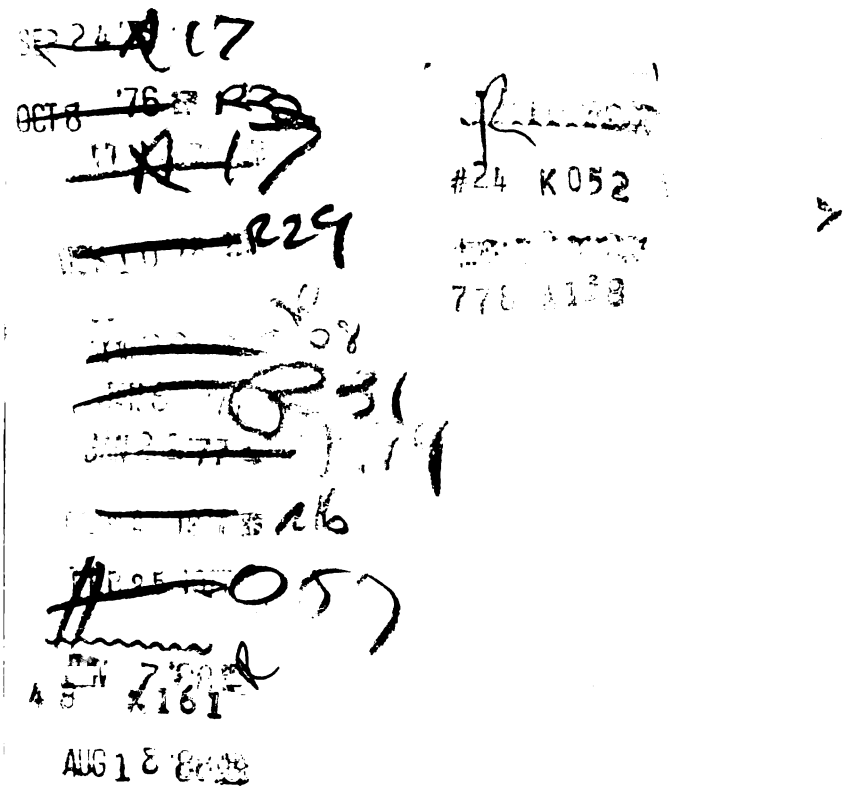
KINETICS OF QXYGEN UPTAKE IN A LIQUID FOOD

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY

Thomas E. Mack

1974


#24 K052
AUG 1 8

ABSTRACT

KINETICS OF OXYGEN UPTAKE IN A LIQUID FOOD

by Thomas E. Mack

The loss of ascorbic acid in liquid foods is due to primarily the oxidative degradation caused by molecular oxygen dissolved in the liquid.

The purpose of this research is to study oxygen uptake from a liquid food. A commercially prepared infant formula was used as the model system. The effects of three variables: light intensity, storage temperature, and initial concentration of dissolved oxygen, on the rate of oxygen uptake were determined.

The results of the study indicated that the uptake of oxygen from liquid foods follows first order rate kinetics. The uptake of oxygen can be described by the equation $dN/dt = -kN$ where N is the concentration of dissolved oxygen and k is the first order rate constant.

In the range of 0 to 4300 lux, it was found that the rate of oxygen uptake increases linearly with increasing light intensity. The rate of oxygen uptake at three different levels of initial dissolved oxygen from 1 ppm to nearly saturation was measured and showed that the rate of oxygen uptake increases linearly with increasing initial concentration of dissolved oxygen.

Thomas E. Mack

The influence of temperature on rate of oxygen uptake indicated that the Arrhenius equation could be used to describe the relationship. The activation energy associated with the Arrhenius relationship was found to increase with increasing light intensity.

KINETICS OF OXYGEN UPTAKE IN A LIQUID FOOD

By

Thomas E. Mack

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of food science and human nutrition

1974

ACKNOWLEDGEMENTS

The author wishes to express his sincere appreciation to Professor Dennis R. Heldman for suggesting the thesis topic and his continuous support, guidance, and interest throughout the course of the study.

The author also wishes to convey his appreciation to Professor J. Kirk for his constructive help and for the vitamin assays his laboratory supplied. He is also grateful to Professor R. Nicholas for his critical review of the manuscript.

A very special thanks is due to his fellow students: R. Paul Singh and David Gorby for their moral and technical support.

The author is deeply indebted to his wife, Kathy, for her encouragement and understanding throughout his studies.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued . . . .)

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

Appendix — Page

INTRODUCTION

The advent of nutritional labeling, the nutrition data bank, and increased interest in the nutritional quality of our foods on the part of both consumer and processor have all served to greatly increase the need for knowledge of nutrient stability. If a processor is to make a claim for the nutritional value of his product he must know the rates of loss of the nutrients claimed. Nutrient stability after processing, is a function of the composition of the product, the package, and the storage conditions. In order to predict the extent of nutrient deterioration the processor must have a knowledge of the degradation rate as a function of both storage variables and variations in product composition.

One of the most important and easily degraded nutrients is ascorbic acid (vitamin C). Ascorbic acid is degraded into non-biologically active forms through an oxidative mechanism. Due to agitation and exposure to the atmosphere, normal processing and storage of liquid foods, results in a high level of dissolved oxygen being present. Unless removed by some deaeration process, this dissolved oxygen promotes oxidative reactions which degrade several important nutrients (ascorbic acid, vitamin B_{12}, and folic acid) (Ford 1967). One logical way to preserve the ascorbic acid is to eliminate oxygen from the system. When oxygen is

removed other catalytic agents, sunlight, trace metals,(Cu^{++} and Fe^{++}), and oxidative enzymes are rendered inactive in regards to ascorbic acid degradation. In the absence of oxygen, vitamin C is stable for a considerable period of time (Kon & Watson,1936; Hand,Guthrie,& Sharp, 1938;Guthrie 1948).

Work with different liquid systems has shown that the losses of ascorbic acid and the uptake of oxygen follow similar patterns (Guthrie,1948; Ford,1967). The rate and amount of ascorbic acid degradation have been shown to be directly proportional to the concentration of dissolved oxygen (Khan and Martell, 1966; Weissberger, et al. 1934; Burton, 1970). The above facts coupled with the fact that ascorbic acid is much more readily oxidized than other food components (i.e. lipids) suggest that the measurement of oxygen uptake rate may be used to predict the loss rates of ascorbic acid.

The rate of oxygen uptake is a function of light intensity, temperature, concentration of dissolved oxygen, and the presence of trace metals and/or oxidative enzymes. In order to predict ascorbic stability under a range of storage conditions and product variations, we must know the rate of oxygen uptake as a function of the above variables. Since oxidative enzymes are easily destroyed, its effect on the rate of oxygen uptake has not been studied, nor has the effect of trace metals been studied.

The specific objectives of this research are:

1) To use chemical kinetics to describe oxygen uptake in a liquid food system.

2) To examine the rate of oxygen uptake as a function of temperature using an Arrhenius-type relationship.

3) To determine the rate of oxygen uptake as a function of both light intensity and initial concentration of dissolved oxygen.

4) To analyze the role of dissolved oxygen in ascorbic acid loss in a liquid food.

REVIEW OF LITERATURE

The loss of several valuable food nutrients during storage and processing is known to be due to oxidative degradation. The literature has been reviewed to determine the relationship between dissolved oxygen and nutrient stability. The factors which influence the rate of oxidative degradation have also been reviewed.

Ascorbic Acid Oxidation

Ascorbic acid (Vitamin C, l-threo-hexono-1,4lactono-2ene) the antiscorbutic vitamin is widespread in the plant kingdom, with certain fruits being exceptionally rich sources (i.e. citrus fruits and cherries). Ascorbic acid can be produced in vivo by most animals: man, monkeys, and guinea pigs being the exceptions. (Braverman, 1963)

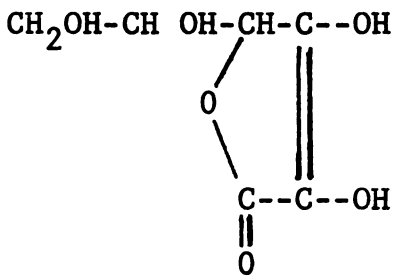


Figure 1. Chemical structure of ℓ-ascorbic acid

Ascorbic acid, which crystallizes in white, odorless plates, melting at 190-192°C, is exceedingly soluble in water (1g./3ml.) and is insoluble in organic solvents. In structure ascorbic acid may be regarded as a derivative of a

simple hexose. Ascorbic acid is a strong reducing agent and is easily oxidized (Braverman, 1963).

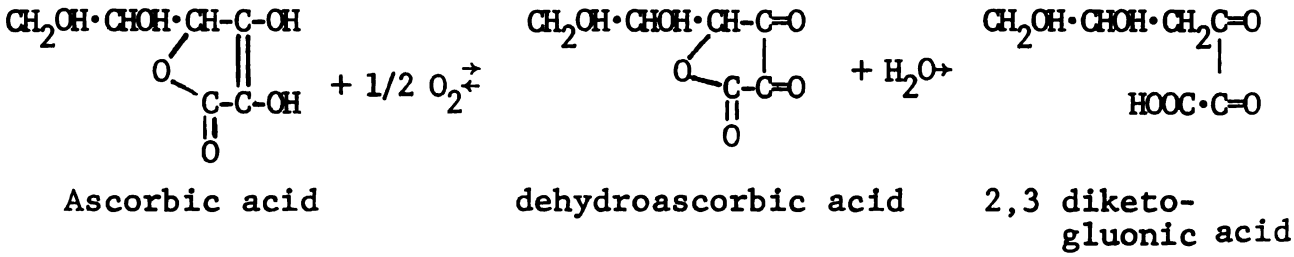


Figure 2. Oxidation of ℓ-ascorbic acid

The general oxidation scheme of ascorbic acid is illustrated in Figure 2. The conversion of l-ascorbic acid to dehydroascorbic acid is reversible; the extent of reversibility being dependent on the catalyst present. If the oxidation proceeds (at pH 4.5) dehydroascorbic acid is irreversibly converted to 2,3 diketogluonic acid. Both ℓ-ascorbic acid and dehydroascorbic acid possess full biological activity while 2,3 diketogluonic acid has no biological activity. The oxidative degradation of ascorbic acid is enhanced by molecular oxygen, irradiation, trace metals (Cu^{++} and Fe^{++}), oxidative enzymes, quinones, hydrogen peroxide, and pH>5.0 (Braverman 1963).

The Influence of Dissolved Oxygen on the Stability of Ascorbic Acid

The deleterious effect of dissolved oxygen on the stability of ascorbic acid in liquid foods has been recognized and investigated since the 1930's. Sharp, et al. (1939) appreciating the effect of dissolved oxygen designed equipment for the continuous deaeration of market milk. The

results of the deaeration were stabilization of ascorbic acid and increased resistance to the development of oxidized flavor.

One of the earliest studies of the influence of dissolved oxygen on vitamin C stability was done by Guthrie, et al. (1938). Working with milk they found that the destruction of ascorbic acid was prevented by the removal of oxygen. In milk which was not deaerated, losses of vitamin C continued until the oxygen supply was depleted, after which further decreases in vitamin C did not occur.

Research by Sharp et al. (1940) showed that even when held in the dark dissolved oxygen still caused large decreases in ascorbic acid content. After seven days at 45°F un-deaerated milk had a 60.7% loss of ascorbic acid while a deaerated sample had a loss of 4.5%. Several other researchers have also shown that in the presence of dissolved oxygen large losses of vitamin C occur and that the ascorbic acid is stabilized by deaeration. (Kon and Watson 1936; Sharp et al. 1941; Hartman 1942; Guthrie 1946; Ford 1967; and Burton 1970).

Researchers (Ford 1967 and Burton 1970) have shown that the presence of dissolved oxygen increases the amount of ascorbic acid loss during thermal processing. Ford (1967) found that while milk flushed with air showed a 50% decrease in vitamin C due to pasteurization milk which had been deaerated showed no loss due to heating.

The rate with which ascorbic acid reacts with oxygen is influenced by several factors. Exposure to light has been demonstrated to greatly accelerate the rate of ascorbic acid oxidation (Kon and Watson 1936; Guthrie 1938, 1946, and Ford 1967). Research by Dunkley et al. (1962) found the rate of ascorbic acid oxidation to be directly proportional to radiant power emitted between 400 and 500nm. Sattar and deMan (1973) found that the rate of oxidation of ascorbic acid increases with increasing light intensity.

