degradation rate has been found to depend on the concentration of ascorbate ions.

Yasutara Shinkawa (15) studied the stabilization of salts of Sorbic Acid. He found that by evacuation and keeping the solution of salts of Sorbic Acid in CO_2 could prevent the loss of antiseptic strength of solutions. The antiseptic strength can be lost by polymerization which is caused by dissolved O_2.

Takasu - et.al (16) found that the addition of O-phenylphenol and/or p-phenylphenol to powdered Sorbic Acid salt was effective for its stabilization.

Heintze K. (17) has found that Sorbic Acid underwent an equal to or less than 3% decomposition after 30 months at 20° in pasterized apple, black current and grape juice and $\approx$ 21% decomposition after 16 months in herring salad and 25% decomposition after one month in aqueous solution.

CHAPTER 2

EXPERIMENTAL METHODS AND RESULTS

2.1 EXPERIMENTAL METHODS

2.1.1 Materials

a) Distilled water

b) Buffer solutions: (18)

1. Acetate buffer solution (pH 4.8) made of glacial acid (chemically pure) and sodium acetate (A.R. Riedel-Dehaenag.)

2. Sorenen phosphate buffer solution (pH 6 and pH 8) made of disodium hydrogen orthophosphate (anhydrous. BDH-laboratory reagents), and potassium dihydrogen phosphate (pure, fluka AG Buchs SG).

3. Clark and Lub's potassium chloride-Hydrochloric acid buffer solution, (pH 1 and pH 2) prepared from hydrochloric acid (Sp. Gr. 1.18, BDH laboratory reagent) and from potassium chloride (E. Merck Darmstadt)

c) Sulfuric Acid Analytical grade (E. Merck)

d) Standard buffer solutions -
pH 4 - citrate
pH 7 - phosphate
} (E. Merck - Darmastadt)

e) Sorbic Acid (CH_3-CH=CHCH=CHCOOH) (E.Merck)

2.1.2 Apparatus and containers used

a) Spectrophotometers

1. Model Q.V. 50

(Shimadzu. Seisakusho Ltd. - Scientific Instrument Plant - Kyoto, Japan).

2. Model Sp. 800 A

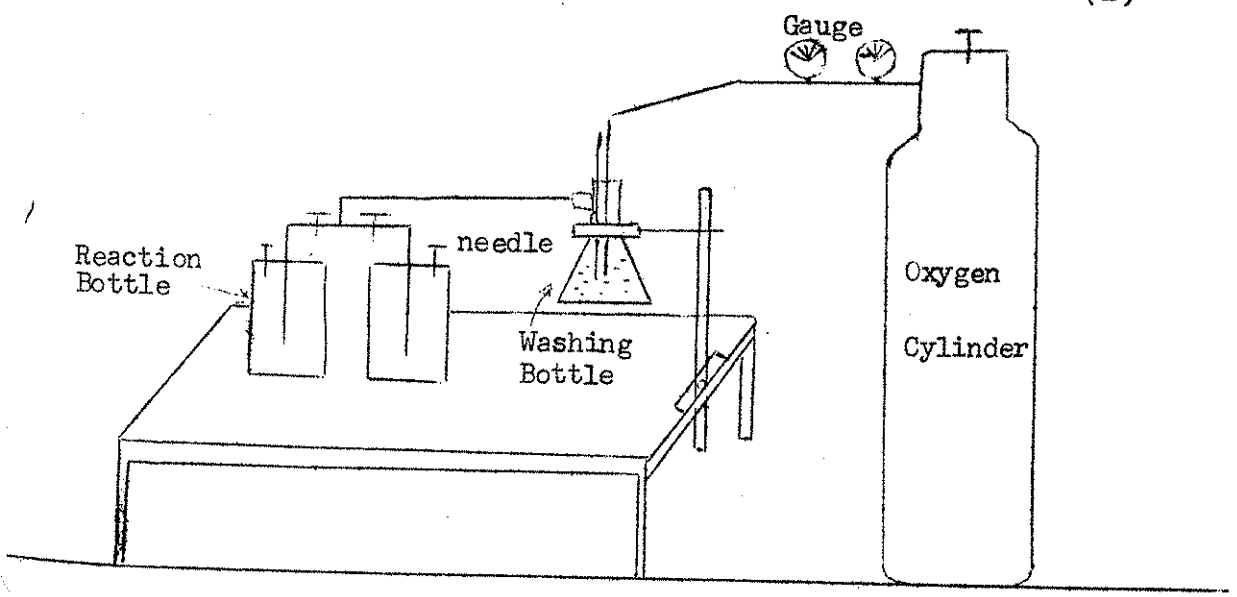
PYE Unicam, England

3. Model Sp. 500

PYE Unicam, England

b) pH - Meter - PYE Unicam - Model 292 MK 2

c) An apparatus designed to study the oxidation of Sorbic Acid as illustrated in the sketch (1)



Sketch (1) Assembly used for oxidation studies

2.1.3 General Method

The Sorbic Acid solutions in the different solvents were prepared as in 2.2 and stored at different temperatures. Samples were withdrawn at different intervals, diluted to appropriate volumes and analyzed for Sorbic Acid content by measuring the absorbence using a spectrophotometer at (262) mu. The absorbence was changed to concentration using a standard curve which was prepared under the same experimental conditions Fig (1).

Any possible inteference by the buffer component to the analytical method was checked experimentally Fig (2). No such interference was observed. The expirements were run in triplicate and the results are representing the mean in each case.