The rate of ascorbic acid oxidation also increases with increasing temperature. Kon and Watson (1936) studied the rate of ascorbic acid oxidation at $0^{o}C$, $21^{o}C$, and $37^{o}C$. Using Van't Hoff's equation they calculated a temperature coefficient of 1.44. Blaug and Hajratwala (1972), working with buffered solutions of ascorbic acid, found that the aerobic oxidation of ascorbic acid follows an Arrhenius relationship. They found that the activation energy varied with pH; they reported an activation energy of 12.2 kcal/mole for a solution at pH 3.52 and an activation energy of 7.8 kcal/mole for a solution at pH 6.60.

Trace metals (Cu^{++} and Fe^{++}) acting as catalysts increase the rate at which oxygen and ascorbic acid react. (Sharp et al. 1936; Sharp 1938, and Guthrie 1938). Guthrie (1938) showed that even at concentrations of 0.1ppm Cu^{++} still accelerated the rate of ascorbic acid loss.

The initial level of dissolved oxygen also effects the rate of ascorbic acid oxidation. Khan and Martell (1967)

studied the uncatalyzed oxidation of ascorbic acid and concluded that the rate of reaction was directly proportional to the amount of oxygen present at concentrations of 20% and greater of molecular oxygen. Weissberger (1943) also found the rate of ascorbic acid oxidation to be proportional to the concentration of dissolved oxygen.

The effect of catalysts on vitamin C stability with no oxygen present has also been studied. Works by Kon and Watson (1934), Guthrie (1938) and Ford (1967) showed that without oxygen vitamin C is stable even when exposed to sunlight, which in the presence of oxygen, greatly increases the rate of loss. Guthrie (1938) observed that in milk with 0.1mg./1. copper added the ascorbic acid was stable if no oxygen was present. Sharp (1941) stated that the elimination of oxygen rendered light, metals, and oxidative enzymes ineffectual in regards to ascorbic acid oxidation.

Theoretically one atom of oxygen should be consumed for each molecule of ascorbic acid which is oxidized. Stated another way, one cm^3 of oxygen can degrade 15.7 mg. of ascorbic acid. (Bayes, 1950) This theoretical relationship of 1 to 1 is observed, however, only when the reaction is enzyme catalyzed. When the oxidation is catalyzed by copper or light the ratio is greater than 1 atom of oxygen per molecule of ascorbic acid. Guthrie (1938), working with a light catalyzed reaction, reported an initial ratio of 1.1 to 1 which increased to 1.4 to 1 as the reaction proceeded.

Hand (1942) reported the ratio for the light catalyzed reaction to be variable from 1.57:1 to 2.0:1 and for the copper catalyzed reaction the ratio of atomic oxygen to ascorbic acid was 1.5:1.

Deviation from the theoretical 1:1 ratio may be due to more than one factor, especially when dealing with a liquid food. Working with an aqueous system and both copper and light catalyzed reactions, Hand (1942) postulated that a ratio of greater than 1:1 was due to formation of hydrogen peroxide and its further reactions. If hydrogen peroxide oxidizes ascorbic acid or is decomposed the ratio remains 1:1. If, however, the hydrogen peroxide accumulates or oxidizes dehydroascorbic acid the ratio becomes 2:1. In his support Hand (1942) observed 1) hydrogen peroxide was present in the solution after the oxidation of ascorbic acid was complete and 2) addition of catalase to the system lowered the ratio to nearly that of the theoretical 1:1.

Weissberger, et al. (1943) stated that the failure to maintain a 1:1 ratio was due to the primary oxidation products of ascorbic acid reacting further with oxygen. Guthrie (1938) working with milk stated that the higher ratio was due to the above two factors plus the oxidation of other milk constituents. The oxidation of these other milk constituents, if it occurred, did not impart any noticeable flavor.

Studying the kinetics of the auto-oxidation of ascorbic acid in sugar solutions with dissolved oxygen present, Joslyn and Miller (1949) concluded the reaction was first order with respect to the ascorbic acid concentration. They also found that with limited oxygen supply the rate of oxidation was reduced. Weissberger (1943) also found the rate of vitamin C oxidation to be first order with respect to vitamin C concentration.

Bayes (1950) concluded from his studies on the effect of dissolved oxygen on vitamin C in aqueous solutions that the depletion of vitamin is directly proportional to the amount of oxygen present. Burton (1970) and Ford (1967) in separate research concluded that the stability of vitamin C is wholly determined by the level of oxygen.

The Influence of Dissolved Oxygen on the Stability of Other Vitamins

Aside from the work on ascorbic acid little has been done on the influence of dissolved oxygen on vitamin stability. Work which has been done does show that dissolved oxygen plays a role in the degradation of other vitamins.

In the presence of dissolved oxygen folic acid is unstable to heating (Ford 1967). Ford (1967) found that nitrogen gassed milk showed no loss of folic acid due to sterilization and subsequent dark storage. An aerated milk sample, however, had a 50% loss of folic acid due to sterilization. Both nitrogen gassed and aerated milk showed losses in folic acid when exposed to sunlight, but with

larger losses in the aerated milk. Thompson (1969) found that UHT processed milk which was not deaerated had a complete loss of folic acid and ascorbic acid in 20 days storage, while the deaerated milk had little loss. The extent of loss of folate is determined by the initial concentration of reduced ascorbic acid; addition of ascorbic acid stabilizes the folic acid.

As with ascorbic acid and folic acid vitamin B_{12} is unstable to heating in the presence of oxygen (Ford 1967). The destruction of vitamin B_{12} is oxidative and consequent upon the oxidative degradation of ascorbic acid (Ford 1957). The destruction of B_{12} is due to reaction with an ascorbic acid breakdown product. The degree of vitamin B_{12} loss is determined by the degree of aeration. Ford (1967) found that the removal of dissolved oxygen offers a practical means of preserving vitamin B_{12}, as well as for stabilizing ascorbic and folic acids.

Luck and Schillinger (1959) heated milk under eight atmospheres of oxygen and found no losses of riboflavin, thiamine, or vitamin B_6. Ford (1967) found similar results when he concluded that the stability of riboflavin, thiamine, nicotinic acid, vitamin B_{12}, and biotin were not influenced by the presence of oxygen.

MATERIALS AND METHODS

Model System

The liquid food used in the experiments as the model system was a commercially prepared infant formula (Similac). The infant formula was received directly from Ross Laboratories in 4 ounce glass bottles and was used without modification. The composition of the infant formula is given in Table 1.

Table 1. Composition of infant formula[1]

fat	36gm/1.
carbohydrate	71gm/1.
protein	15.5gm/1.
minerals	3.7gm/1.
calcium	0.6gm/1.
phosphorous	0.44gm/1.
magnesium	0.04gm/1.
iron	trace
copper	0.4mg/1.
iodine	0.04gm/1.
water	901.8gm/1.
Vitamin A	2500 USP units/1.
Vitamin D	400 USP units/1.
Vitamin E	9 Int. units/1.
Vitamin C	55mg/1.
Vitamin B_1	0.65mg/1.
Vitamin B_2	1.0mg/1.
niacin	7 mg equiv./1.
Vitamin B_6	0.4mg/1.
folic acid	0.05mg/1.
pantotheic acid	3mg/1.
Vitamin B_{12}	1.5mcg/1.

[1]Compositional data based upon label claims.

The infant formula was chosen as the model system because of 1) its close compositional similarity to milk, 2) high initial Vitamin C content, 3) uniform composition from container to container, and 4) the product is sterile so there is no problem with microbial stability or microbial consumption of oxygen. Compositional similarity to milk is desirable because milk, though a potential good source of vitamin C, delivers little to the consumer due to oxidative degradation. Milk upon leaving the cows udder contains 22.2 mg/l. ascorbic acid while market milk has very little.

Exposure Cells

Cells in which the infant formula was exposed were made of Plexiglass with the dimensions indicated in Figure 3. The cells have an exposed surface area of 31.67 $cm.^2$ and a volume of 31.67 $cm.^3$. The bottom and sides of the cells were made of opaque Plexiglass while the top was made of clear Plexiglass. The spectral transmission pattern of the clear Plexiglass (Figure 4) shows that in the region of 400-700 nm. the percent transmission of the Plexiglass is constant at 82%, i.e., 82% of the light is transmitted through the surface of the cell and reaches the infant formula. The region of 400-700 nm. is the spectral area responsible for the light catalyzed oxidation of ascorbic acid. Two 1mm. holes were drilled on the side for filling and emptying the cells. During the exposure the holes were covered with surgical tape.

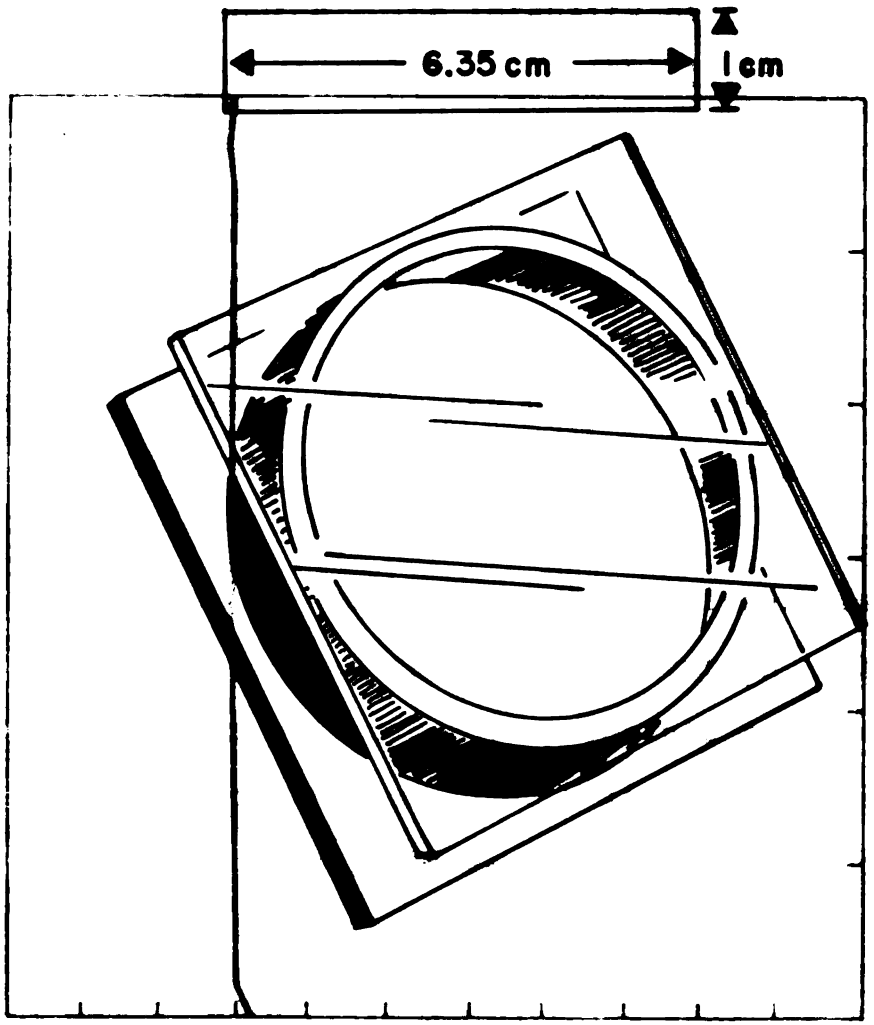


Figure 3. Sample cell.

Figure 3. Sample cell.