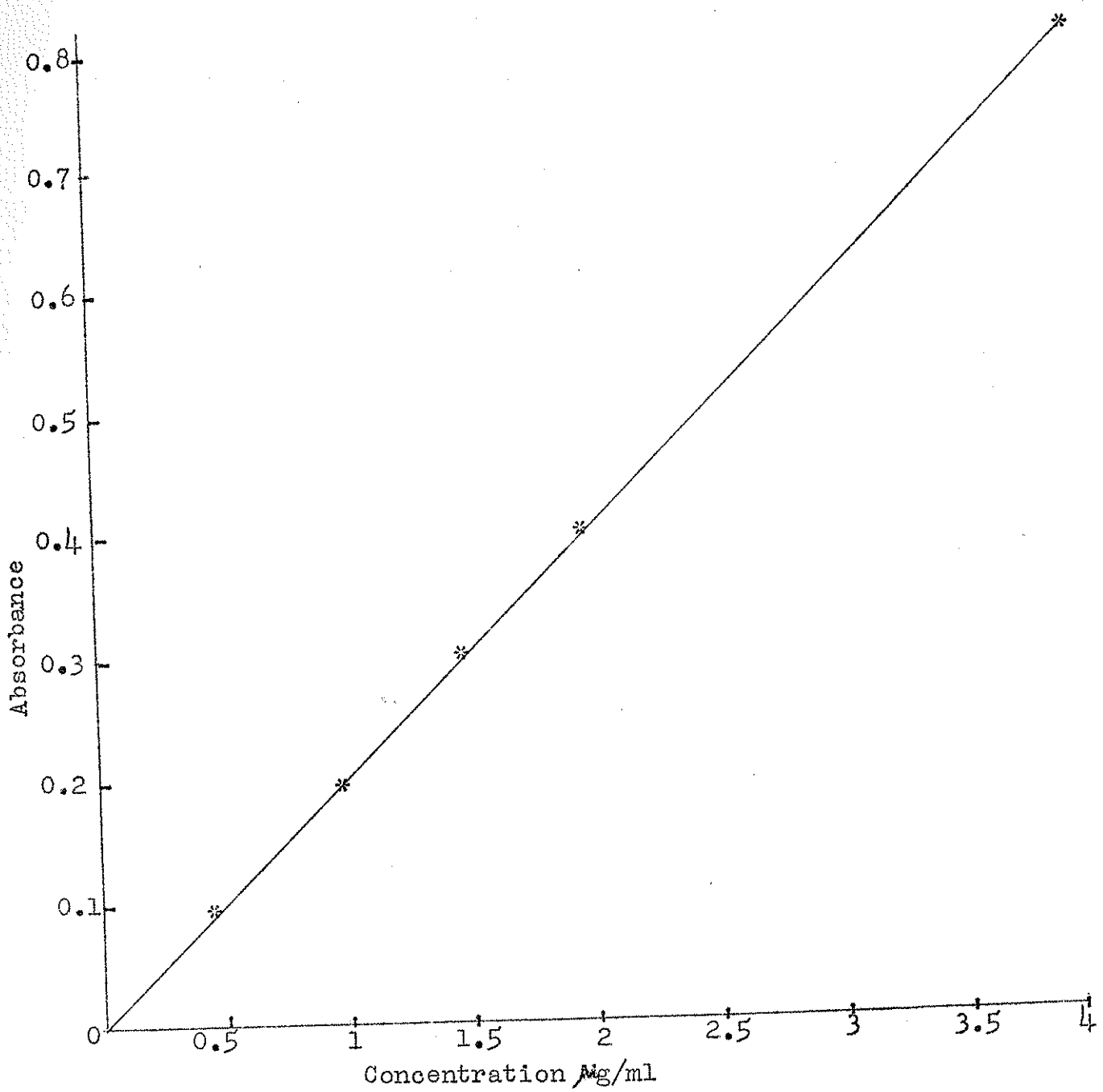


Fig (1) Standard U.V. Curve for sorbic acid at 262 mu

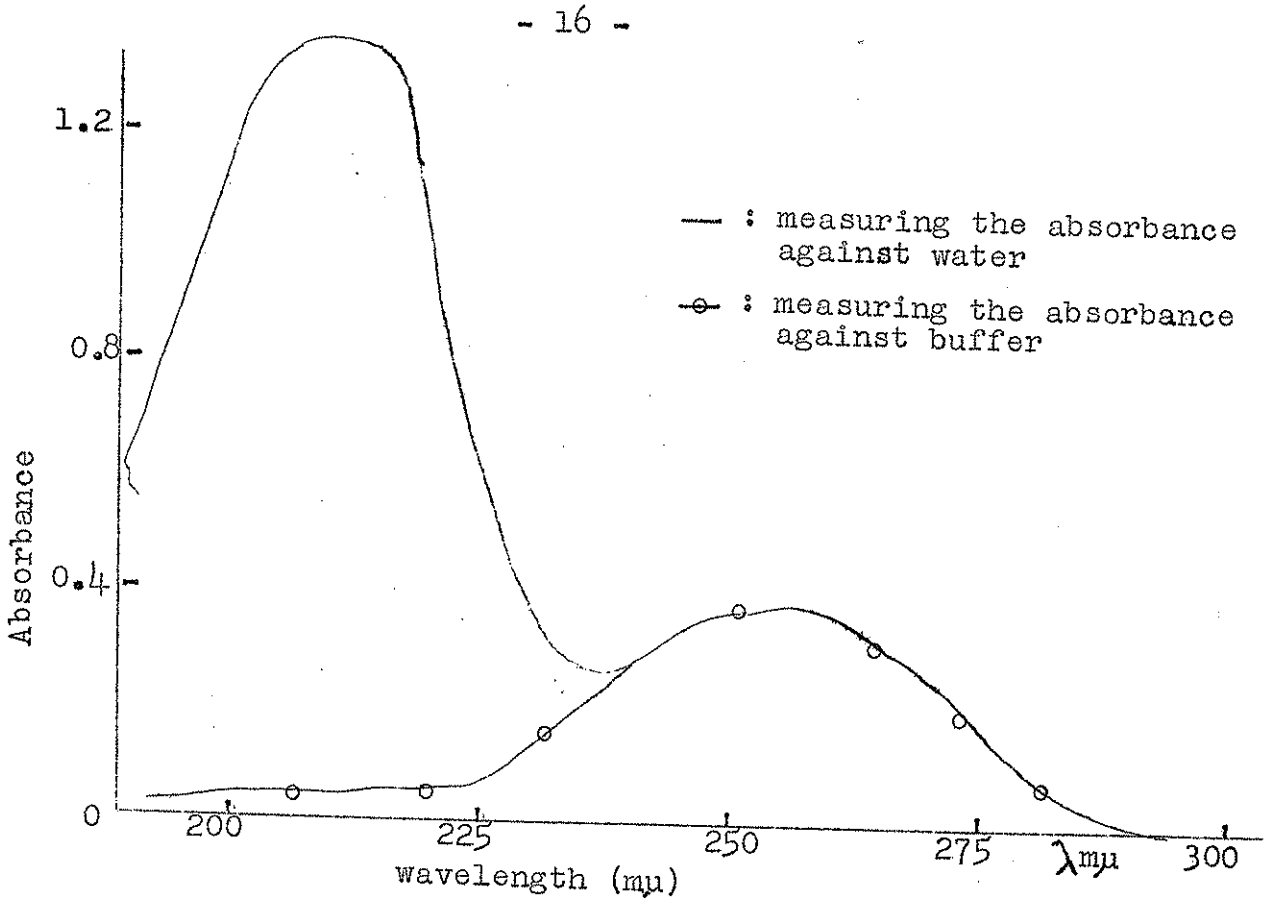


Fig (2a) Acetate buffer

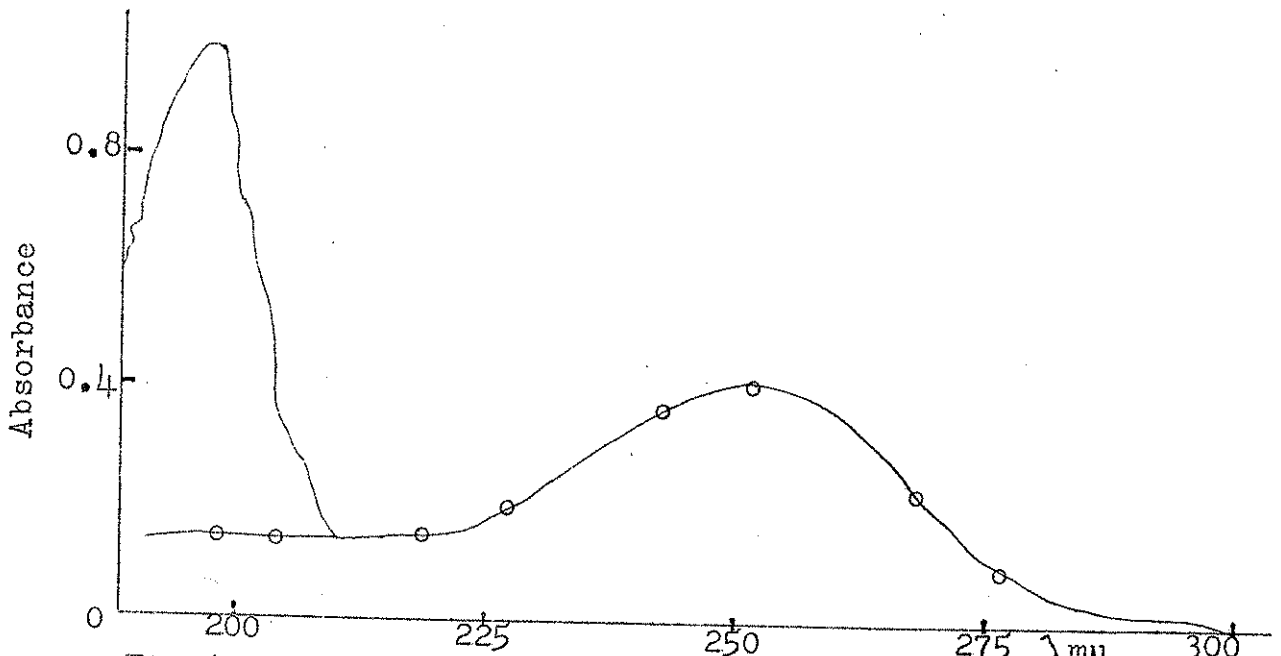


Fig (2b) Phosphate buffer (pH 6 and pH 8)

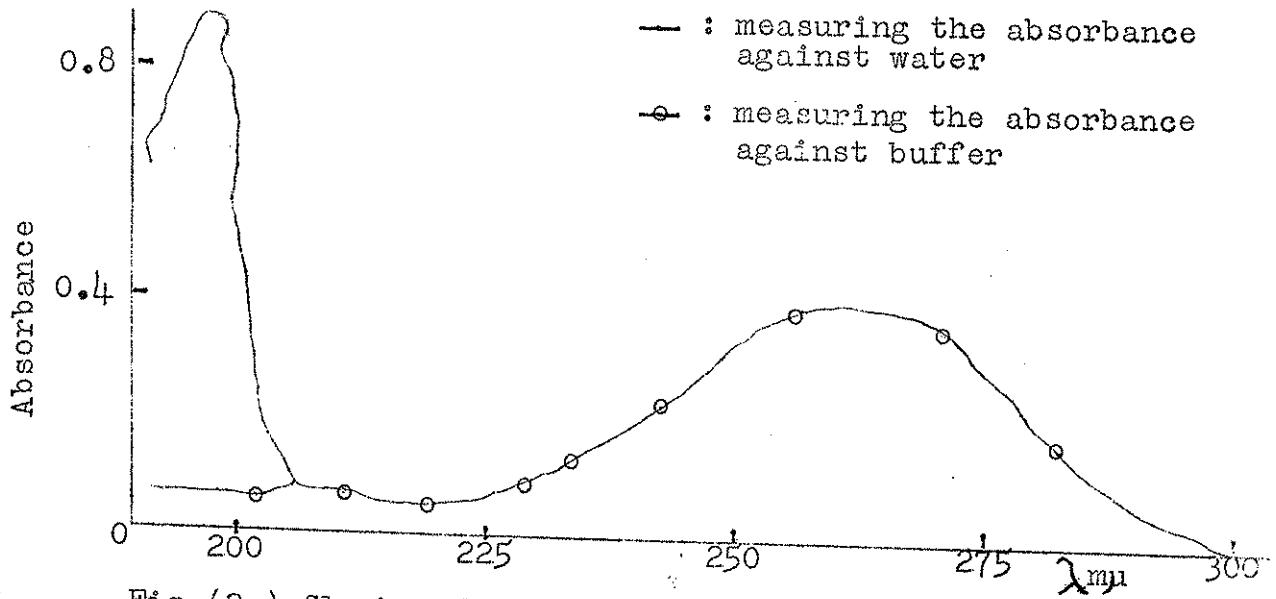


Fig (2c) Clark and Lubus KCl-HCl buffer (pH 1 & pH 2)
Fig (2a-c) Effect of pH on U.V. absorption by sorbic acid

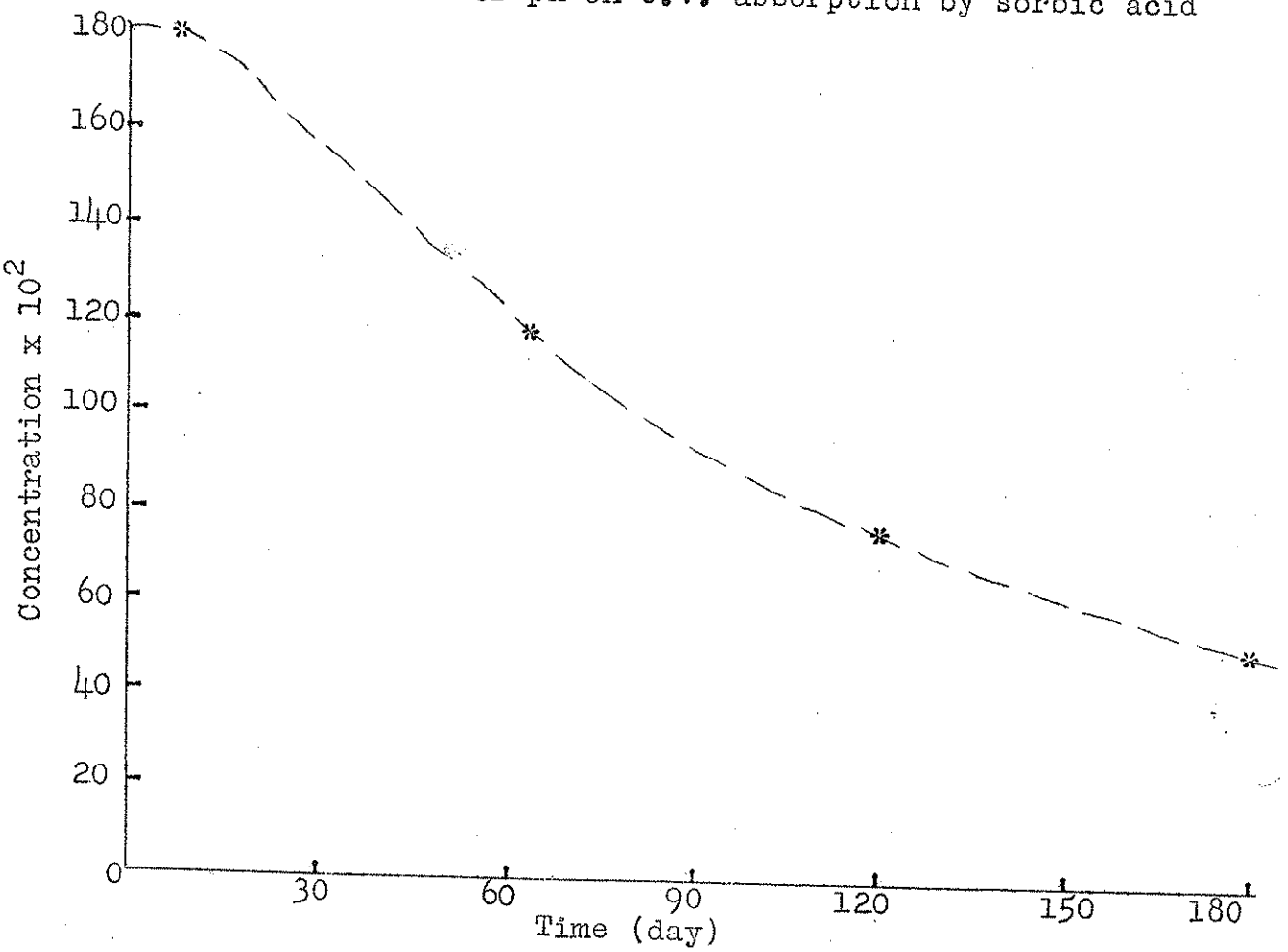


Fig (3) Concentration of sorbic acid as a function of time at pH2 and 65° at fixed ionic strength

2.2 RESULTS

2.2.1 Effect of Ionic Strength (μ)

The effect of ionic strength on the stability of sorbic acid solutions was studied under different experimental conditions as follows:

a) Effect of Ionic Strength on the Stability of Sorbic Acid at pH 2 and 65° :

Sorbic acid solutions (0.1%) at pH 2 and different ionic strength (0.1. 0.2, 0.3, 0.4 and 0.5) were prepared. The ionic strength was adjusted by the addition of Potassium Chloride (KCl) as shown in Table 2.

The amount of (KCl) added for this purpose was calculated from the following equation (19):

$$\mu = \tfrac{1}{2} \sum (C \times \text{Valence}^2)$$

where

C = Molar concentration of all ions in the solution

V = Valence

μ = Ionic strength

Twenty five milliliter (ml) solutions were placed in (30) ml amber colored bottles and stored at 65°.

Samples were then withdrawn and analyzed at different intervals. Fig (3) shows the relationship between the concentration of Sorbic Acid and time. Fig (4) shows the straight line relationship between the logarithm of concentration of Sorbic Acid and time which indicate that the decomposition was according to first order kinetics. From the graph the decomposition was very slow initially and became faster after about a week. The concentration was about similar in all ionic strengths as shown in Fig 4. This indicates that the ionic strength has no effect on the decomposition of Sorbic Acid. The rate constant for the decomposition was 0.007 day^{-1}.

b) <u>Effect of Ionic strength on the stability of Sorbic Acid at pH 4.8 at 65°</u>

This was studied under the same experimental conditions as in (a) except that the pH of the different solutions was 4.8. The results obtained are shown in Fig (4). The graph representing the decomposition of Sorbic Acid at pH 4.8 was similar to that at pH 2.