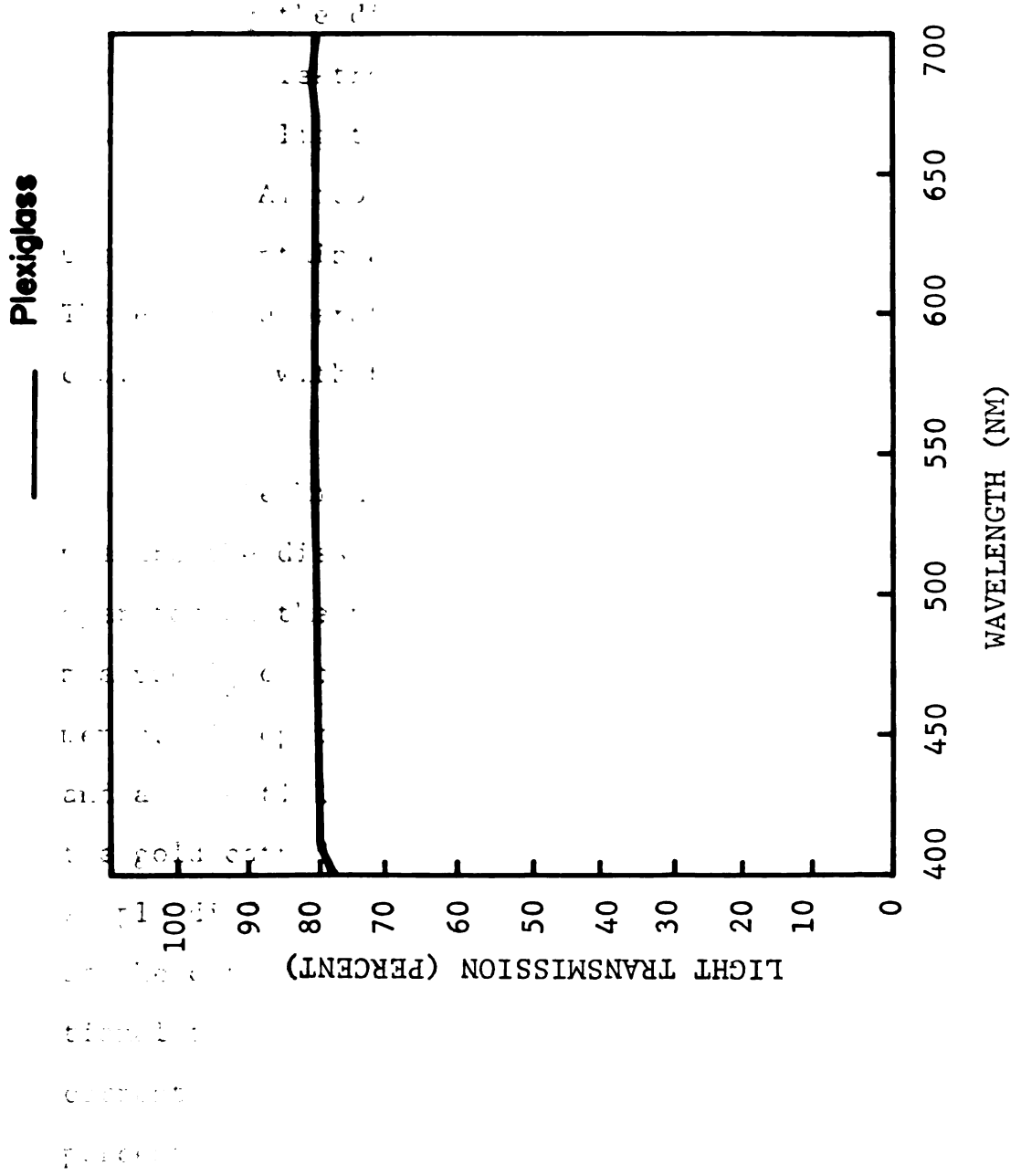


Figure 4. Light transmission through plexiglass

Figure 4. Light transmission through plexiglass

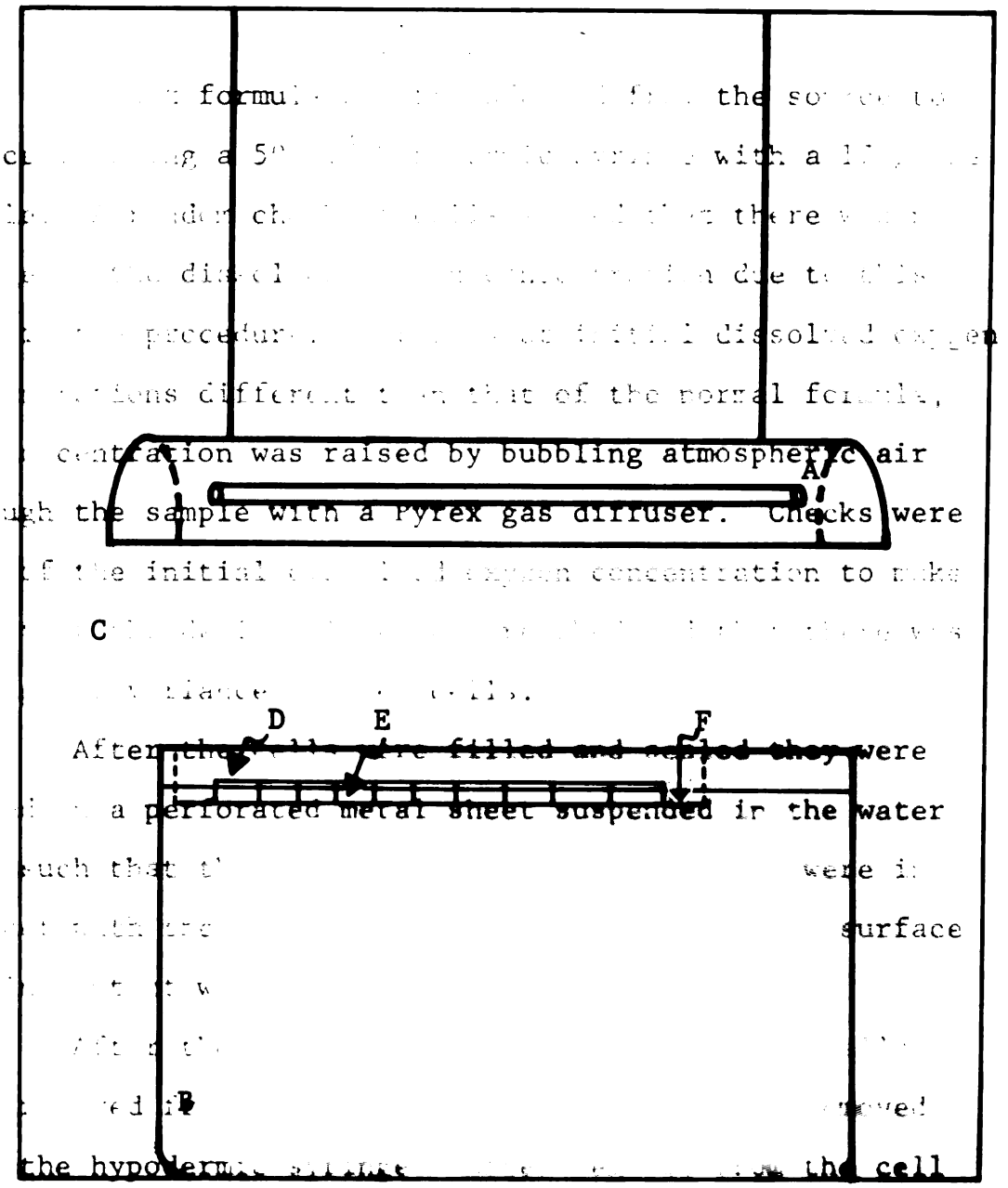
Experimental Set-up

Figure 5 is an illustration of the complete experimental set-up. Two General Electric 40 watt cool white fluorescent bulbs in a curved reflector shield (A) were used as the light source. The intensity of the light was varied by changing the distance between the lights and the cell surface. A Tektronic J16 digital photometer was used to measure the light intensity.

An Aminco controlled temperature water bath (B) was used to maintain a constant temperature around the cells. The entire apparatus was set up in a controlled temperature cubicle (C) with the same air temperature as the water bath.

Oxygen Meter

A Beckman model 777 oxygen analyzer was used to measure the dissolved oxygen concentration. The analyzer operates on the polarographic principle and can be used to measure 0_2 concentration in both liquid and gaseous environments. In operation, the sensor is placed in the sample and a potential of approximately 0.8 volt is applied between the gold cathode and the silver anode. The oxygen in the sample diffuses through the Teflon membrane and is reduced at the cathode. The reduction causes a current flow proportional to the partial pressure of oxygen in the sample. The current passes through an amplifier and a scale reading of percent saturation can be read directly.



A Two 40 watt cool white fluorescent bulbs in deflector shield (height above cells adjustable).
B Aminco controlled temperature water bath.
C Controlled temperature cubicle.
D Sample cells
E Coolant surface
F Perforated metal sheet

Figure 5. Experimental set-up

A Two 40 watt cool white fluorescent bulbs in deflector shield (height above cells adjustable).
B Aminco controlled temperature water bath.
C Controlled temperature cubicle.
D Sample cells
E Coolant surface
F Perforated metal sheet

Figure 5. Experimental set-up

Sample Exposure

Infant formula was transferred from the source to the cells using a 50cm.^{3} hypodermic syringe with a 17 gauge needle. A random check of cells showed that there was no change in the dissolved oxygen concentration due to this step in the procedure. For runs at initial dissolved oxygen concentrations different than that of the normal formula, the concentration was raised by bubbling atmospheric air through the sample with a Pyrex gas diffuser. Checks were made of the initial dissolved oxygen concentration to make sure that the desired level was reached and that there was negligible variance between cells.

After the cells were filled and sealed they were placed on a perforated metal sheet suspended in the water bath such that the bottom and sides of the cells were in contact with the water while the top was above the surface and in contact with the air in the cubicle.

After the desired length of exposure, the cells were removed from the water bath and the formula removed with the hypodermic syringe. After removal from the cell the sample was used for either dissolved oxygen measurements or vitamin assay.

Storage Conditions

The storage conditions studied as well as the sampling schedules followed are given in Table 2.

Table 2. Storage conditions tested

light intensity (lux)	/DO/ (mg./1.)	1.00	4.86		8.71		
	Temp (°C)	7.2	7.2	7.2	12.8	18.3	23.9
0		A	A	A	C	C	C
1,076		A	A	A	C	C	C
2,153		C	A	A	C	A	B
3,229		A	A	A	A	A	B
4,306		A	A	A	A	A	B

A - samples taken at 0,4,8,16,24 hrs.
B - samples taken at 0,2,4,8,16,24 hrs.
C - samples taken at 0,2,4,6,8,16,24 hrs.

Dissolved Oxygen Measurements

After the desired length of exposure, duplicate samples were measured for dissolved oxygen using the Beckman analyzer. To convert the dissolved oxygen readings from the percent of saturation given by the Beckman to a more usable unit a standard curve of percent saturation versus mg./1. was prepared. The concentrations in mg./1. were determined using the ascorbic acid oxidase method (Sharp, Hand, and Guthrie, 1941).

The procedure used can be summarized as follows: 1) The dissolved oxygen concentration was raised or lowered from that of the formula as it is in the original container by bubbling air or nitrogen through the sample. 2) The dissolved oxygen concentration was measured in terms of percent of saturation on the Beckman analyzer. 3) The concentration of reduced ascorbic acid was determined by

2,6 dichlorophenolindophenol titration. 4) an aliquot of the sample was placed in a test tube; ascorbic acid oxidase was added; and the tube sealed so that there was no air inclusion. 5) After reacting for 15 minutes the sample was placed in 0.1 N H_2SO_4 to inactivate the enzyme. 6) The concentration of reduced ascorbic acid was again determined.

Previous research (Hand, 1940; Weissberger, 1941) has shown that when catalyzed by the oxidase enzyme, one atom of oxygen is consumed per molecule of ascorbic acid oxidized. The oxygen concentration in mg/l. is therefore obtained by multiplying the difference in dye titration before and after adding the oxidase by the dye factor and then dividing by 11. Division of eleven is because the molecular weight of ascorbic acid is eleven times the molecular weight of oxygen.

Vitamin Assay

Total and reduced ascorbic acid were determined by the microfluorometric o-phenylene diamine method developed by Kirk and Ting, 1974.

RESULTS AND DISCUSSION

In the following sections the rate kinetics of oxygen uptake from a liquid food will be examined. The rate of oxygen uptake as a function of light intensity, initial concentration of dissolved oxygen, and temperature is investigated.

A prediction of vitamin C loss rates from the rate of oxygen uptake using a second order model is also attempted.