Table 2

Amounts of Potassium Chloride needed for adjusting the ionic strength

(μ) ionic strength of solution	Potassium Chloride (Mole/litter of solution)		
	pH 4.8	pH 2	pH 1
0.1	0.0388	0.0394	-
0.2	0.1388	0.1394	-
0.3	0.2388	0.2394	0.153
0.4	0.3388	0.3394	-
0.5	0.4388	0.4394	0.353
0.6	-	-	-
0.7	-	-	0.553

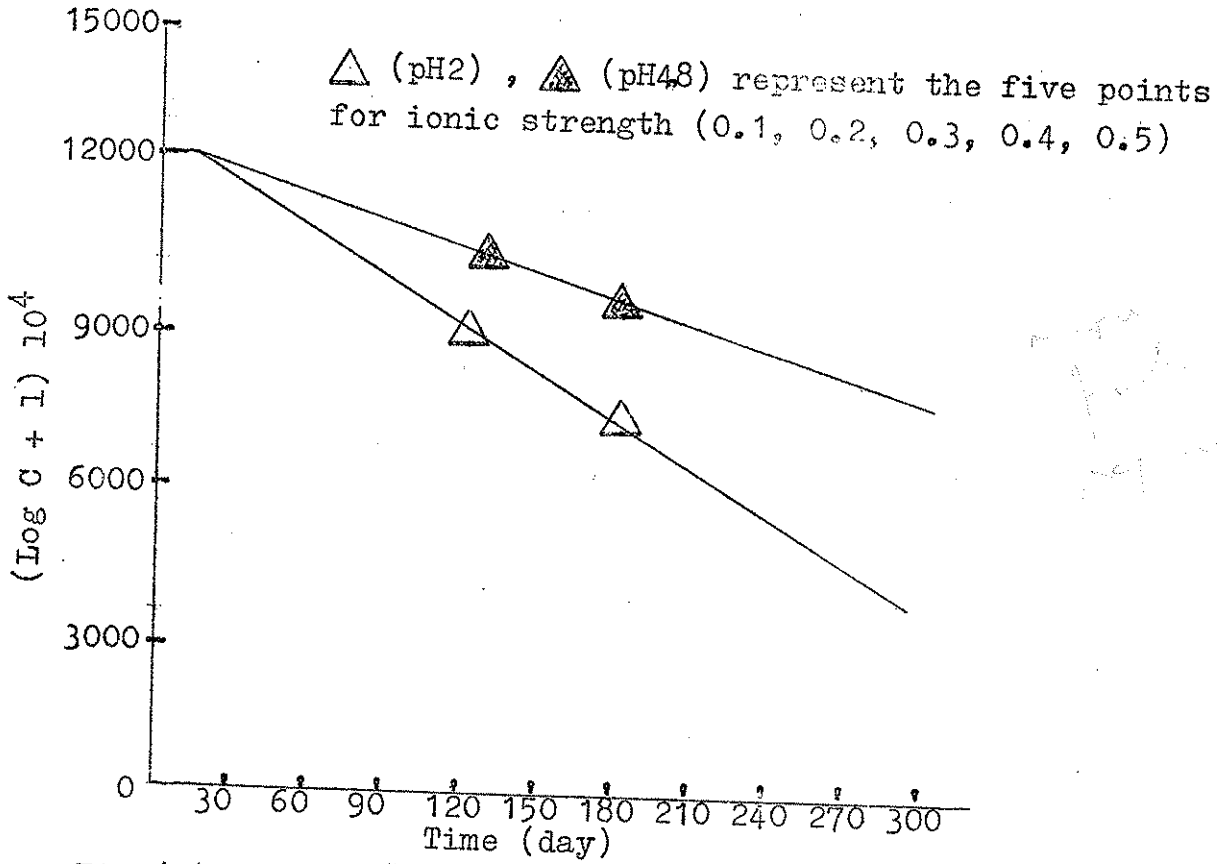


Fig (4) Effect of ionic strength on the rate of decomposition of sorbic acid at pH2 & pH4.8

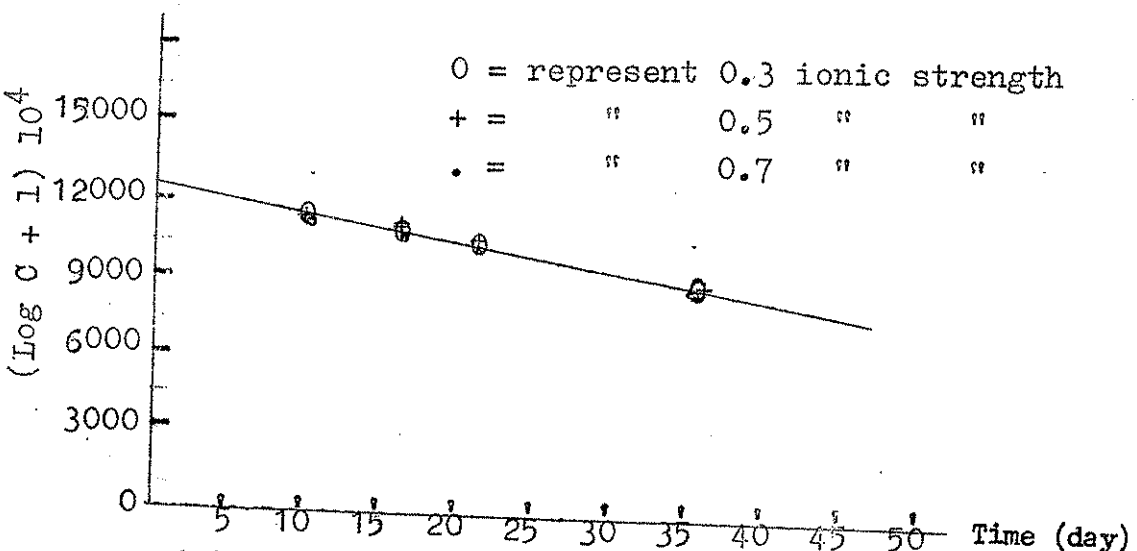


Fig (5) Effect of ionic strength on the rate of decomposition of sorbic acid at pH1 and 65° and under sealed conditions

Here again the ionic strength showed no effect on decomposition. The rate constant for the decomposition was 0.0036 day^{-1}.

c) Effect of Ionic strength on the stability of Sorbic Acid at pH 1 and under sealed conditions:

Different Sorbic Acid solutions were prepared as described under(a) and (b) except that the solutions here were at pH 1 and that the ionic strengths selected were 0.3, 0.5, 0.7. Two milliliters from each solution was introduced separately in 10 ml ampoules, using a pipette. The ampoules were then sealed, using a sealing apparatus and tested for leakage. Those ampoules that are properly sealed were stored at 65°. Each sample was analyzed at zero time at room temperature. Ampoules from each ionic strength were then opened at different intervals after cooling to room temp., and 1 ml from each was withdrawn, diluted to 500 ml in a volumetric flask and analyzed for Sorbic Acid content. The amount of (KCl) added for adjusting the ionic strength are shown in Table 2.

Fig (5) shows the results obtained. These results indicate that the ionic strength has no effect on the decomposition. The rate constant for the decomposition was 0.02 day^{-1}.

d) Effect of Ionic strength on the stability of Sorbic Acid at pH 4.8 in the presence of excess oxygen:

Solutions of sorbic acid 0.1% were prepared. The pH of these solutions was 4.8. The ionic strength was adjusted to 0.1, 0.3 and 0.5 by using KCl. Fifteen milliliters portions from the prepared solutions were placed separately in 30 ml white bottles and stored at room temperature. The solutions were analyzed at zero time for sorbic acid and oxygen was then passed in each solution for 15 minutes using an apparatus which was constructed for this purpose. A sketch of this apparatus is shown in sketch (1). The oxygen pressure was equal to 10 cm water column. Samples were then withdrawn at different time intervals and analyzed for sorbic acid. After each analysis, oxygen was passed in the same manner described above.

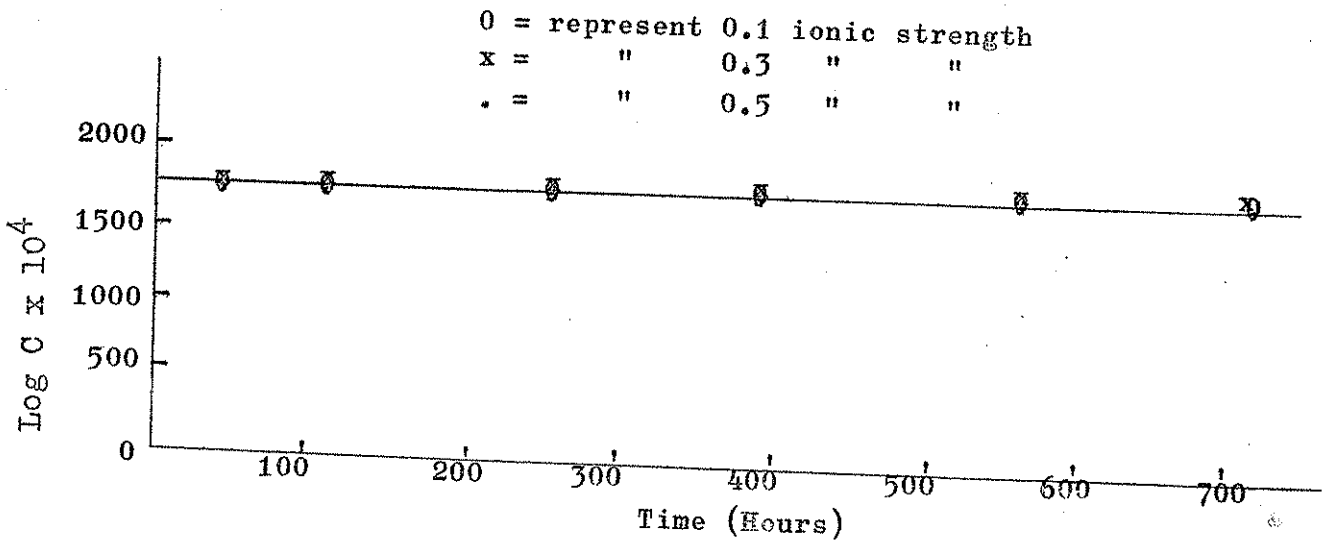


Fig (6) Effect of ionic strength on the rate of decomposition of sorbic acid at pH 4.8 and room temperature in presence of excess of oxygen

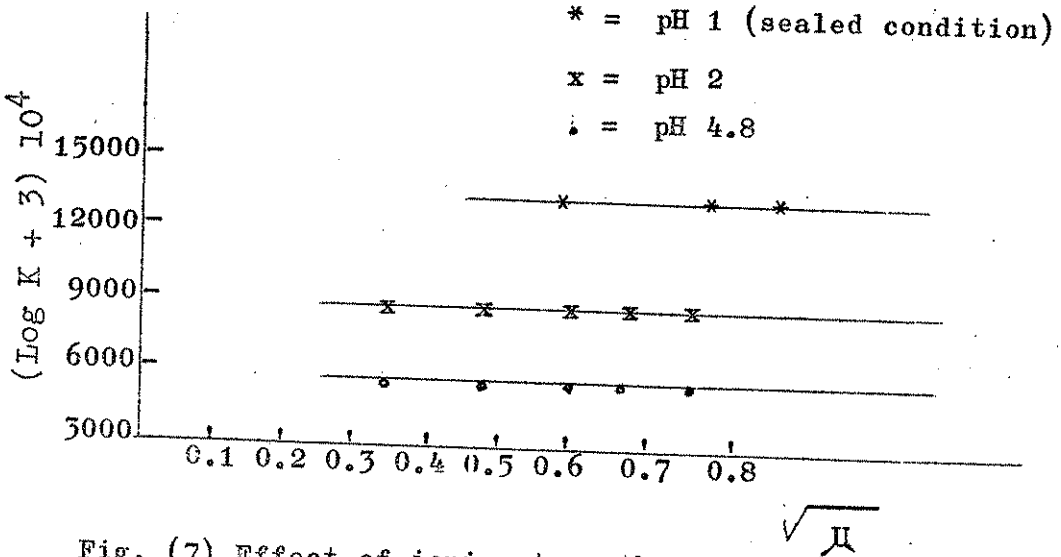


Fig. (7) Effect of ionic strength on the oxidation of sorbic acid at 65° and at various pH values.