Conversion of Dissolved Oxygen Measurements from Percent of Saturation to Concentration

The dissolved oxygen values obtained when using the Beckman analyzer were in terms of percent of saturation. The magnitude of these values was relative to the amount of oxygen present in the product at saturation. In order to convert the dissolved oxygen measurements to more meaningful units, a standard curve of percent of saturation versus concentration (mg./l.) was developed.

The concentrations were determined using the ascorbic acid oxidase method (Sharp, Hand, and Guthrie; 1941). This method was chosen because it was specifically designed for milk and did not use strong reagents which can react with organic compounds.

The error involved in the oxygen determinations was 0.1 to 0.2 mg./l. Comparison of the ascorbic acid oxidase

and Winkler methods for oxygen determinations in water showed good agreement. (Sharp, Hand and Guthrie, 1941) The ascorbic acid oxidase method has been previously used by Guthrie (1946), Herreid (1949)

The standard curve obtained is given in Figure 6. The least square fit equation of the line is $y = 6.898 + 9.0778x$ with a correlation factor of 0.999 where y equals dissolved oxygen in percent of saturation and x equals the concentration of dissolved oxygen in mg./l. All dissolved oxygen data presented in the thesis are in terms of mg./l.

Order of Oxygen Uptake

Semilogarithmic plots of dissolved oxygen concentration versus time resulted in curves which appeared to be linear indicating that the rate of oxygen uptake was first order. In order to analyze the experimental data using a first order function a least squares fit of the data to the equation $y = ae^{bx}$ was conducted. From each least squares fit the associated correlation coefficient (r) was obtained, where:

$$r = \frac{n\Sigma(x_i \log e\ y_i) - (\Sigma x_i)(\Sigma \log e\ y_i)}{\sqrt{\{n\Sigma x_i^2 - (\Sigma x_i)^2\}\{n\Sigma(\log e\ y_i)^2 - (\Sigma \log e\ y_i)^2}}$$

The correlation coefficients, which equal one with a perfect linear fit, are given in Table 3 for each condition tested. From each correlation coefficient the statistic:

$t = \frac{r}{\sqrt{(1-r^2)/(n-2)}}$ which has a t-distribution, was obtained. The calculated "t" values were compared with t-test table values to determine the level of significance (Table 3).

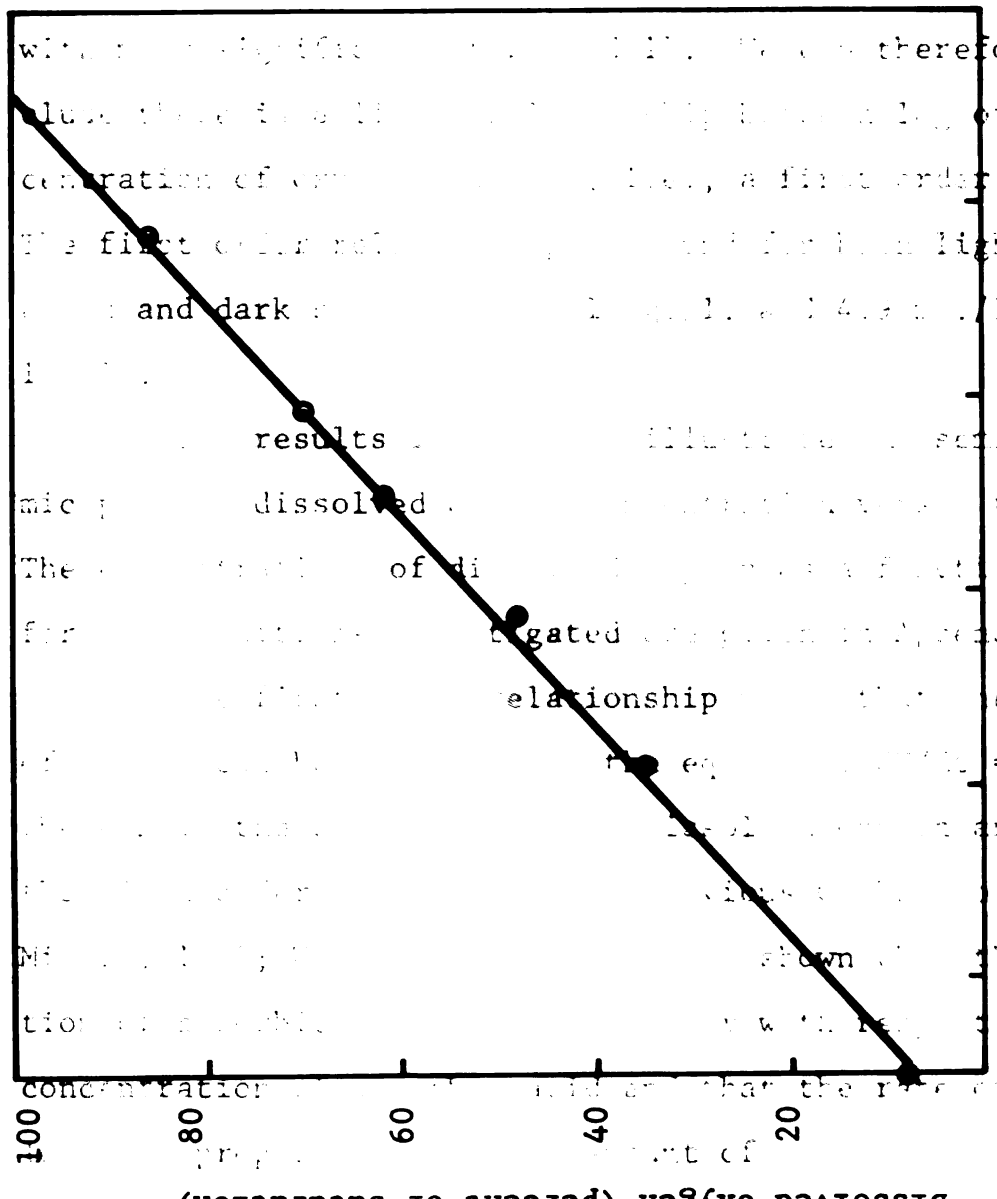


Figure 6. Standard curve for conversion of dissolved oxygen measurements from percent of saturation to concentration in mg/l.

Figure 4. Example of saturation curve for conversion of percent dissolved oxygen measurements from percent of saturation to concentration in mg/L.

The results indicate that for all cases, except for the experiments with an initial oxygen concentration of 1.00 mg./l. at 7.2°C and 2153 lux, 3229 lux, and 4306 lux, the t values calculated are significant at the 5% level with many significant at 2% and 1%. We can therefore conclude there is a linear relationship between log of the concentration of oxygen and time, i.e., a first order function. The first order relationship is found for both light exposed and dark samples at 8.71 mg./l. and 4.9 mg./l. oxygen levels.

The results in Figure 7 illustrate a semilogarithmic plot of dissolved oxygen concentration versus time. The concentrations of dissolved oxygen as a function of time for all conditions investigated are given in Appendix 1.

The first order relationship means that the uptake of oxygen can be described by the equation: $dN/dt = -kN$ where N is the concentration of dissolved oxygen and k is the first order rate constant. Previous works (Joslyn and Miller, 1949; Weissberger, 1934) have shown that the oxidation of ascorbic acid was first order with respect to the concentration of ascorbic acid and that the rate of vitamin loss was proportional to the amount of oxygen present. Coupling these two facts with the equation for the oxidation of ascorbic acid (Figure 2), one would expect that since ascorbic acid and atomic oxygen react on a 1:1 ratio the uptake of oxygen would also be first order.

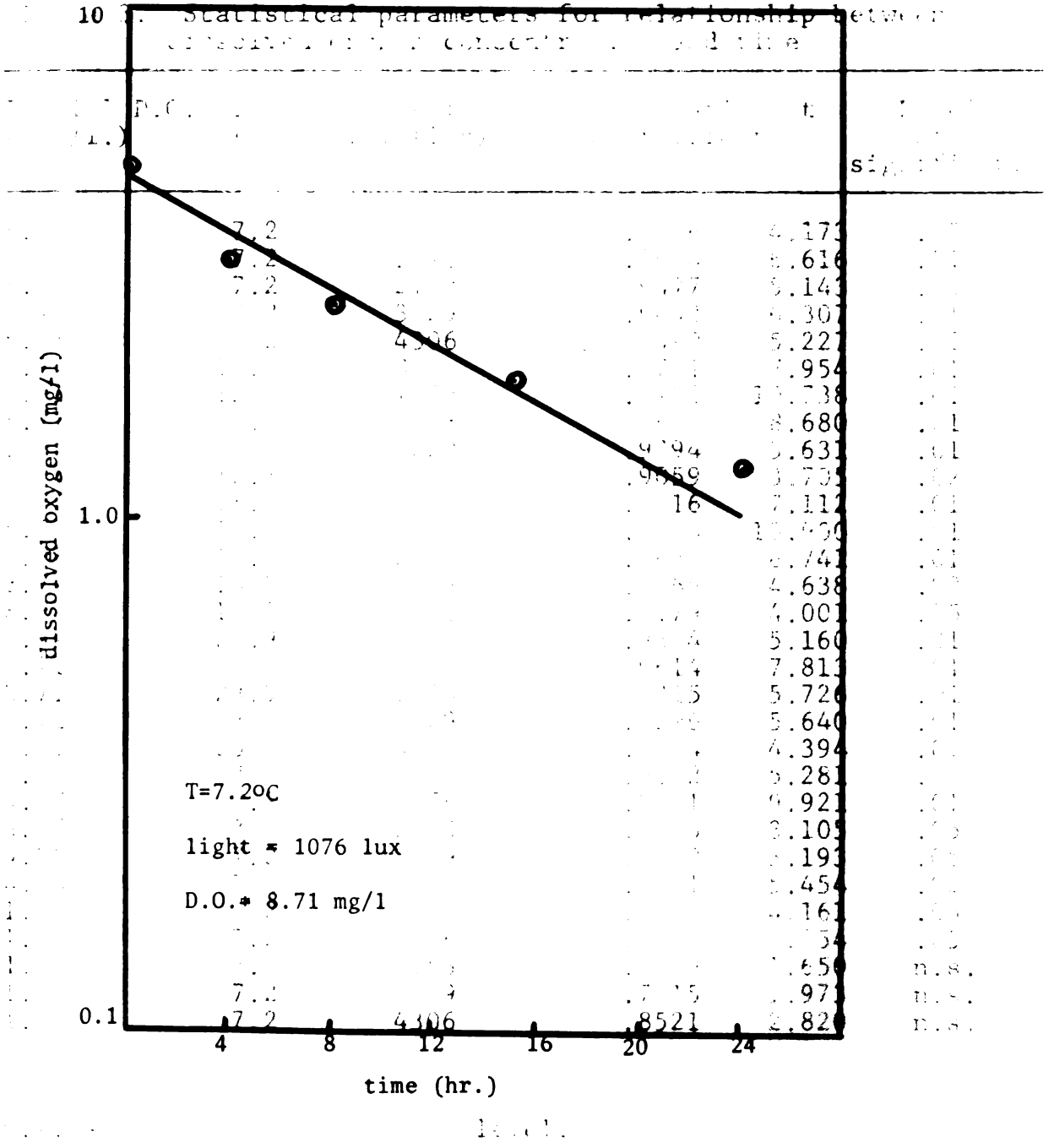


Figure 7. Semilogarithmi- plot of the log of concentration of dissolved oxygen versus time.

Figure 7. Semilogarithmic plot of the log of concentration of dissolved oxygen versus time.