The results are illustrated in Fig (6). These results indicate that the ionic strength has no effect on the stability of Sorbic Acid. The relationship between the ionic strength and rate constant, is usually expressed by the following equation:

$$\log K = \log Ko + 1.02\ Z_a\ Z_b\ \sqrt{\mu}$$

Fig (7) represents the graphs obtained by plotting the logarithm of the rate constant against the square root of the ionic strength in each case.

2.2.2 <u>The effect of pH on the stability of Sorbic Acid at room temperature:</u>

Sorbic Acid solutions of the same concentration 0.1% but different pH (1, 2, 4.8, 6, 8) were prepared. Fifteen milliliter portions of these solutions were placed separately in 30 ml white bottles and stored at room temperature. Samples were then taken from each bottle at different intervals and analyzed for sorbic acid. The results shown in table (3) and depicted in Figs (8-11).

Table 3

Kinetic Parameters for the decomposition of Sorbic Acid at different pH and room Temperature

pH	K (day $^{-1}$)		t 1/2 (day) for rapid stage
	slow stage	rapid stage	
1	0.004	0.0492	14.09
2	0.003	0.0126	55.00
4.8	0	0	-
6	0	0	-
8	0.0026	0.014	49.5

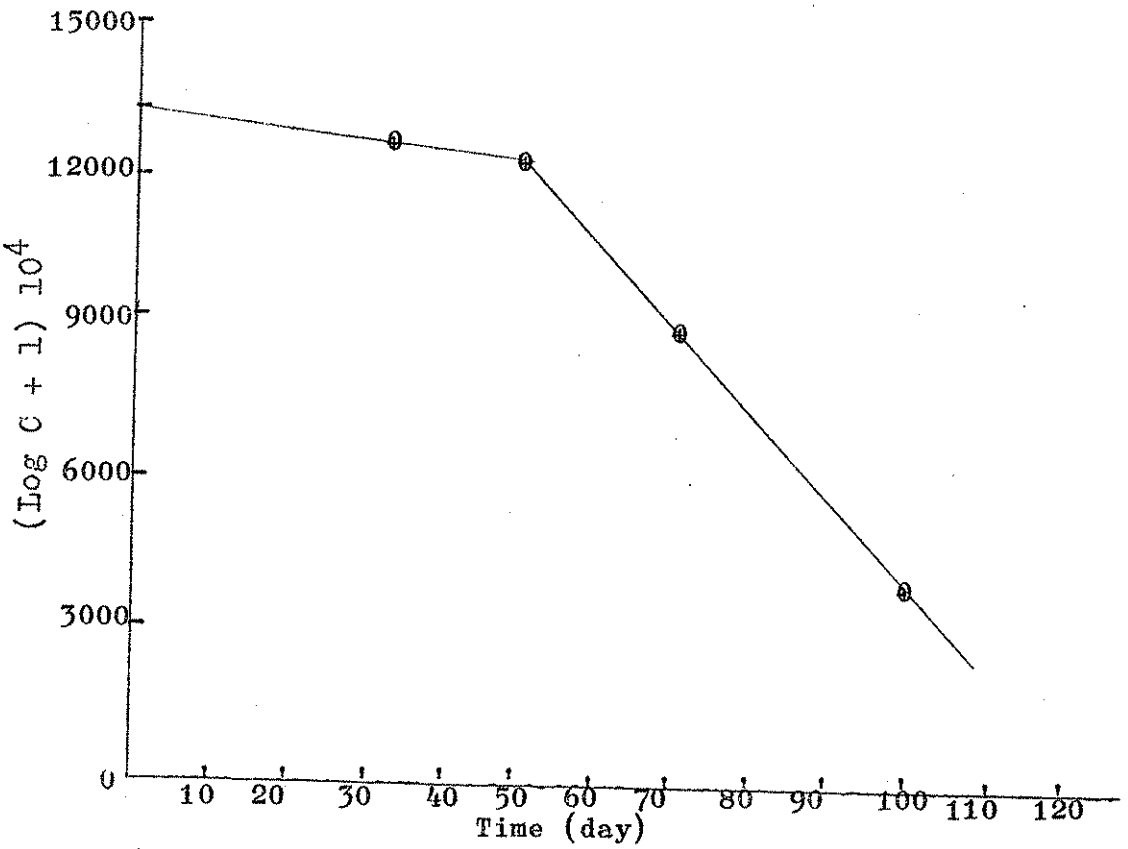


Fig (8) Oxidation progress of sorbic acid at pH1 & room temperature

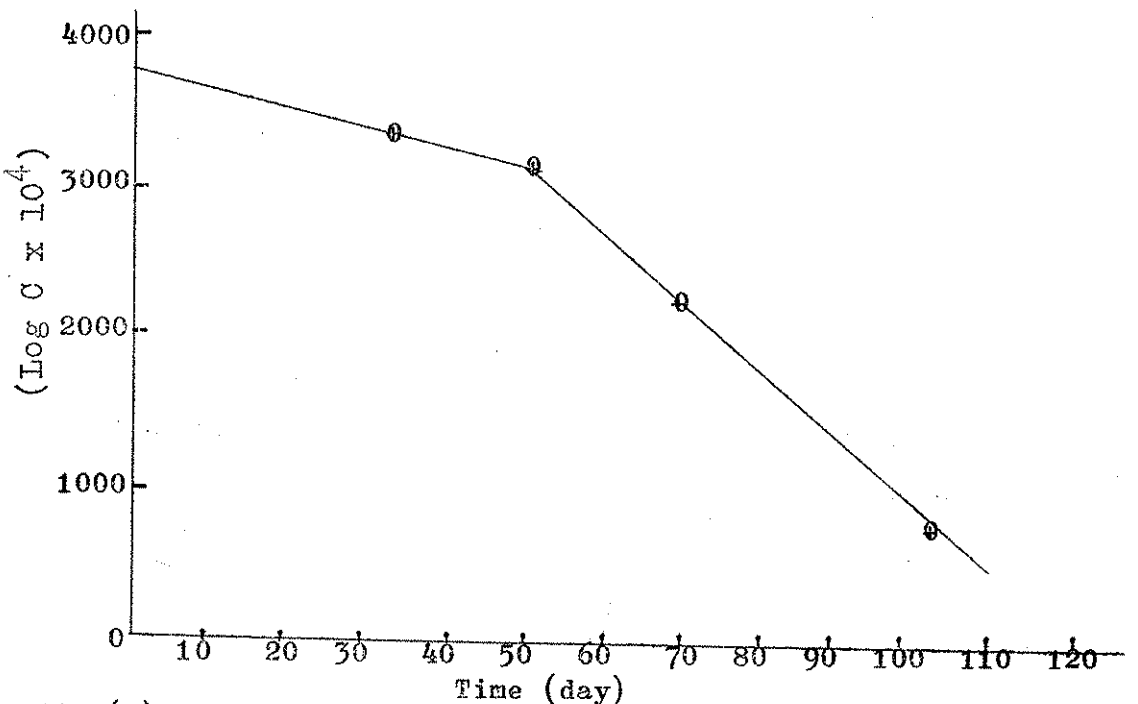


Fig (9) Oxidation progress of sorbic acid at pH 2 & room temperature

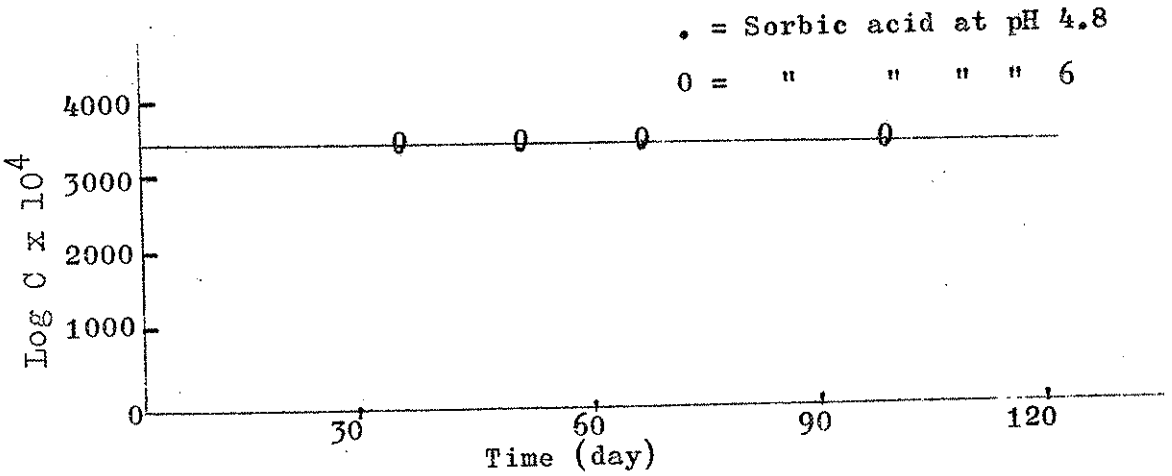


Fig (10) Effect of pH 4.8 and pH 6 on decomposition of Sorbic acid at room temperature

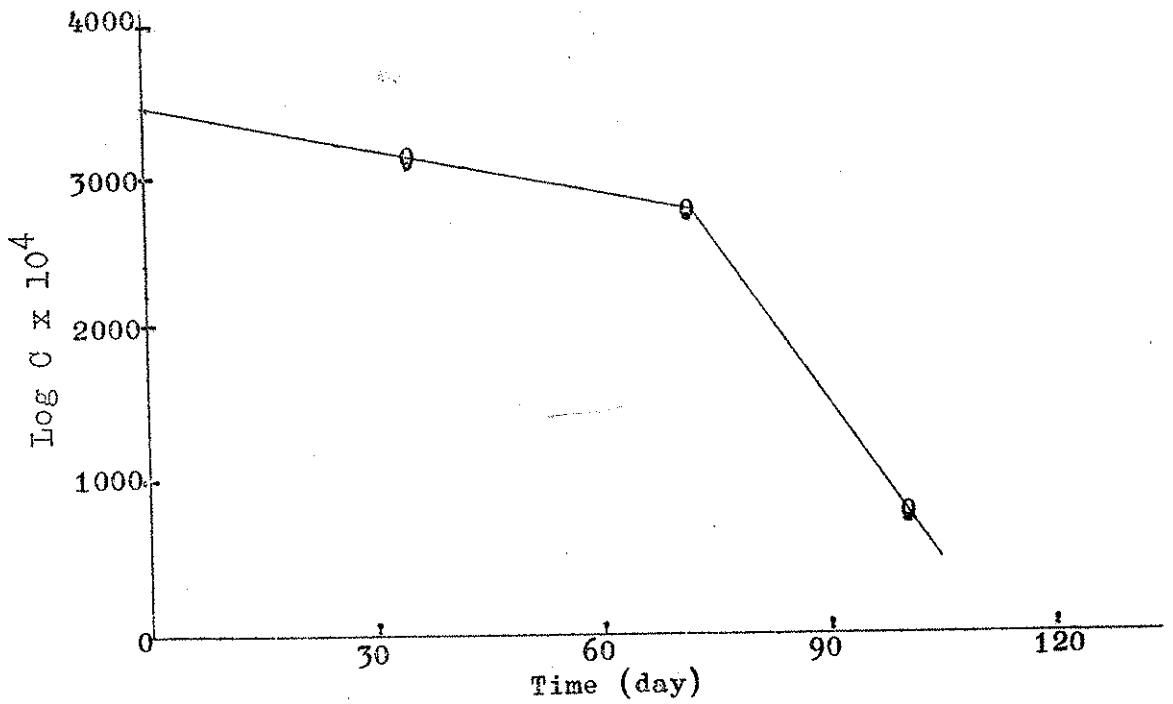


Fig (11) Oxidation progress of sorbic acid at pH 8 and room temperature

These figures indicate that:

At pH 1: The decomposition was slow at the beginning and then became faster after 52 days. The rate constant for the decomposition during the rapid stage was calculated from the slope of the graph constructed and found to be 0.0492 day^{-1}.