Table 3. Statistical parameters for relationship between dissolved oxygen concentration and time

Initial D.O. (mg./l.)	Temp. (°C)	Light intensity (lux)	Correlation coefficient	t	Level of significance
8.71	7.2	0	.9236	4.173	.05
8.71	7.2	1076	.9674	6.616	.01
8.71	7.2	2153	.9477	5.143	.02
8.71	7.2	3229	.9643	6.307	.01
8.71	7.2	4306	.9490	5.227	.02
8.71	12.8	0	.9771	7.954	.01
8.71	12.8	1076	.9790	10.738	.01
8.71	12.8	2153	.9684	8.680	.01
8.71	12.8	3229	.9294	5.631	.01
8.71	12.8	4306	.9059	3.705	.02
8.71	18.3	0	.9716	7.112	.01
8.71	18.3	1076	.9796	10.900	.01
8.71	18.3	2153	.9688	8.741	.01
8.71	18.3	3229	.9368	4.638	.02
8.71	18.3	4306	.9179	4.001	.05
8.71	23.9	0	.9324	5.160	.01
8.71	23.9	1076	.9614	7.813	.01
8.71	23.9	2153	.9315	5.726	.01
8.71	23.9	3229	.9296	5.640	.01
8.71	23.9	4306	.8904	4.394	.02
4.86	7.2	0	.9502	5.281	.02
4.97	7.2	1076	.9851	9.921	.01
4.97	7.2	2153	.8733	3.105	.05
4.86	7.2	3229	.8790	3.193	.05
4.86	7.2	4306	.9531	5.454	.02
1.00	7.2	0	.9232	4.161	.05
1.00	7.2	1076	.9080	3.754	.05
1.00	7.2	2153	.6869	1.650	n.s.
1.00	7.2	3229	.7515	1.973	n.s.
1.00	7.2	4306	.8521	2.820	n.s.

n.s. = not significant at 5% level.

Based on the conclusion that oxygen uptake follows first order rate kinetics, the value of the rate constant (k) for each experimental condition was determined (Table 4). A computer program, KINFIT, was used to fit the data to the first order equation. The program differs from the usual least squares fitting techniques since it does not "linearize". Numerical integration procedures are used to provide the fit to the desired differential equation (Dye and Nicely, 1971). The program output gives the calculated values for the rate constant and initial oxygen concentration with their respective standard deviations. The input variables, time (hours) and concentration of dissolved oxygen (mg./l.), are accompanied with their variances. This approach assists in accounting for the internal errors of oxygen measurements and small variations in exposure time. The program was specifically written for chemical reactions and for evaluating the kinetic parameters of these reactions.

Rate of Oxygen Uptake as a Function of Light Intensity

The rate of oxygen uptake as a function of light intensity was measured at five points in the range 0 to 4,306 lux. This range of light intensities represents the range of light intensities typical for supermarket display cases. A least squares fitting method was used to determine the relationship between rate constants and light intensity using a straight line ($y = a+bx$) function. The results of the least squares fit and the correlation coefficient are given in Table 5.

Table 4. Oxygen uptake rate constants (k) as determined by KINFIT

Initial D.O. (mg./l.)	Temp. (°C)	Light intensity (lux)	Rate constant (k)	Standard deviation
1.00	7.2	0	.020044	.0060
1.00	7.2	1076	.092113	.0341
1.00	7.2	2153	.111123	.0351
1.00	7.2	3229	.053002	.0305
1.00	7.2	4306	.143743	.0600
4.86	7.2	0	.036721	.0098
4.97	7.2	1076	.063109	.0088
4.97	7.2	2153	.147708	.0421
4.86	7.2	3229	.151250	.0305
4.86	7.2	4306	.219894	.0323
8.71	7.2	0	.013063	.0042
8.71	7.2	1076	.142851	.0161
8.71	7.2	2153	.199752	.0369
8.71	7.2	3229	.275851	.0720
8.71	7.2	4306	.286340	.0736
8.71	12.8	0	.073318	.0158
8.71	12.8	1076	.157670	.0129
8.71	12.8	2153	.237069	.0140
8.71	12.8	3229	.316799	.0328
8.71	12.8	4306	.333286	.0740
8.71	18.3	0	.103587	.0349
8.71	18.3	1076	.161975	.0123
8.71	18.3	2153	.239958	.0134
8.71	18.3	3229	.362158	.0585
8.71	18.3	4306	.424329	.0551
8.71	23.9	0	.108846	.0347
8.71	23.9	1076	.170149	.0122
8.71	23.9	2153	.254356	.0198
8.71	23.9	3229	.387389	.0447
8.71	23.9	4306	.536992	.0505

Table 5. Coefficients describing linear relationship between oxygen uptake rate constants and light intensity

Initial D.O. (mg./l.)	Temp. (°C)	a	b	r	t
8.71	7.2	.04768	.0000631	.9603	7.60*
8.71	12.8	.08783	.0000631	.9819	9.01*
8.71	18.3	.09008	.0000782	.9926	13.61*
8.71	23.9	.07685	.0000997	.9844	9.69*
4.86	7.2	.03284	.0000422	.9729	5.94*
1.00	7.2	.04235	.0000194	.6786	1.60 ns

* = (P.01) ns = not significant

Though the high values of the correlation coefficients would indicate that there is a linear relationship between the rate of rate constant and light intensity, a t-test, the same as used to show that oxygen uptake was first order, was also run. The values of t obtained were all significant at the 1% level, except for the t obtained for 1.00 mg./l. and 7.2°C. It is therefore safe to conclude that the rate of oxygen uptake increases linearly with light intensity. The lack of linear fit of the data for 1.00 mg./l. initial concentration of dissolved oxygen and 7.2°C. is not unexpected when considering that the data used to generate the rate constants did not statistically fit the first order equation. Figure 8 is an example of a plot of rate of oxygen uptake as a function of light intensity.

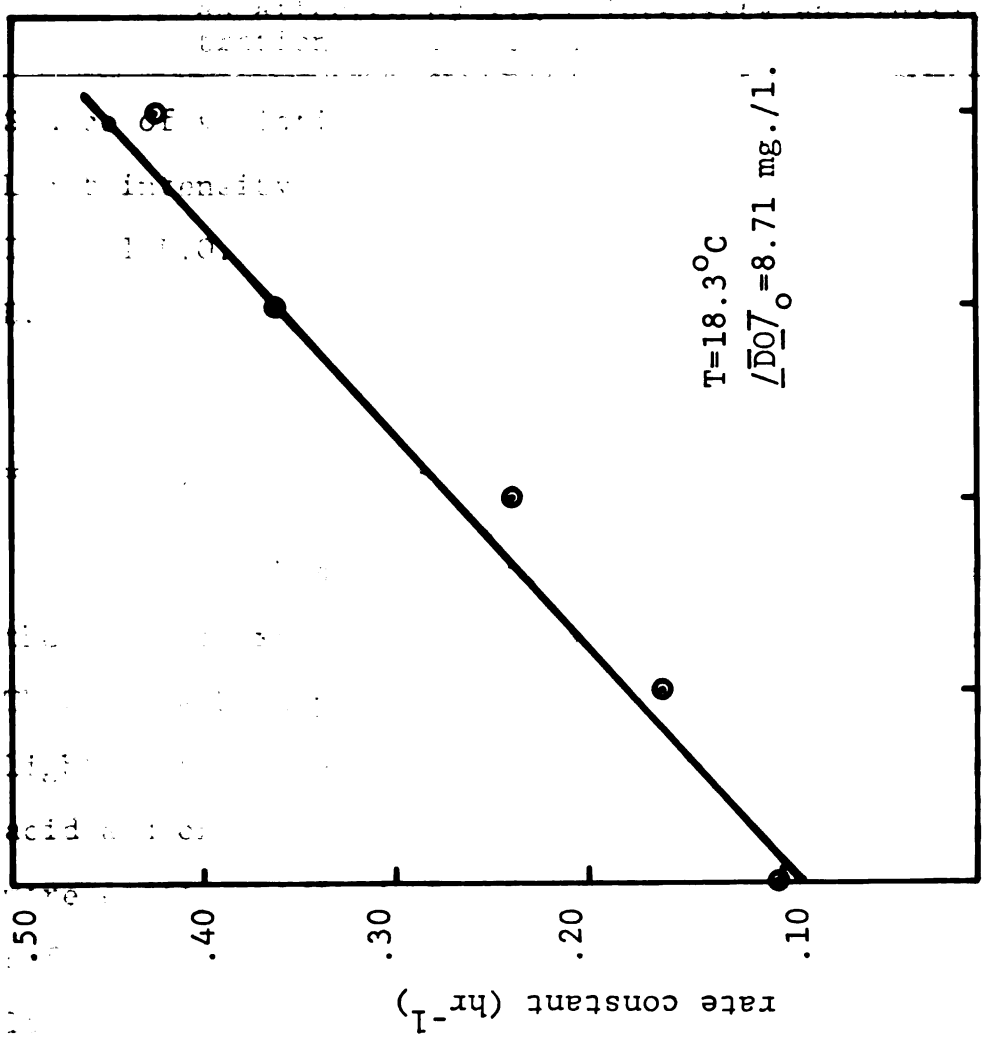


Figure 8. Rate of oxygen uptake as a function of incident light intensity.

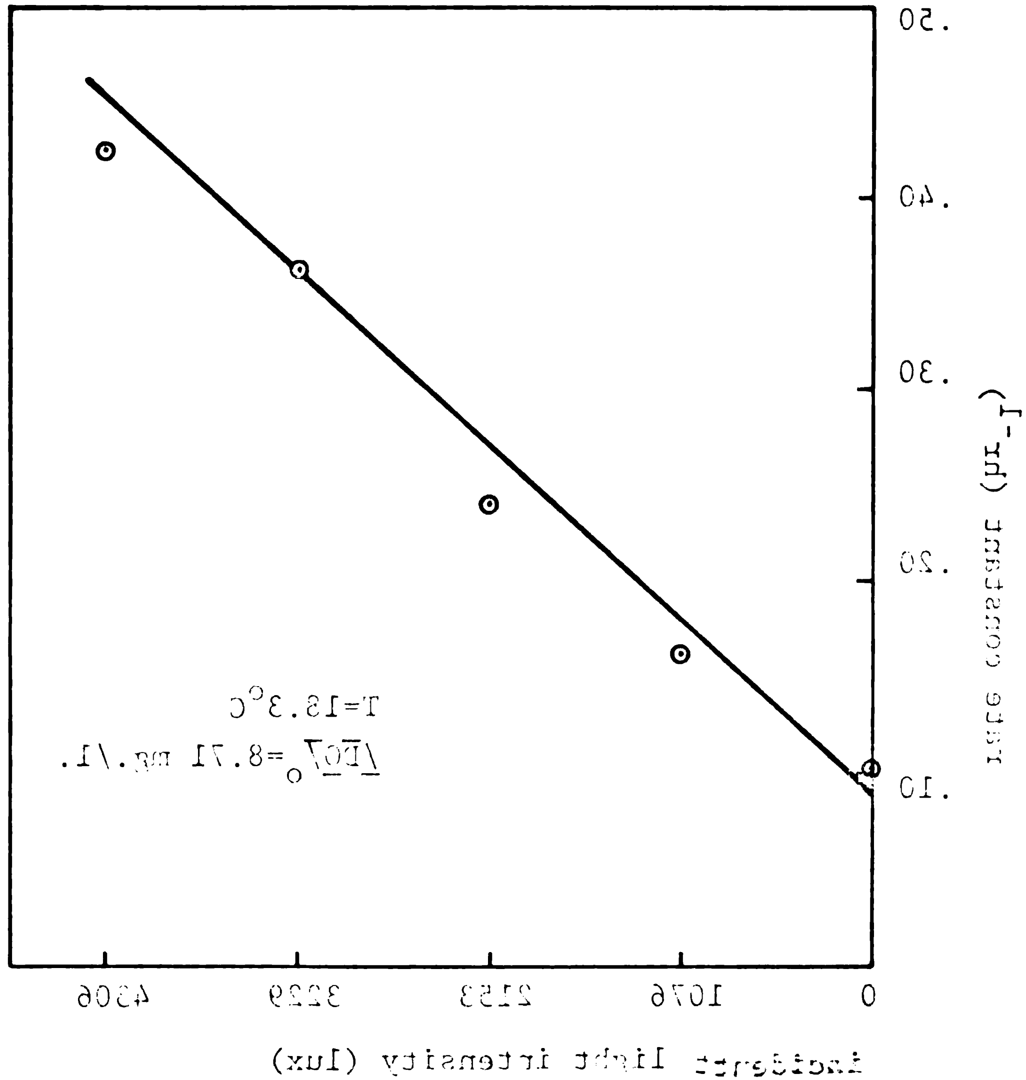


Figure 9. Rate of oxygen uptake as a function of incident light intensity.

An analysis of variance of the rate of oxygen uptake as affected by light intensity and initial concentration of dissolved oxygen (Table 6) indicates that both light intensity and initial concentration of dissolved oxygen have a highly significant influence on the rate of oxygen uptake.