At pH 2: The shape of the graph is similar to that at pH 1 but the decomposition was slower. The rate constant for the decomposition was 0.0126 day^{-1}.

At pH 4.8 No decomposition could be observed at this pH.

At pH 6: The results were similar to those at pH 4.8.

At pH 8: As in the case of pH 1 and pH 2 there were two stages for the decomposition of sorbic acid but at this pH the slower stage was longer. The faster stage started after 72 days. The rate constant for the decomposition was 0.0142 day^{-1}.

The rate-pH profile for the decomposition of sorbic acid at room temperature is further illustrated in Fig (12) where the rate constant for the decomposition was plotted against pH. From the graph it could be seen that the decomposition was more rapid at pH 1 and slower at pH 2 and pH 8. No decomposition was observed at pH 4.8 and pH 6.

2.2.3 Effect of pH on stability of Sorbic Acid under sealed condition at 65°:

Sorbic Acid solutions 0.1% were prepared at different pH 1, 2, 4.8, 6, 8. Two milliliter portions from each solution were placed separately in 10 ml ampoules, sealed and tested for leakage. Those ampoules with no leakage were stored at 65°. The zero time analysis was done at room temperature. Sample ampoules are then cooled to room temperature at different time intervals and then analyzed for sorbic acid. The results of this experiment are shown in Table (4) which are illustrated in Figs (14 - 18). These results indicate the following:

At pH 1: Decomposition was slow at the beginning then became faster after 23 days. The rate constant for the decomposition at rapid stage was 0.0087 day^{-1}.

At pH 2: The results for this pH were about similar to that for pH 1 but the rate of decomposition was slower in both stages. The rate constant for the rapid stage was 0.0057 day^{-1}.

At pH 4.8 No decomposition could be observed during the first 23 days, but decomposition started after this period. The rate constant for this decomposition was 0.005 day^{-1}.

At pH 6: At this pH again there was no decomposition during the first 23 days but it proceeded faster than that at pH 4.8 after this period. The rate constant for the decomposition was 0.017 day^{-1}. The solutions which were colorless initially became yellow during the last period of the experiment.

At pH 8: Here the decomposition started from the beginning but became faster after 30 days. The rate constant for the rapid stage of the decomposition was 0.0027 day^{-1}.

Table 4

Kinetic Parameters for the decomposition of Sorbic Acid at different pH under sealed condition at 65°

pH	K (day $^{-1}$)		t 1/2 (day) for rapid stage
	slow stage	rapid stage	
1	0.0057	0.0087	79.66
2	0.0034	0.0057	121.58
4.8	0	0.005	138.60
6	0	0.017	40.76
8	0.0013	0.0027	256.67

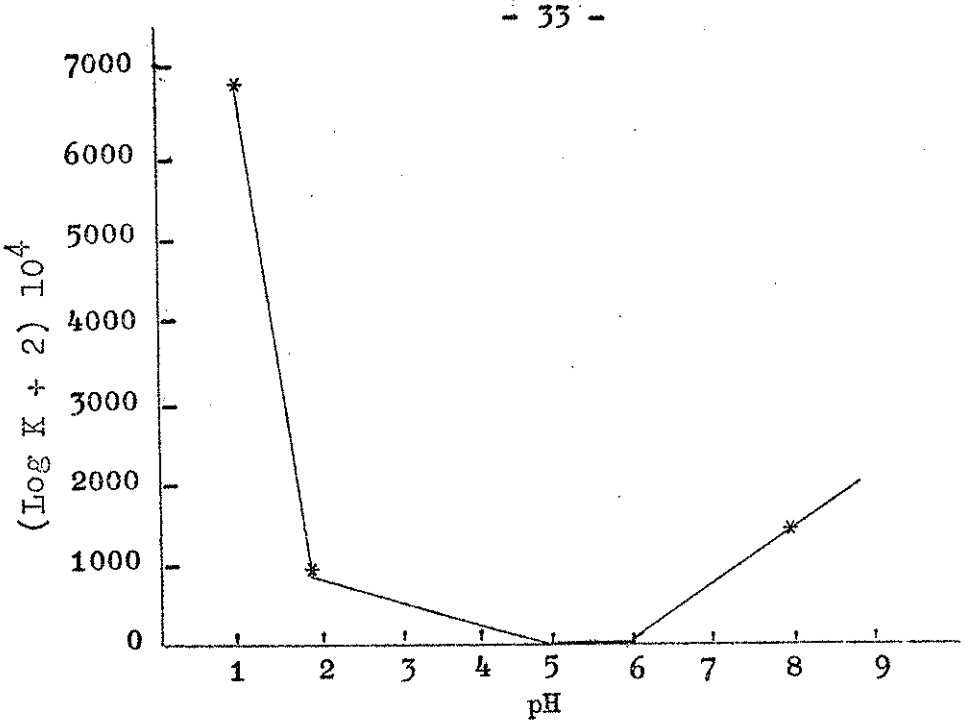


Fig (12) pH-rate profile for the decomposition of Sorbic acid at room temperature.

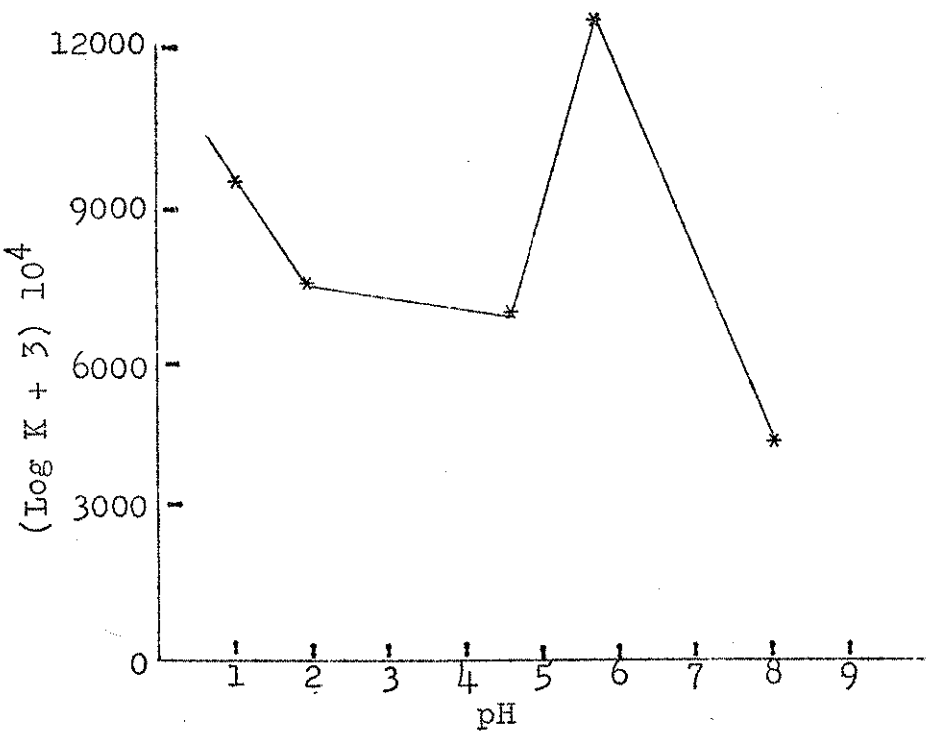


Fig (13) pH-rate profile for the decomposition of sorbic acid at 65° under sealed conditions

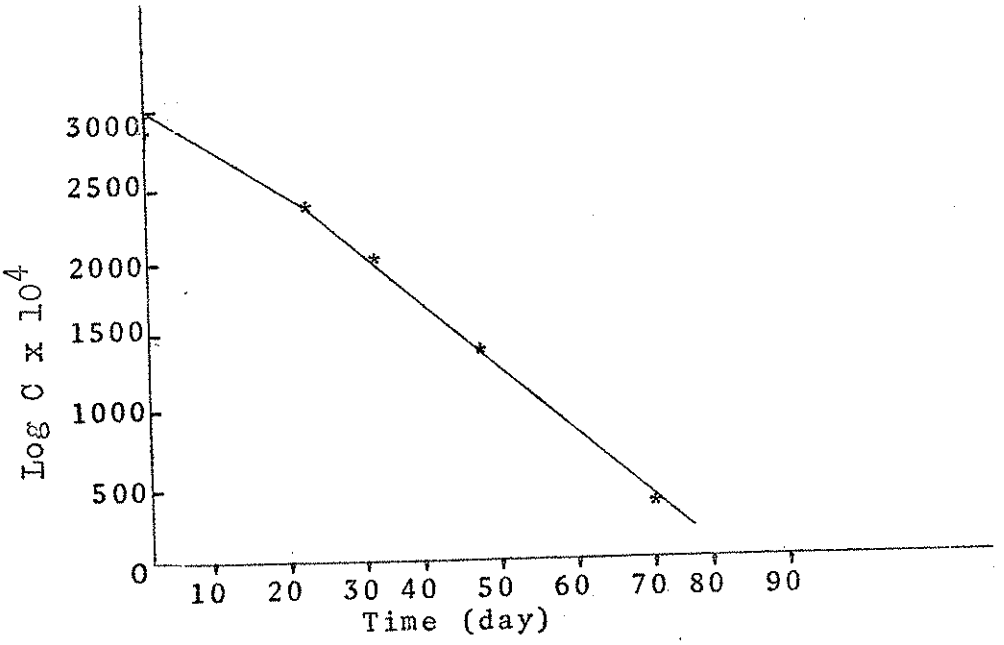


Fig (14) Oxidation progress of sorbic acid at pH 1 and 65° under sealed condition

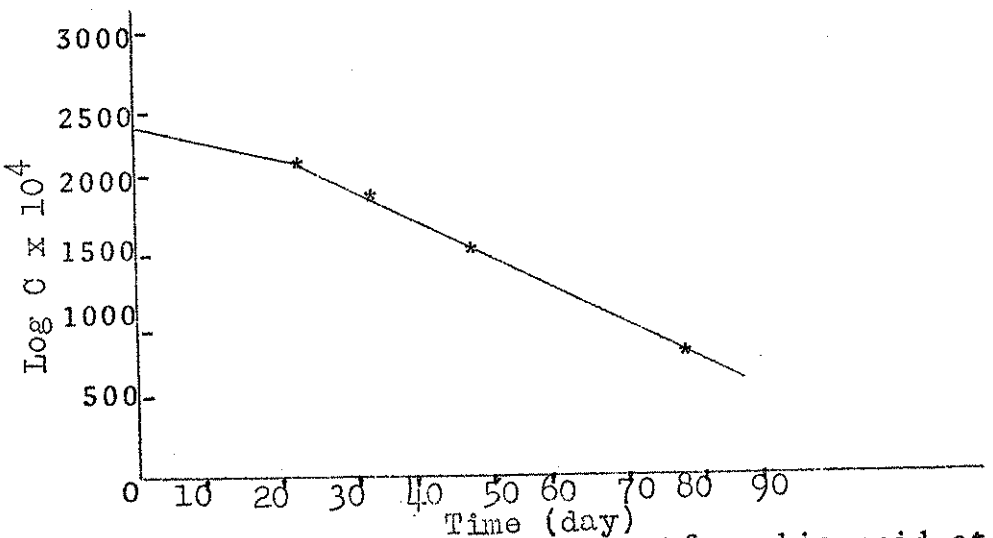


Fig (15) Oxidation progress of sorbic acid at pH2 and 65° under sealed condition

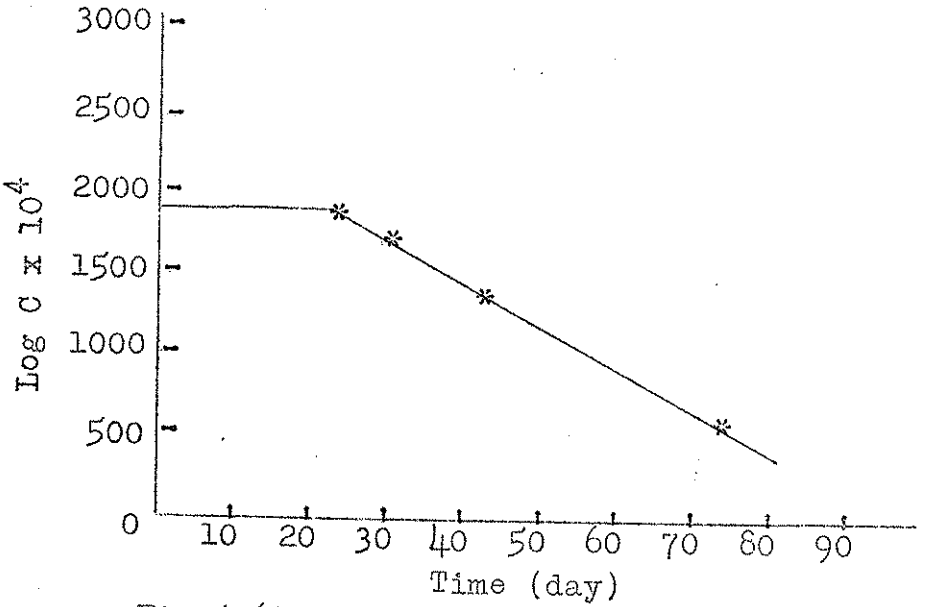


Fig (16) Oxidation progress of sorbic acid at pH 4.8 and 65° under sealed condition