Table 6. Analysis of variance for the rate of oxygen uptake as affected by light intensity and initial concentration of dissolved oxygen.

Source of variation	D.F.	SS	MSS	F-Ratio
Light intensity	4	.063787	.015947	7.278*
Initial D.O.	2	.025120	.012560	5.732**
Error	8	.017530	.002191	
	14	.106432		

* = (P.01) ** = (P.05)

There has been no previous research which dealt with the rate of oxygen uptake as a function of light intensity. There have been previous investigations on the influence of light on the rate of ascorbic acid loss. Since ascorbic acid and oxygen react on a 1:1 ratio and ascorbic acid is more easily oxidized than other food components, it should be safe to extrapolate the results of research on ascorbic acid loss to oxygen uptake. In order for this assumption to be acceptable, the product must be in a closed container with no oxygen transmission; these conditions were satisfied in the experiment.

The low rate constants in the dark conditions and the significant increase in the rate constant when the product was exposed to light is similar to results obtained for ascorbic acid degradation (Kon & Watson, 1934; Guthrie, 1938; 1946; Ford, 1967). Work by Dunkley et al. (1962) indicated that the rate of ascorbic acid degradation was directly proportional to radiant power emitted between 400-500nm. Sattar and deMan (1973) revealed the rate of loss of ascorbic acid increases with light intensity; only two light intensities were tested so the exact relationship was not determined. The results of the present investigation indicate that the oxygen uptake rate constant increases linearly with light intensity and follows a pattern similar to the rate of ascorbic acid loss with changing light intensity.

Rate of Oxygen Uptake as a Function of the Initial Concentration of Dissolved Oxygen

The rate of oxygen uptake as a function of initial concentration of dissolved oxygen was investigated at three levels. (Table 7) The levels chosen represent the wide range of possible conditions, from 1 mg./l. to nearly saturation.

By plotting oxygen uptake rate constants versus initial concentration of dissolved oxygen (Figure 9) the results indicate that the rate of oxygen uptake is directly proportional to the amount of oxygen present. The linear relationship was found for 2153, 3229, and 4306 lux. For the dark condition, the rate constants were within the standard

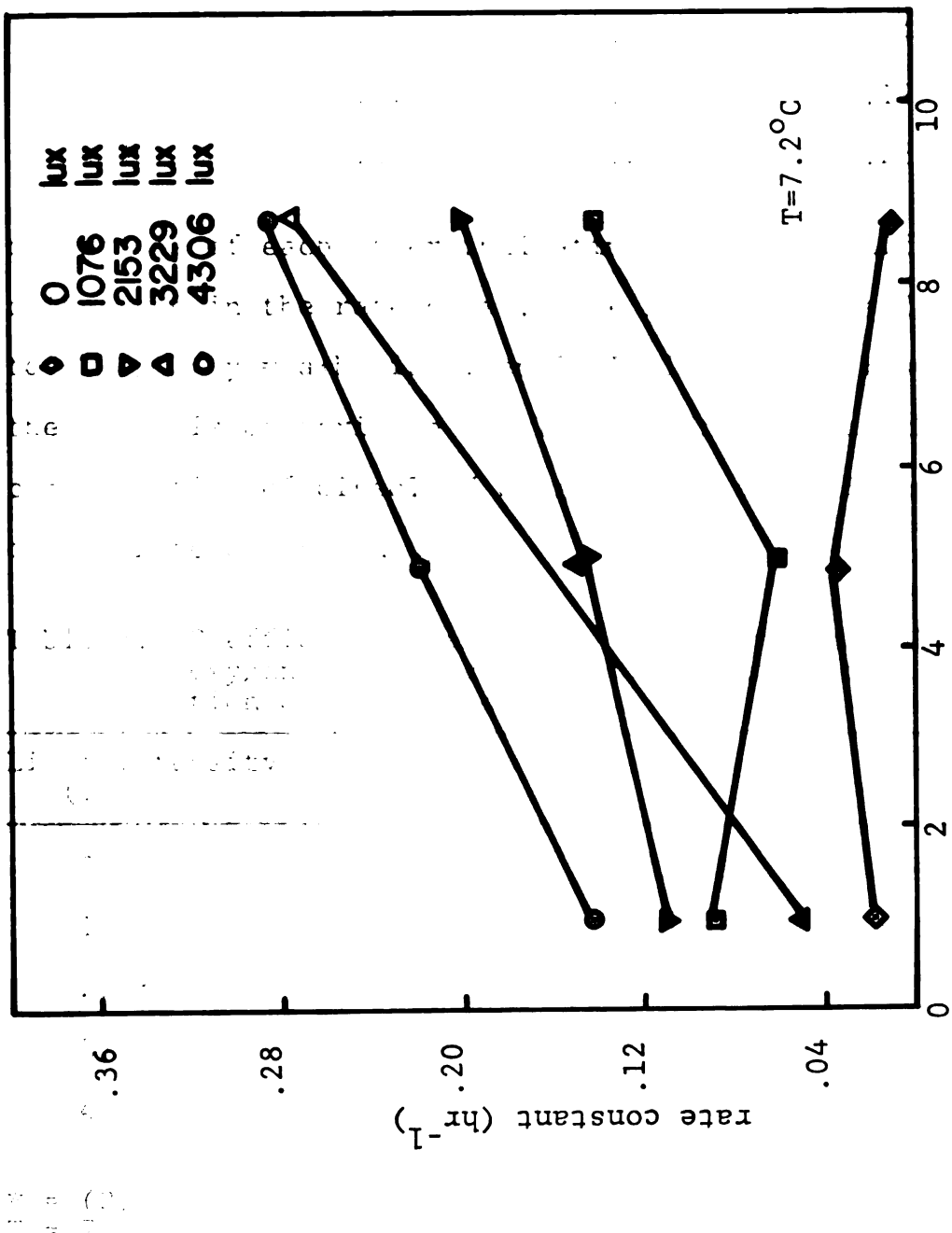


Figure 9. Rate of oxygen uptake as a function of the initial concentration of dissolved oxygen.

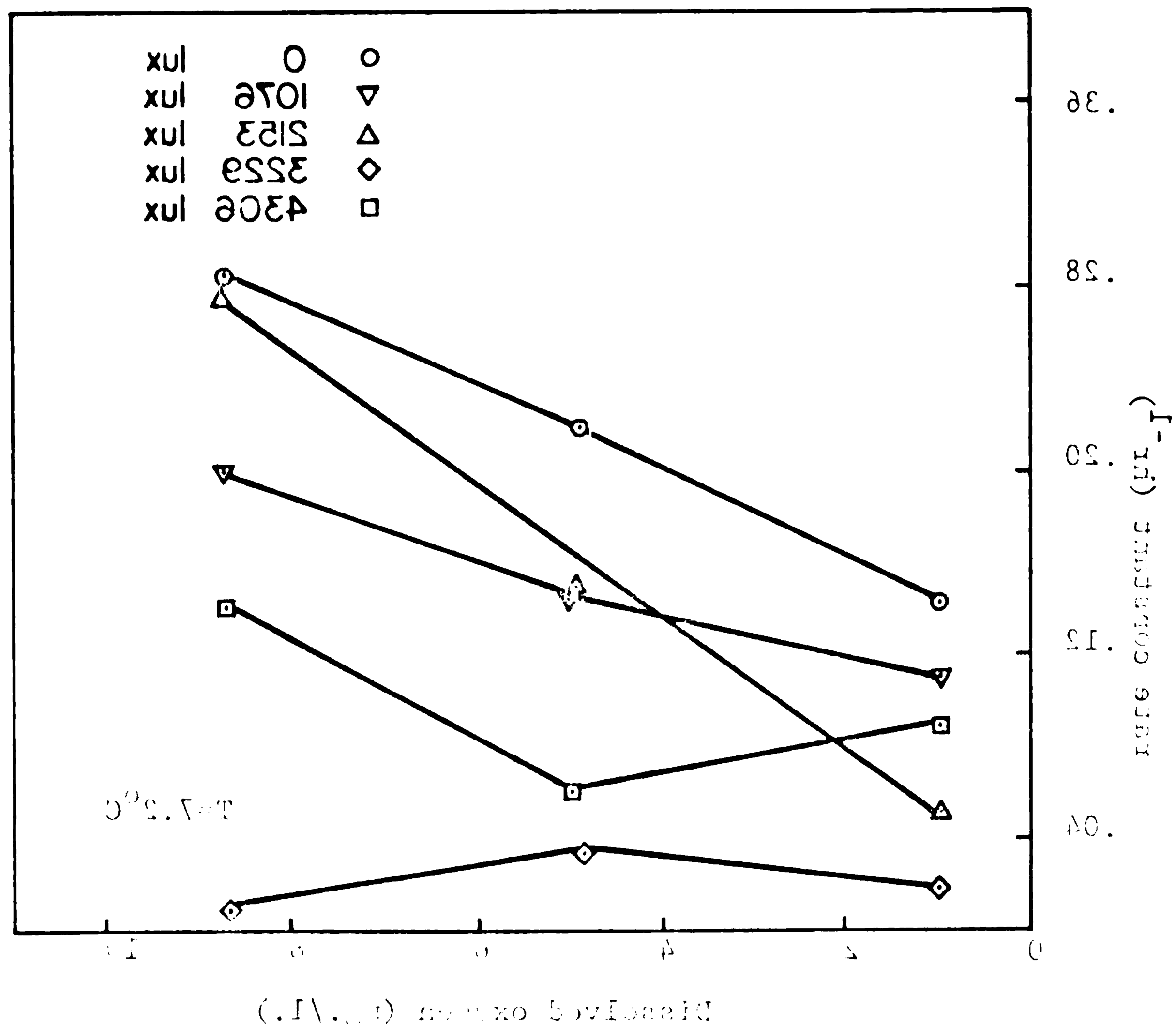


Figure 17. Rate of oxygen uptake as a function of the initial concentration of dissolved oxygen.

Table 7. Oxygen uptake rate constants as a function of initial concentration of dissolved oxygen

Light intensity (lux)	Initial concentration of D.O. (mg./l.) 1.00	4.86	8.71
1076	.020044	.036721	.013063
2153	.092113	.063109	.142851
3229	.111123	.147708	.199752
4306	.143743	.219894	.286340

deviations of each other indicating that no significant difference in the rate exist. The least squares fit of data to the line $y = a+bx$ is given in Table 8. The results of the analysis of variance (Table 6) indicate that the initial concentration of dissolved oxygen has a significant effect on the rate of oxygen uptake.

Table 8. Coefficients describing linear relationship between oxygen uptake rate constants and initial concentration of dissolved oxygen

Light intensity (lux)	a	b	r	t
0	.02766	.000903	.286	.248ns
1076	.06785	.006439	.615	.780ns
2153	.09672	.011472	.993	8.41*
3229	.01966	.03890	.997	12.88*
4306	.12680	.01849	.999	22.34*

* = (P.05) ns = not significant at 5% level
T = 7.2°C.

The direct proportionality between rate of oxygen uptake and initial concentration of oxygen agrees with the results of Khan and Mantell (1967) and Weissberger (1934). These reports indicated that the rate of destruction of ascorbic acid was directly proportional to the initial concentration of dissolved oxygen.

Rate of Oxygen Uptake as a Function of Temperature

In order to analyze the rate of oxygen uptake as a function of temperature, an Arrhenius plot was utilized. By plotting the rate constants for the four temperatures tested (7.2°C, 12.8°C, 18.3°C, 23.9°C) versus the inverse of the absolute temperature a straight line was obtained for all experiments except dark conditions (Figure 10). The straight line plots indicate that the rate of oxygen uptake follows the common Arrhenius relationship,

$$d\ln k/dt = \frac{E}{RT^2}$$

where R = gas constant, E = activation energy. The data were fit to the function y = a+bx using a least squares method (Table 9). The high correlation factors (all greater than 0.9) and the "t" test show that there is a linear relationship between the rate of oxygen uptake and the inverse of the absolute temperature, an Arrhenius relationship.

The oxygen uptake rate constants in dark conditions as a function of temperature did not follow an Arrhenius relationship. Increasing the temperature from 7.2°C resulted

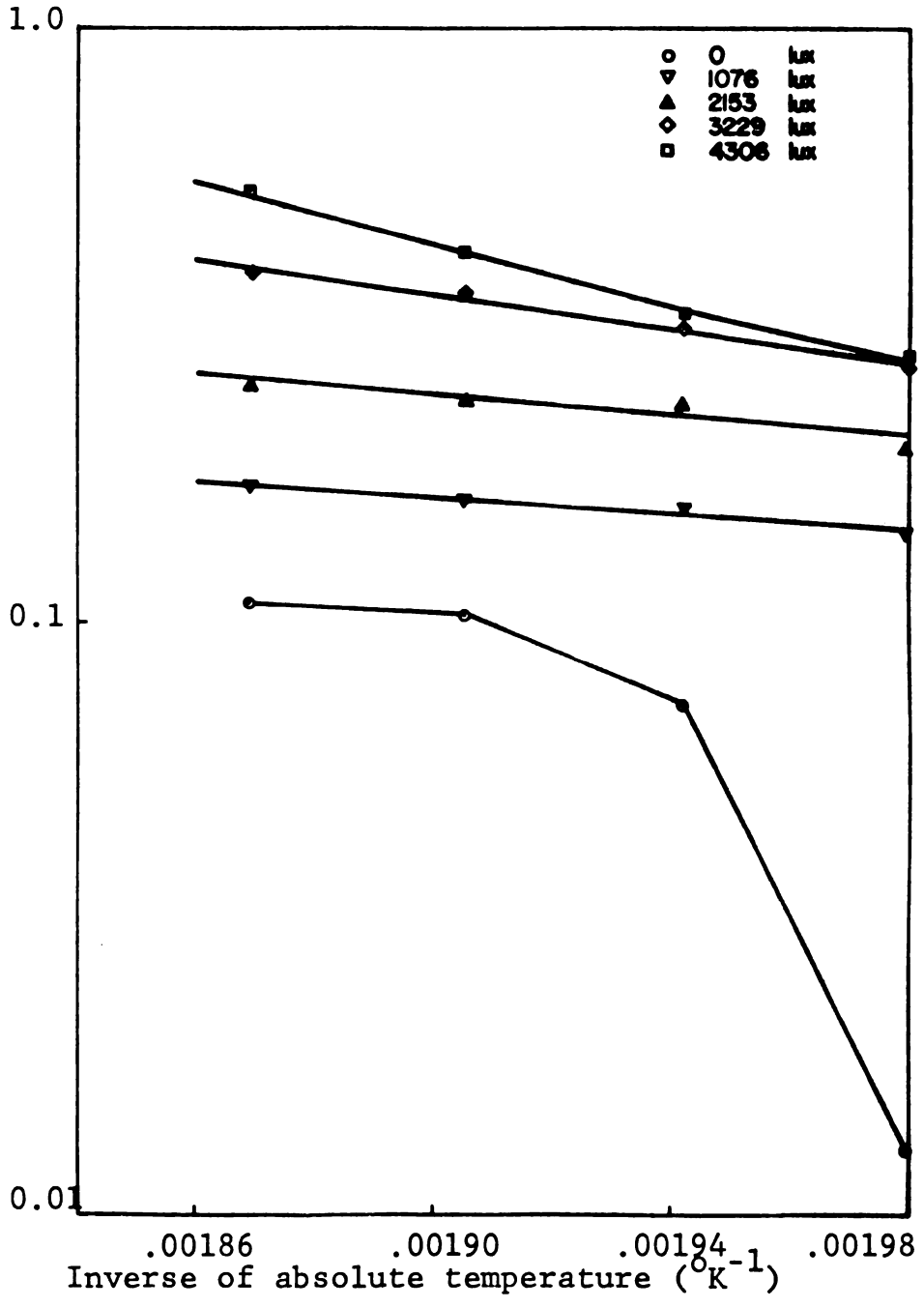


Figure 10. Arrhenius plot for oxygen uptake at several light intensities.

Table 9. Coefficients describing linear relationship between oxygen uptake rate constants and the inverse of absolute temperature

Light intensity (lux)	a	b	r	t
0	.446	-648.87	.969	5.547*
1076	1.036	-868.63	.918	3.27*
3229	2.549	-1149.72	.993	12.56**
4306	4.38	-2494.8	.994	12.83**

* = (P.05) initial $\overline{D.O.}$ = 8.71 mg./1.
** = (P.01)

in a drastic increase on the rate of oxygen uptake. Vitamin C assays indicated that the accelerated oxygen uptake rate was accompanied by only a slight increase in the rate of ascorbic acid loss (Table 10).

Table 10. Reduced Vitamin C as a function of time for infant formula held in the dark at 7.2°C and 23.9°C

	Concentration of reduced vitamin C (mg./1.)	
Time (hr)	7.2°C	23.9°C
0	92.39	99.69
4	84.95	92.08
8	83.12	92.32
16	81.86	85.65
24	76.70	82.07
k = .00664		k = .00750

No follow up tests were conducted to determine the cause of the large increase in rate of oxygen uptake. One could assume that in the dark conditions, the oxygen is being consumed in another oxidative reaction in the product. The type of reaction involved is difficult to predict without additional investigations. The sensitivity to increasing temperature is indicative of lipid oxidation although many other oxidation reactions are possible.

From each Arrhenius plot, which was significant, the associated activation energy (E) was computed (Table 11).

Table 11. Activation energies associated with oxygen uptake in liquid formula at initial dissolved oxygen of 8.71 mg./l.

Light intensity (lux)	Activation energy (cal/mole)
1076	2967
2153	3973
3229	5258
4306	11406

The activation energy, which is a measure of the dependence of the rate of reaction on temperature, is equal to the slope of the Arrhenius plot times the gas constant /(R)x2.303/. The activation energy increased with light intensity indicating that at a higher light intensity the rate of oxygen uptake is more sensitive to temperature. The increase in activation energy with higher light intensity would appear to be

opposite of what would be expected. Since light acts as a catalyst for the oxidative reaction it would be expected that the higher light intensity would decrease the amount of energy which must be supplied in order for the reaction to proceed. We see, however, what appears to be a synergistic relationship between light and temperature.

Prediction of Losses of Vitamin C from Dissolved Oxygen Uptake Data

An attempt was made to predict by use of a computer model the losses of vitamin C from the dissolved oxygen uptake data. The prediction was a two step process using a second order model (Singh, 1974) with slight modification. The Singh (1974) model when given the initial concentrations of dissolved oxygen, and vitamin C, diffusion constants, and the second order rate constant gives the concentrations of vitamin C and dissolved oxygen as a function of time.

The first step in the prediction was to supply the second order model with the oxygen uptake data and an estimate of the second order rate constant. A subroutine added to the model then computed the sum of squares of the differences between the dissolved oxygen values computed on the basis of the given rate constant and the experimental dissolved oxygen data. The second order rate constant was then varied to give a minimum value to the sum of squares.

In the second step of the prediction the rate constant which gave the dissolved oxygen values closest to the

experimental data was placed in the second order model to predict the concentration of vitamin C as a function of time.

The vitamin values obtained by the computer predictions as well as the experimental values are given in Table 12.

Table 12. Experimental and computer predicted values of vitamin C as a function of time

Time (hr)	Experimental values (mg./l.)	Computer predicted values (mg./l.)	
		Boundary condition (I)	Boundary condition (II)
0	94.91	94.91	94.91
4	83.71	91.43	56.42
8	77.66	89.42	44.44
15	69.15	87.69	27.97
24	56.16	86.87	15.46

D.O. = 8.71 mg./l. T = 7.2°C light = 1076 lux

In using the Singh (1974) model two different boundary conditions were tried. In the first attempt the boundary condition set the values of dissolved oxygen and vitamin C at the first node equal to the values at the second node (I). With this boundary condition the predicted vitamin values were considerably higher than the experimental values.

Noting that the first boundary condition gave vitamin values considerably higher than the experimental

values a second boundary condition setting the values of vitamin and dissolved oxygen at the first node equal to zero (II). This condition resulted in two problems: 1) the vitamin values obtained were considerably lower than the experimental data and 2) the second order rate constant had very little effect on the vitamin values obtained.

In order to obtain predicted values close to the experimental values the boundary condition must be set between the two conditions tried.

CONCLUSIONS

1. The uptake of dissolved oxygen from a liquid food is adequately described by first order rate kinetics.
2. The rate of oxygen uptake increases linearly with increasing light intensity in the range of 0 to 4,300 lux.
3. The rate of oxygen uptake as a function of temperature follows an Arrhenius relationship for light exposed samples.
4. The rate of oxygen uptake is directly proportional to the amount of dissolved oxygen initially present in the liquid.
5. The activation energy associated with oxygen uptake increases with increasing light intensity.

SUGGESTIONS FOR FURTHER STUDY

1. Investigation of the uptake of oxygen which occurred in the dark at higher temperatures.

2. Continue work on computer prediction of vitamin C losses from oxygen uptake data with emphasis on finding a workable boundary condition.

BIBLIOGRAPHY

Bayes, A.L. 1950. Investigations on the use of nitrogen for the stabilization of perishable food products. Food Technology 4:151.

Blaug, S.M. and Hajratwala, B. 1972. Kinetics of aerobic oxidation of ascorbic acid. Journal of Pharmaceutical Sciences 61(4) 556-62.

Braverman, J.B.S. 1963. Introduction to the Biochemistry of Foods. New York. Elsevier Publishing Co.

Burton, H. et al. 1970. Comparison of milk processed by the direct and indirect methods of ultra-high-temperature sterilization. IV. The vitamin comparison of milk sterilized by different processes. Journal of Dairy Research 37(3) 529.

Dunkley, W.L., Franklin, J.D., and Pangborn, R.M. 1962. Effects of fluorescent light on flavor, ascorbic acid, and riboflavin in milk. Food Technology 16:112.

Dye, J. and Nicely, V. 1971. A general purpose of curve fitting program for class and research use. Journal of Chemical Education (48) 443.

Ford, J.E. 1957. Factors influencing the destruction by heat of vitamin B_{12} in milk. Journal of Dairy Research 24:360-365.

_____. 1967. The influence of the dissolved oxygen in milk on the stability of some vitamins towards heating and during subsequent exposure to sunlight. Journal of Dairy Research 34(3) 239-247.

Guthrie, E.S., Sharp, P.F., and Hand, D.B. 1938. Oxygen in milk and its relation to the destruction of vitamin C and the development of oxidized flavor. 12th Annual Report, New York State Association of Dairy and Milk Inspectors. pp. 101-110.

_____. 1946. The results of deaeration on the oxygen, vitamin C, and oxidized flavors of milk. Journal of Dairy Science 29(6) 359-369.

Hamburg, M. 1970. Statistical Analysis for Decision Making. New York. Harcourt, Brace and World, Inc.

Hand, D.E., Guthrie, E.S., and Sharp, P.F. 1938. Effect of oxygen, light, and lactoflavin on the oxidation of vitamin C in milk. Science 87:439.

Hand, D.B. and Greisen, E.C. 1942. Oxidation and reduction of vitamin C. Journal of the American Chemical Society 64:358-361.

Hartman, G.H. and Garrett, O.F. 1942. The factors affecting the stability of certain milk properties. VI. Relation of the concentration of dissolved oxygen to oxidation of ascorbic acid and to the development of oxidized flavor in milk. Journal of Dairy Science 26(4) 337-342.

Herreid, E.O. and Francis, J. 1949. The effect of handling and processing of milk on its oxygen content. Journal of Dairy Science 32(3) 202-208.

Joslyn, M.A. and Miller, J. 1949. The kinetics of autoxidation of vitamin C in sugar solution in the presence of dissolved oxygen. Food Research 14:325.

Khan, M.M.J. and Martell, A.E. 1966. Metal ion and metal chelate catalyzed oxidation of ascorbic acid by molecular oxygen. I. Cupric and ferric ion catalyzed oxidation. Journal of the American Chemical Society 89(16) 4176-4185.

Kirk, J.R. and Ting, N. 1974. Total vitamin C using continuous flow analysis. Presented at the 34th IFT Meeting, New Orleans.

Kon, S.K. and Watson, M.B. 1936. The effect of light on the vitamin C content of milk. Biochemical Journal 30: 2273.

Lück, H. and Schillinger, A. 1959. Untersuchungen uber den Einflufb von Sauerstoff auf verschiedene Milchbestandteile. Zertschrift für lebensmittel - untersuchung und - forschung 110:267.