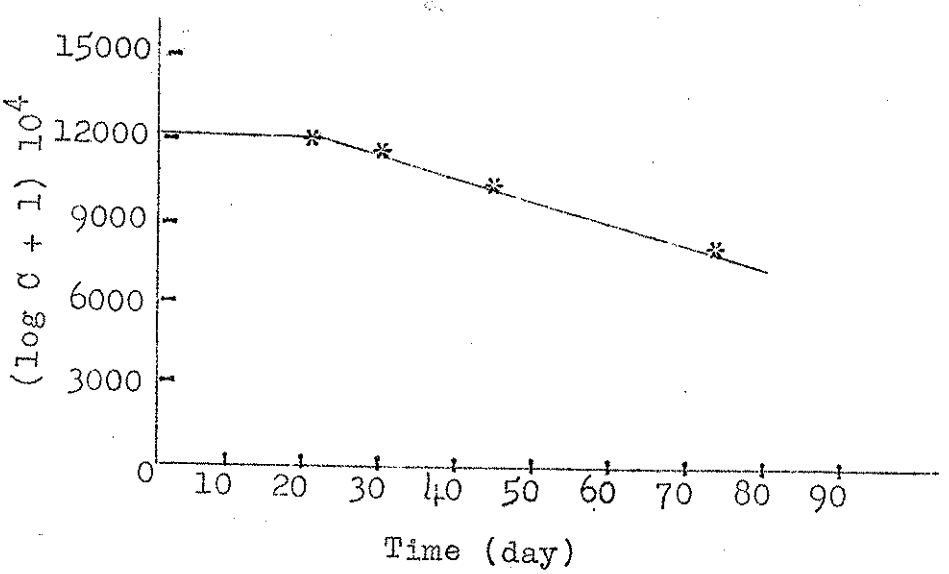


Fig (17) Oxidation progress of sorbic acid at pH 6.0 and 65° under sealed condition

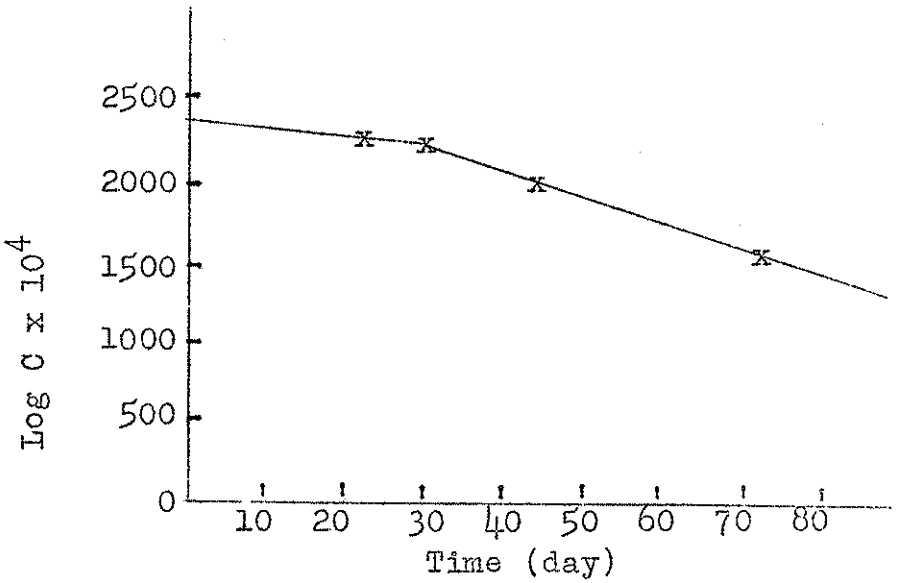


Fig (18) Oxidation progress of sorbic acid at pH 8 and 65° under sealed condition

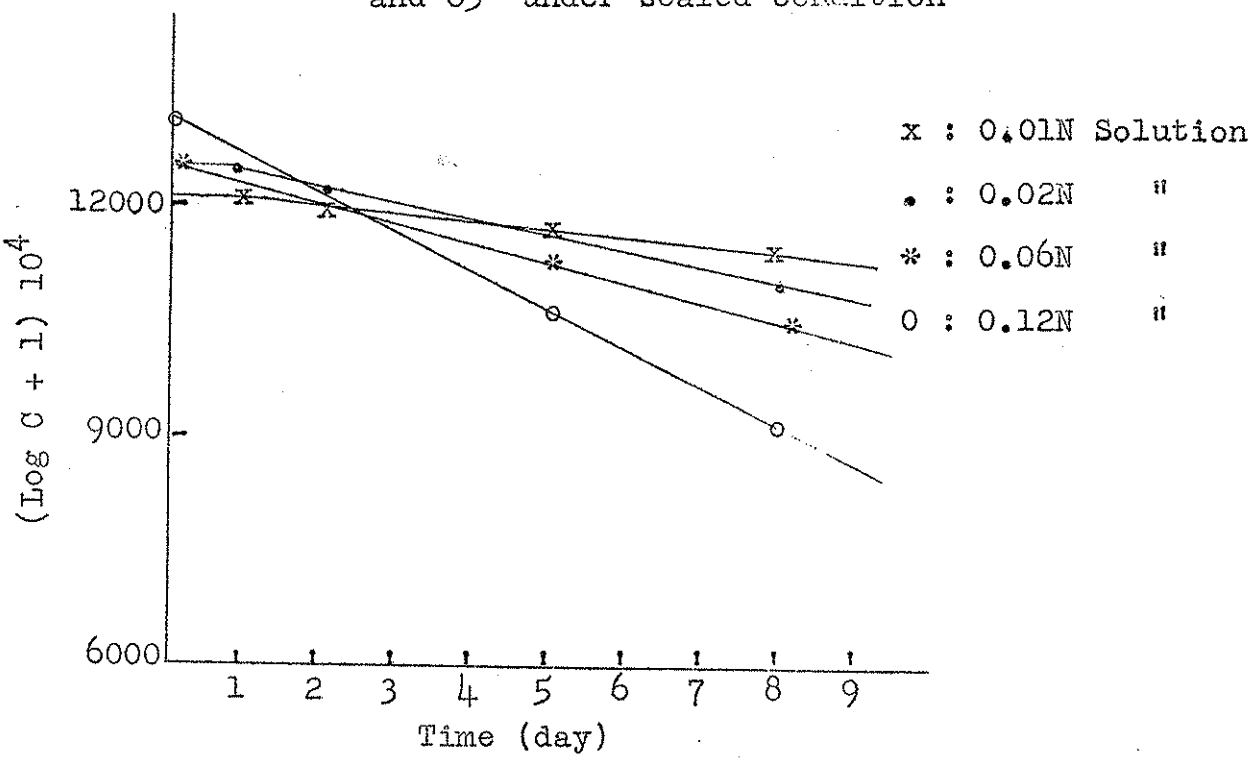


Fig (19) Effect of sulfuric acid concentration on the rate of decomposition of sorbic acid at 55°

When the rate constant in each pH value was plotted against the pH as in Fig (13) the rate-pH profile for the decomposition of sorbic acid at 65° became obvious. In the acidic pH the acid was more stable in the range between pH 2 and pH 4.8 with a maximum stability at the second end of the range. The maximum unstability was at pH 6. At pH 8 the acid was more stable than at the acidic pH values.

2.2.4 Effect of Sulfuric Acid concentration on the decomposition of Sorbic Acid

Sulfuric Acid was found to possess a stronger effect on the decomposition of Sorbic Acid than other acids (20, 21). In order to study the effect of the concentration of sulfuric acid on the decomposition at a fixed temperature, Sorbic Acid solutions (0.1%) in different concentrations of sulfuric acid solutions in water (0.01N, 0.02 N, 0.06 N and 0.12 N) were prepared. Fifteen milliliter portions from each solution were placed separately in 30 ml amber colored bottles and stored at 55°. The zero time analysis was done

at room temperature. At different time intervals the bottles were cooled to room temperature a sample withdrawn for analysis and the bottles are returned back to 55^{o}. Table (5) shows the results which are illustrated in Fig (19). These results indicate the following:

In 0.01 N Sulfuric Acid

No decomposition was observed after one day but started after this period. The rate constant for the decomposition rate was 0.023 day^{-1}.

In 0.02 N Sulfuric Acid

The pattern of decomposition was about similar to that in 0.01 N but was faster. The rate constant for the decomposition was 0.057 day^{-1}.

In 0.06 N Sulfuric Acid

The analysis of the first sample withdrawn after one day indicated that the decomposition has already started in this concentration of Sulfuric Acid. The rate of decomposition was still higher than

in 0.02 N sulfuric acid, as is obvious from the rate constant which was 0.069 day^{-1}.

In 0.12 N Sulfuric Acid

The rate of decomposition of sorbic acid has increased further in this higher concentration of sulfuric acid. The rate constant for the decomposition was 0.115 day^{-1}.

The above results indicate that rate of decomposition of sorbic acid increases as the concentration of sulfuric acid is increased. They also indicate that the increase in sulfuric acid concentration decreases the lag time for the decomposition.

2.2.5 Effect of Sorbic Acid concentration on the decomposition:

In order to investigate the possible effect of the concentration of sorbic acid on the rate of its decomposition, different concentration of sorbic acid solutions (0.05 and 0.1%) in 0.12 N sulfuric acid were prepared. Fifteen milliliter portions were placed separately in 30 ml amber

color bottles and stored at 55°. The solutions analyzed at room temperature before storage. The bottles were cooled to room temperature at different time intervals, samples withdrawn for analysis and then the bottles were returned back to 55°. The results obtained are shown in Table (6) which are depicted in Fig (20). The results indicate that the concentration of sorbic acid in solution affects the rate of decomposition. The higher the concentration of the acid the higher the rate of its decomposition. The results also indicate that the lag time for decomposition is shorter as the concentration of sorbic acid is increased.

2.2.6 Effect of light on stability of Sorbic Acid

Sorbic acid solution (0.1%) was prepared using 0.12 N sulfuric acid as a solvent. Fifteen milliliter portions of this solution were placed separately in 30 ml white and colored bottles. Fig (21) illustrates the results obtained. These results indicate that light has no effect on the decomposition of sorbic acid. The rate constant for the decomposition in both cases was 0.172 day^{-1}.

Table 5

Kinetic Parameters for decomposition of Sorbic Acid as a function of Sulfuric Acid concentration at 55°

Concentration of H_2SO_4 (v)	K (day^{-1})	t 1/2 (day)
0.01	0.023	30.13
0.02	0.057	12.16
0.06	0.069	10.04
0.12	0.115	6.00

Table 6

Kinetic Parameters for decompositon of Sorbic Acid as a function of Sorbic Acid concentration at 55°

concentration of Sorbic Acid (%)	K (day^{-1})	t 1/2 (day)
0.05	0.057	12.16
0.1	0.115	6.00

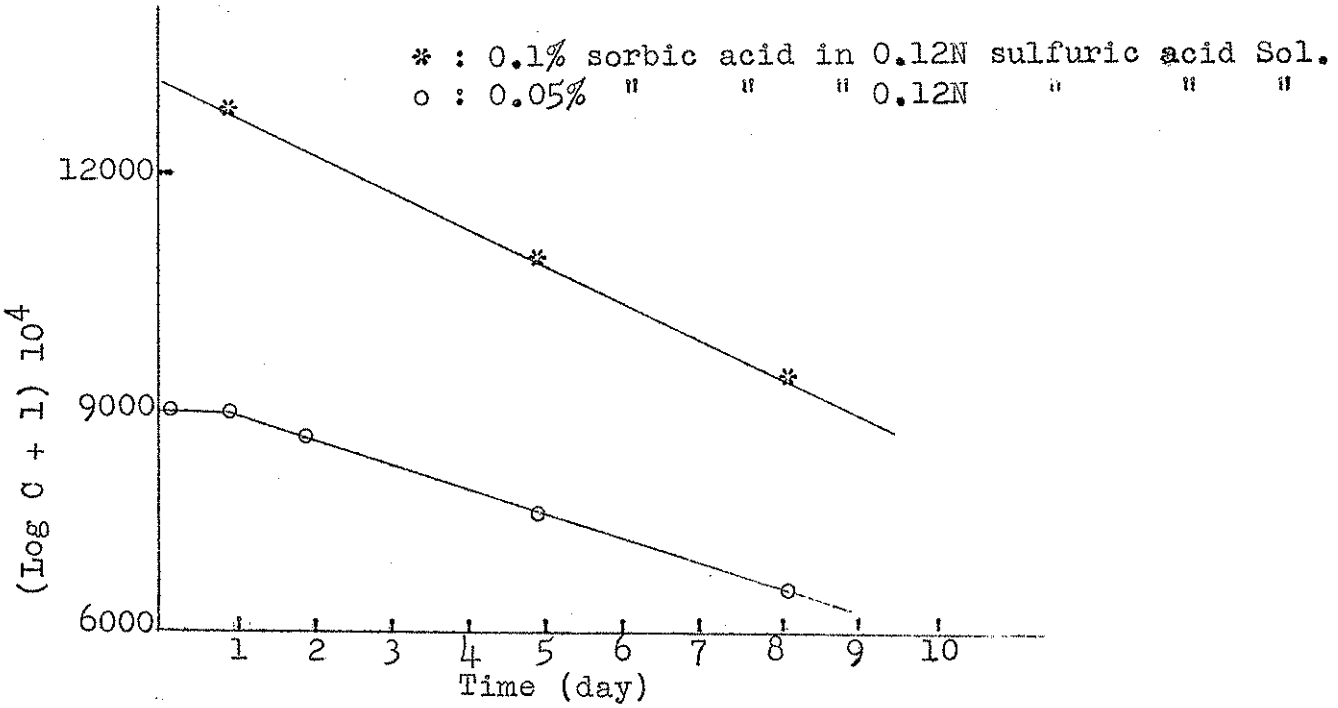


Fig (20) Effect of initial concentration of sorbic acid on its rate of decomposition at 55°

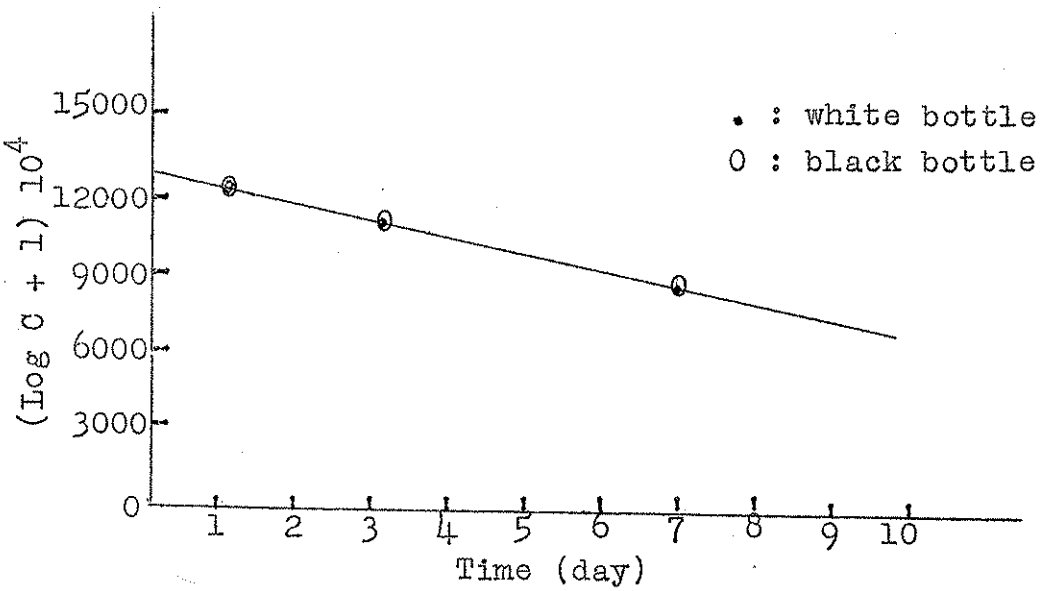


Fig (21) Effect of light on the rate of decomposition of sorbic acid at room temperature

2.2.7 Effect of temperature on the rate of decomposition of Sorbic Acid

Sorbic acid solution (0.1%) in 0.12 N sulfuric acid was prepared and analyzed spectrophotometrically at room temperature. Fifteen milliliter portions are placed in 30 ml amber colored bottles. The bottles are divided into three groups. The first group was stored at 45°, the second at 55° and the third at 65°. The bottles were cooled to room temperature and at different time intervals, samples were withdrawn for analysis and the bottles were then returned back to the corresponding temperature of the experiment. Table (7) shows the results obtained for this experiment which were illustrated in Fig (22). These results show that the rate of decomposition decreases as the temperature is increased.

The graph obtained by plotting the logarithm of the rate constant against the reciprocal of absolute temperature is shown in Fig. (23). It is obvious that the graph is of the anti-Arrhenius type. The activation energy (Ea) which was calculated from the slope of the graph was - 6106 cal/mol.

The free energy of activation $\triangle F^{\ddagger}$, the enthalpy of activation $\triangle H^{\ddagger}$ and the entropy of activation $\triangle S^{\ddagger}$ are calculated from the following equations (22)

$$\triangle F^{\ddagger} = - RT \ln (Krh/bkT)$$

$$\triangle H^{\ddagger} = Ea - RT$$

$$\triangle S^{\ddagger} = (\triangle H^{\ddagger} - \triangle F^{\ddagger}) / T$$

Where

b = 1 = transmission coefficient

Kr= rate constant

h = Planck constant

k = Boltzman constant

Table 7

Kinetic and Thermodynamic Parameters for the Decomposition of Sorbic Acid

Temperature	K day^{-1}	$\triangle F^{\ddagger}$ Cal/mol.	$\triangle H^{\ddagger}$ Cal/mol.	$\triangle S^{\ddagger}$ Cal/mol. degree
45^{o}	0.138	-	-	-
55^{o}	0.115	-	-	-
65^{o}	0.075	20686.4	- 6748.3	-81.1

(Activation Energy) Ea = Calculated from slop of graph - 6106 cal/mole

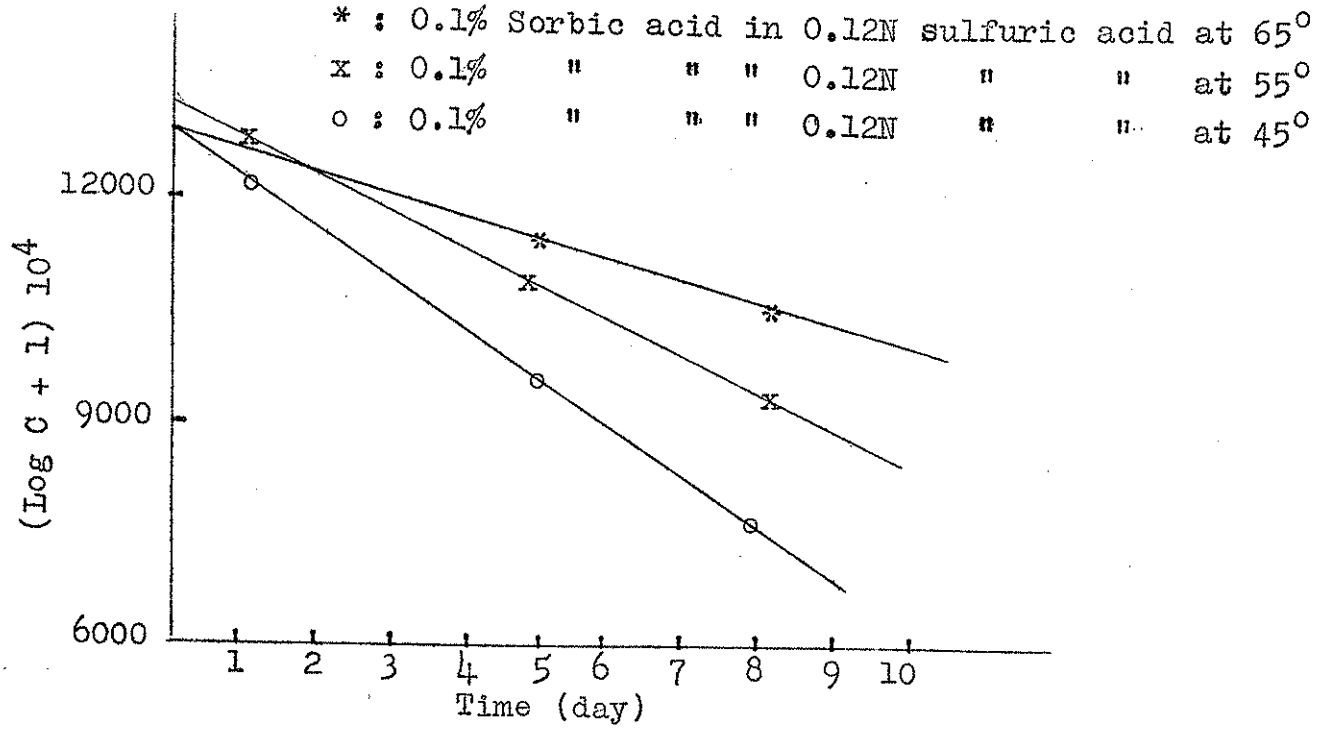


Fig (22) Effect of temperature on the rate of decomposition of sorbic acid

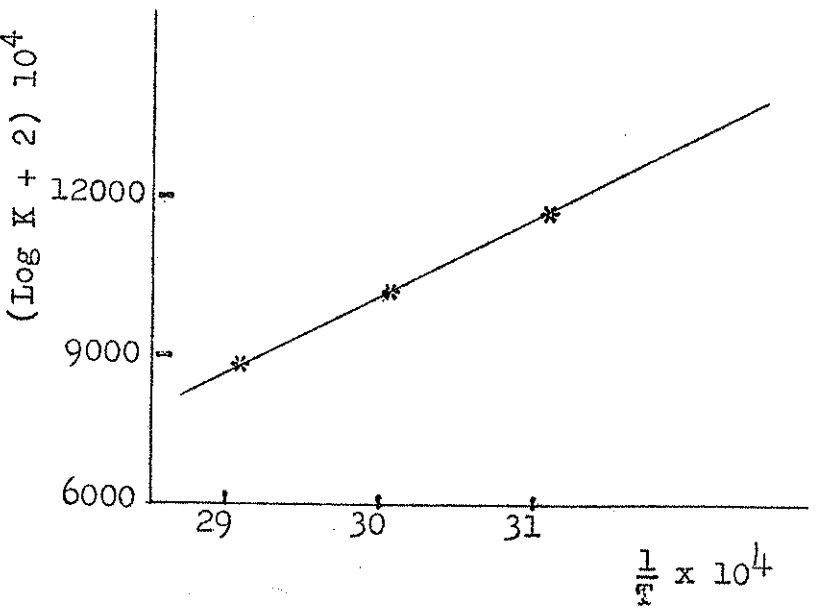


Fig (23) Arrhenius plot for the oxidation of sorbic acid

CHAPTER 3

D I S C U S S I O N

3. DISCUSSION

3.1 Effect of Ionic Strength on the rate of decomposition of Sorbic Acid

Figures (4 - 6) show the effect of ionic strength on the rate of oxidation of sorbic acid at different experimental conditions. The results indicate the independence of the decomposition rate on the ionic strength. Fig (7) is a graph representing the Bronsted-Bjeaum equations (19)

$$\text{Log } K = \text{Log } Ko + 1.02\ Z_a\ Z_b \sqrt{\mu}$$

Where

K = rate constant

Ko= the rate constant in an infinitely dilute solution where $\mu = 0$

$Z_a Z_b$ = the charges of reactants

μ = ionic strength

The graph shows that the slope of the straight line obtained in each case is zero. It is obvious from the equation that zero slop indicates the reaction of positive or negative ions with a neutral molecule (19, 23). The sorbic acid at

pH 1 and pH 2 mainly occur in protonated and in unionized form. The pKa of sorbic acid is about 4.8 and so at pH 4.8 half of the acid occurs in the ionized and half is the unionized form. Zero slope at pH 1 and pH 2 indicates reaction of protonated and unionized forms with a neutral molecule(which is oxygen in this case) and at pH 4.8 reaction of neutral and ionized forms of sorbic acid with a neutral species. Similar zero effect of ionic strength were found in many kinetic studies (24-29).