Sattar, A. and deMan, J.M. 1973. Effect of packaging material on light induced quality degradation in milk. Canadian Institute of Food Science and Technology Journal 6:170-174.

Sharp, P.F., Trout, G.M., and Guthrie, E.S. 1936. Vitamin C, copper and the oxidized flavor of milk. 10th Annual Report, New York State Association of Dairy and Milk Inspectors. pp. 153-164.

Sharp, P.F., Hand, D.B., and Guthrie, E.S. 1939. Preliminary report on the deaeration of market milk. Journal of Milk Technology 3:137-143.

_____, _____, and _____. 1940. Deaeration as a means of retarding oxidized flavor and the vitamin C in milk. 14th Annual Report, New York State Association of Dairy and Milk Inspectors, pp. 63-76.

_____, _____, and _____. 1941. How to conserve vitamin C in a liquid. Food Industry 13(6) 50-52.

_____, _____, and _____. 1941. Quantitative determination of dissolved oxygen, ascorbic acid oxidase method. Industrial and Engineering Chemistry 13(9) 593-597.

Singh, R.P. 1974. Unpublished Doctoral dissertation, Michigan State University, Department of Agricultural Engineering.

Stadhouders, J. 1965. The polargraphic measurement of dissolved oxygen in milk as an index of the bacteriological quality of milk. Netherlands Milk and Dairy Journal 19(2) 119-138.

Thompson, S.Y. 1969. Nutritional aspects of UHT processed products. Proceedings of a seminar Agricultural Institute, Republic of Ireland, May 21-22, 1969, pp. 46-52.

Weissberger, A., LuValle, J.E., and Thomas Jr., D.R. 1943. Oxidation processes. XVI. The autoxidation of ascorbic acid. Journal of American Chemical Society 65:1934-1939.

APPENDICES

APPENDIX 1. Concentration of dissolved oxygen as a function of time for each condition tested.

initial /D.O./ = 1.00 mg./1.
light intensity = 0 kyx
temperature = 7.2°C

exposure time (hr.)	D.O. (mg./1.)
0	1.00
4	1.11
8	1.00
16	0.84
24	0.62

initial /D.O./ = 1.00 mg./1.
light intensity = 1076 lux
temperature = 7.2°C

exposure time (hr.)	D.O. (mg./1.)
0	1.00
4	0.40
8	0.40
16	0.34
24	0.18

initial /D.O./ = 1.00 mg./1.
light intensity = 2153 lux
temperature = 7.2°C

exposure time (hr.)	D.O. (mg./1.)
0	1.00
4	0.45
8	0.34
16	0.23
24	0.23

initial /D.O./ = 1.00 mg./1.
light intensity = 3229 lux
temperature = 7.2°C

exposure time (hr.)	D.O. (mg./1.)
0	1.00
4	0.40
8	0.45
16	0.56
24	0.23

initial /D.O./ = 1.00 mg./1.
light intensity = 4306 lux
temperature = 7.2°C

exposure time (hr.)	D.O. (mg./1.)
0	1.00
4	0.45
8	0.18
16	0.40
24	0.01

initial /D.O./ = 4.86 mg./1.
light intensity = 0 lux
temperature = 7.2°C

exposure time (hr.)	D.O. (mg./1.)
0	4.86
4	4.75
8	4.64
15	3.21
24	1.66

initial $\overline{D.O.}$ = 4.97 mg./1.
light intensity = 1076 lux
temperature = 7.2°C

exposure time (hr.)	D.O. (mg./1.)
0	4.97
4	3.27
8	2.66
15	1.88
24	1.28

initial $\overline{D.O.}$ = 4.97 mg./1.
light intensity = 2153 lux
temperature = 7.2°C

exposure time (hr.)	D.O. (mg./1.)
0	4.97
4	2.21
8	1.11
15	1.06
24	0.78

initial $\overline{D.O.}$ = 4.86 mg./1.
light intensity = 3229 lux
temperature = 7.2°C

exposure time (hr.)	D.O. (mg./1.)
0	4.86
4	2.27
8	0.89
15	1.17
24	0.56

initial $\overline{D.O.}$ = 4.86 mg./1.
light intensity = 4306 lux
temperature = 7.2°C

exposure time (hr.)	D.O. (mg./1.)
0	4.86
4	1.88
8	0.73
15	0.56
24	0.23

initial $\overline{D.O.}$ = 8.71 mg./1.
light intensity = 0 lux
temperature = 7.2°C

exposure time (hr.)	D.O. (mg.1.)
0	8.71
4	8.00
8	7.72
15	7.50
24	4.97

initial $\overline{D.O.}$ = 8.71 mg./1.
light intensity = 1076 lux
temperature = 7.2°C

exposure time (hr.)	D.O. (mg./1.)
0	8.71
4	5.58
8	2.49
15	0.95
24	0.67

initial $\overline{D.O.}$ = 8.71 mg./1.
light intensity = 2153 lux
temperature = 7.2°C

exposure time (hr.)	D.O. (mg./1.)
0	8.71
4	3.54
8	1.44
15	0.89
24	0.51

initial $\overline{D.O.}$ = 8.71 mg./1.
light intensity = 3229 lux
temperature = 7.2°C

exposure time (hr.)	D.O. (mg./1.)
0	8.71
4	1.88
8	1.28
15	0.84
24	0.12

initial $\overline{D.O.}$ = 8.71 mg./1.
light intensity = 4306 lux
temperature = 7.2°C

exposure time (hr.)	D.O. (mg./1.)
0	8.71
4	1.88
8	1.17
15	0.95
24	0.18

initial $\overline{D.O.}$ = 8.71 mg./1.
light intensity = 8 lux
temperature = 12.8°C

exposure time (hr.)	D.O. (mg./1.)
0	8.71
4	7.39
8	6.62
15	2.54
24	0.89

initial $\overline{D.O.}$ = 8.71 mg./1.
light intensity = 1076 lux
temperature = 12.8°C

exposure time (hr.)	D.O. (mg./1.)
0	8.71
2	7.06
4	5.52
6	3.76
8	2.54
16	0.34
24	0.23

initial $\overline{D.O.}$ = 8.71 mg./1.
light intensity = 2153 lux
temperature = 12.8°C

exposure time (hr.)	D.O. (mg./1.)
0	8.71
2	6.18
4	3.43
6	2.21
8	1.22
16	0.34
24	0.23

initial [D.O.] = 8.71 mg./1.
light intensity = 3229 lux
temperature = 12.8°C

exposure time (hr.)	D.O. (mg./1.)
0	8.71
2	4.30
4	1.77
6	1.33
8	1.00
16	0.23
24	0.12

initial [D.O.] = 8.71 mg./1.
light intensity = 4306 lux
temperature = 12.8°C

exposure time (hr.)	D.O. (mg./1.)
0	8.71
4	1.66
8	1.00
16	0.45
24	0.34

initial [D.O.] = 8.71 mg./1.
light intensity = 0 lux
temperature = 18.3°C

exposure time (hr.)	D.O. (mg./1.)
0	8.71
4	7.17
8	6.29
16	0.56
24	0.12

initial [D.O.] = 8.71 mg./1.
light intensity = 1076 lux
temperature = 18.3°C

exposure time (hr.)	D.O. (mg./1.)
0	8.71
2	6.84
4	5.41
6	3.54
8	2.43
16	0.34
24	0.23

initial [D.O.] = 8.71 mg./1.
light intensity = 2153 lux
temperature = 18.3°C

exposure time (hr.)	D.O. (mg./1.)
0	8.71
2	5.85
4	3.54
6	1.88
8	1.33
16	0.34
24	0.23

initial [D.O.] = 8.71 mg./1.
light intensity = 3229 lux
temperature = 18.3°C

exposure time (hr.)	D.O. (mg./1.)
0	8.71
4	1.55
8	0.89
16	0.12
24	0.12

initial /D.O./ = 8.71 mg./1.
light intensity = 4306 lux
temperature = 18.3°C

exposure time (hr.)	D.O. (mg./1.)
0	8.71
4	1.44
8	0.45
16	0.34
24	0.12

initial /D.O./ = 8.71 mg./1.
light intensity = 0 lux
temperature = 23.9°C

exposure time (hr.)	D.O. (mg./1.)
0	8.71
2	7.72
4	6.95
8	6.18
16	0.12
24	0.12

initial /D.O./ = 8.71 mg./1.
light intensity = 1076 lux
temperature = 23.9°C

exposure time (hr.)	D.O. (mg./1.)
0	8.71
2	6.84
4	4.97
6	3.54
8	2.10
16	0.34
24	0.34

initial /D.O./ = 8.71 mg./1.
light intensity = 2153 lux
temperature = 23.9°C

exposure time (hr.)	D.O. (mg./1.)
0	8.71
2	6.07
4	3.32
6	1.99
8	1.00
16	0.34
24	0.45

initial /D.O./ = 8.71 mg./1.
light intensity = 3229 lux
temperature = 23.9°C

exposure time (hr.)	D.O. (mg./1.)
0	8.71
2	3.54
4	1.55
8	0.67
16	0.12
24	0.12

initial /D.O./ = 8.71 mg./1.
light intensity = 4306 lux
temperature = 23.9°C

exposure time (hr.)	D.O. (mg./1.)
0	8.71
2	3.10
4	0.89
8	0.34
16	0.12
24	0.12

APPENDIX 2. Analysis of variance for the rate of oxygen uptake as affected by light intensity and initial concentration of dissolved oxygen.

light intensity (lux)	initial D.O. (mg./l.) 1.00	4.86	8.71	
0	.020044	.036721	.013063	= .069828
1076	.092113	.063109	.142851	= .298073
2153	.111123	.147708	.199752	= .458583
3229	.053002	.151250	.275851	= .480103
4306	.143743	.219894	.286340	= .649977
	.420025	.618682	.917857	

Temperature = 7.2°C

$CM = (\Sigma y_i)^2/n = (1.956564)^2/15 = .255210$

$SS(light) = \sum_{i=1}^{5} T_i^2/n - CM = [(.069828)^2 + (.298073)^2 + (.458583)^2 + (.480103)^2 + (.649977)^2] 3 - .255210 = .063787$

$SS\ (D.O.) = \sum_{i=1}^{3} T_i^2/n - CM = [(.420025)^2 + (.618682)^2 + (.917857)^2] 5 - .255210 = .025120$

$y_i^2 = .361647 \quad TSS = y_i^2 - CM = .361647 - .255210 = .106437$

$SS(error) = TSS - SS(light) - SS(D.O.) = .106432$

source of variation	D.F.	SS	MSS	F-ratio
light intensity	4	.063787	.015947	7.278*
initial D.O.	2	.025120	.012560	5.732**
error	8	.017530	.002191	
	14	.106432		

* = (P.01)
**=(P.05)

APPENDIX 3. Addition made to Singh (1974) program to compute sum of squares and vary rate constant.

```
IF (TIME.EQ.2.0.)     CALL SUMSQ(1)

IF (TIME.E.Q.4.0)     CALL SUMSQ(2)

IF (TIME.E.Q.6.0)     CALL SUMSQ(3)

IF (TIME.E.Q.8.0)     CALL SUMSQ(4)

IF (TIME.E.Q.16.0)    CALL SUMSQ(5)

IF (TIME.E.Q.24.0)    CALL SUMSQ(6)

RK1 = RK1*3600
PRINT 1000, RK1, DIFF, SQ

1000 FORMAT (*O*, 3(3X,E12.5))

IF (DIFF. GT. 0.0)  GO TO 30

K2 = RK1

RK1 = (K1-RK1)/2.0+RK1

GO TO 40

30 K1 = RK1

RK1 = (K1 - RK1)/2.0+RK1

40 CONTINUE

IF (SQ.LT. 1.0E - 20) STOP

GO TO 50

SUBROUTINE  SUMSQ

  SUBROUTINE SUMSQ

  DIMENSION DATA (6)

  COMMON /c/ DATA, SQ, DIFF, BVCON
```

APPENDIX 3 (Continued . . .)

```
IF (DATA(I).EQ 0.0)  GO TO 1
SQ = SQ (ABS(BVCON - DATA (I)))**2.0
DIFF = DIFF (DATA (I)) - BVCON
CONTINUE
RETURN
END
```