3.2 Effect of pH on the decomposition of Sorbic Acid

The effect of pH on the oxidation of sorbic acid was studied at room temperature and at 65°. As shown in Figures (8-18) the decomposition of sorbic acid occurs in two stages, a slow stage and a rapid stage except at pH 4.8 and pH 6 at room temperature where no decomposition has been observed. At pH 4.8 and pH 6 and 65°, there was no decomposition during the first stage. The appearance of a second stage for the decomposition may be due to the formation of a peroxidic compound and that the degradation rate is a function of the concentration of this intermediate (30).

The linear relationship between Log c and time shown in the Figures indicates an apparent first order decomposition.

3.2.1 The pH-rate profile for the decomposition of sorbic acid at room temperature

Fig (12) shows the Log K-pH profile for sorbic acid at room temperature which was constructed from the data in table (3). This figure indicates that the degradation process is catalyzed markedly at pH values less than 4.8 and greater than 6. The rate of degradation below pH 2 is higher than that between pH 2 and 4.8. Sorbic acid appears stable over the range of pH 4.8-6. The shape of pH-rate profile indicates that the degradation is subject to acid-base catalysis. The rate of degradation is apparently influenced by the type of sorbic acid species present and the catalysis depends on the hydrogen ion concentration. Below pH 2 the acid is present in the undissociated form which is protonated and the catalysis is obviously high. Between pH 2 and pH 4.8, it exists in the undissociated and dissociated forms.

As shown in Figure (12) both forms undergo degradation proportional to the hydrogen ion concentration. The reason for the stability of sorbic acid over the pH range of 4.8-6 may be a complex formation between ionized and unionized forms of sorbic acid. As shown in Fig. (12) the degradation of unionized and ionized forms of sorbic acid when are present separately is subjected to acid base catalysis. When both forms are present together (4.8-6) no degradation is observed. Many examples of complex formation between an organic acid molecules and its anions have appeared in the literature (23). Above pH 6 sorbic acid mainly occurs in the dissociated form. The shape of the graph in this region indicates a base catalyzed degradation.

3.2.2 <u>The pH-rate profile for the decomposition of Sorbic Acid under sealed conditions at 65°</u>

Fig. (13) shows the Log K-pH profile for sorbic acid degradation under sealed conditions and at a temperature of 65°. The sorbic acid species present in solution are similar to those present under unsealed conditions over the same pH regions.

The graph indicates that the rate of degradation of the acid is high below pH 2. The rate of degradation is slower between pH 2 and pH 4.8. The acid undergoes some degradation at pH 4.8, although it is relatively stable at this pH. The shape of the graph above pH 4.8 is different from that under unsealed conditions and room temperature. The acid shows a high rate of degradation at pH 6 but a low rate at pH 8.

Higuchi et.at (29) found that the anaerobic degradation of ascorbic acid at 96^{o} is catalyzed by the phosphate buffer at pH 6 but not at pH 7.5. The authors believe that the phosphate ions are non catalytic to the acid present at the pH region above neutrality at the conditions of the experiment which are about similar to the conditions of this experiment.

3.3 Effect of sulfuric acid on the decomposition of Sorbic Acid

The effect of sulfuric acid on the rate of decomposition was studied at different sulfuric acid and sorbic acid concentration. The effect of light and temperature has also been studied.

3.3.1 Effect of sulfuric acid concentration on the decomposition of Sorbic Acid

Figure (19) shows the effect of increasing the sulfuric acid concentration on the rate of decomposition of sorbic acid. The increase in the sulfuric acid concentration has increased the rate and decreased the lag time for decomposition. The rate of decomposition in the presence of sulfuric acid is higher than that at a similar concentration of other acids example hydrochloric acid (20, 21). This was referred to a joined effect of both the hydrogen ions and the sulfate ions.

3.3.2 Effect of sorbic acid concentration on the decomposition

Figure (20) shows the effect of initial sorbic acid concentration on the decomposition. It is clear that the rate of degradation is increased with the increase in initial concentration. The lag time was also observed to decrease by such an increase in initial concentration. This was expected as the reaction is apparently first order.

3.3.3 Effect of temperature on the rate of decomposition of Sorbic Acid

The effect of temperature was studied at 45°, 55° and 65°. Fig. (22) shows that the rate of decomposition of sorbic acid in the presence of sulfuric acid decreases with an increase in temperature. The anti-arrhenius type of graph gained for this reaction was also obtained for the reaction between nitrogen dioxide (NO_2) and oxygen (31). Thermodynamic data were calculated and shown in table (7). It is observed that Ea and $\triangle H^{\ddagger}$ have negative values. This may indicate that the reaction is exothermic and may explain the obtained result concerning the lower rate of decomposition at higher temperature. Another factor may be playing part in the decrease of decomposition rate on increasing the temperature. It is the effect of temperature on the solubility of oxygen in the solution. The decomposition is an oxidation reaction with oxygen. The rate of the reaction depends of course on the concentration of oxygen in the solution. The increase in temperature lowers the solubility of oxygen in

the solution and this may lower the rate of the reaction. The negative value of entropy of activation $\triangle S^{\ddagger}$ indicates that the degree of disorder in the activated complex and in the reaction is great (32), similar result gained in oxidation of ascorbic acid.

3.3.4 Effect of light on the rate of decomposition of Sorbic Acid

Figure (21) indicates that light has no effect on the rate of sorbic acid decomposition in the presence of sulfuric acid. The results are in line with those obtained from the effect of temperature on the decomposition. As the effect of heat energy on the rate of decomposition is negative, it is not strange that rate of decomposition does not depend on the light energy under the conditions of the experiment.

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٣) دراسة سرعة تحلل حامض السوربيك بوجود حامض الكبريتيك وتشمل :

أ — دراسة تاثير تركيز حامض الكبريتيك باستعمال محاليل لحامض السوربيك المختلفة بتركيز حامض الكبريتيك فيها وقد اجريت هذه التجارب بدرجة حرارة ٥٥ مئوية وكانت نتائجها زيادة سرعة تحلل الحامض بزيادة تركيز حامض الكبريتيك •

ب — دراسة تاثير تركيز حامض السوربيك على سرعة تحلله ، وقد اجريت هذه التجارب بدرجة حرارة ٥٥ مئوية وظهر ان درجة التحلل تزداد بزيادة تركيز حامض السوربيك •

جـ — دراسة تاثير الضوء باستعمال قنانى شفافة غير ملونة لحفظ جزء من المحاليل وقنانى داكنة لحفظ الجزء الاخر من نفس المحاليل •

وقد اجريت هذه التجارب بدرجة حرارة الغرفة وكانت النتائج تدل ان لا تاثير للضوء على درجة ثبات الحامض •

د — تاثير درجة الحرارة حيث اجريت التجارب على محاليل متشابهة لحامض السوربيك بدرجات حرارة مختلفة (٤٥ و ٥٥ و ٦٥ مئوية) وقد ظهر بان سرعة تحلل الحامض تقل بارتفاع درجة الحرارة في ظروف التجارب •

ان التجارب قد اجريت ثلاث مرات لكل منها وتمثل النتائج المعدل في كل حالة • ان التجارب التي اجريت لدراسة درجة ثبات حامض السوربيك تشمل :

١) دراسة تاثير تركيز الايونات (Ionic strength)في محاليل حامض السوربيك وذلك باستعمال محاليل منظمة (Buffer solutions) عديدة مختلفة في درجة تركيز الايونات ، ودرس ذلك بدرجة حرارة الغرفة ودرجة ٦٥ مئوية وعلى عدة محاليل مختلفة بالاس الهايدروجيني (pH) وقد وجد ان لا تاثير لتركيز الايونات على سرعة تحلل حامض السوربيك في تلك المحاليل •

٢) دراسة تاثير اختلاف الاس الهايدروجيني على سرعة تحلل حامض السوربيك وذلك باستعمال محاليل منظمة مختلفة في الاس الهايدروجيني •

وقد اجريت هذه التجارب في درجة حرارة الغرفة ودرجة ٦٥ مئوية فظهر ان اقصى درجة ثبات للحامض كان في محاليل ذات اس هايدروجيني يتراوح بين ٨ ر٤ — ٦ في درجة حرارة الغرفة وفي محاليل ذات اس هايدروجيني يساوي (٨) في درجة حرارة ٦٥ مئوية •

دراســـــــة

ثبات حامـض السوربيك فـي المحاليل

يتكون حامض السوربيك من بلورات بيضاء تستعمل منذ خمسة وعشرين سنة لمحافظة المواد الغذائية (المعجنات ، المنتوجات السمكيـــــة ، الفواكه المجففة ، الجبن ، لحوم الدواجن وغيرها ، عصير الفواكه ، الجلي ، المشروبات الغير كحولية والخمر والمخللات) والادوية ومواد التجميل وبعـــض المواد الاخرى كالتبغ والحبر ولذلك لسبب فاعليتها لمحافظة المـــــواد المذكورة من التلف وقلة مضارها للجسم •

استخرج الحامض لاول مرة من زيت ثمرة شجرة السمّن (Rowan berry) في سنة ١٨٥٩ ـ (١) ، وانه من فصيلة الحوامض الدهنية الغير مشبعـــة (Unsaturated fatty acid) ذات ذرات الكاربون القليلة (Short chain) وتحتوى على اصرتين غير مشبعتين وهما سببان رئيسيان لنشاط حامض السوربيك ضد الجراثـــيم •

رغم اهمية هذه المادة لم تجر حتى الان دراسة كافية لمعرفة درجـــة ثباتها واستقرارها في المحاليل وفي ظروف معينة (controlled condition) وعليه قمت بدراسة ذلك حسب الطريقة التالية :

حضرت محاليل حامض السوربيك وحفظت في درجات حرارة مختلفة وقرأ تركيزها بواسطة جهاز مقياس الطيف (u.v. spectrophotometer) - الاشعة فوق البنفسجية ـ في اوقات متفاوتة ورسم الخط البياني الذى يمثل علاقة التركيز بالزمن والذى استخدم لاستخراج سرعة تحلل حامض السوربيك فيها •

دراســـــة

ثبات حامض السوربيـــك في المحاليـــل

رسالـــة مقدمـــة

مـــن

فكـرت وهـــبي عزالديـــــن
ـ بكلوريوس علوم في الصيدلة ـ

الـى

فرع الصيدلـــة ولجنة الدراسات العليـــا فـــي
كليـــة الصيدلة كجزء من متطلبـــات الحصـــول
علـــى درجـة ماجســـتير علـــــوم فـــي
الصيدلـــة

شبـــاط ١٩٧٦