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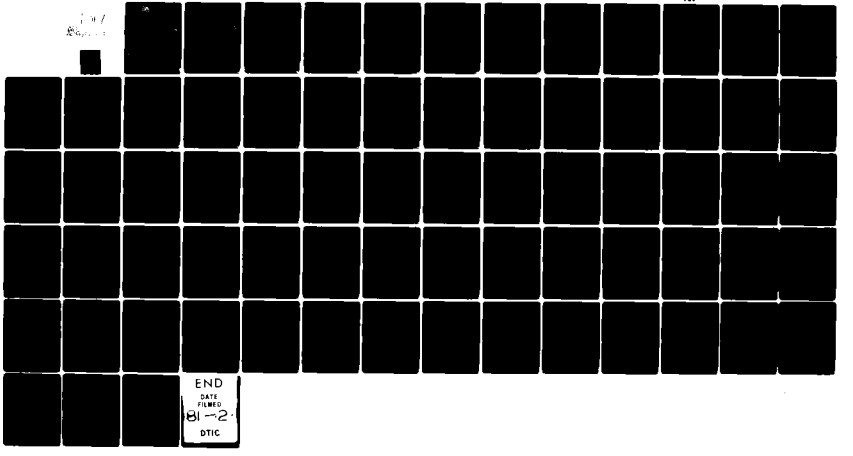

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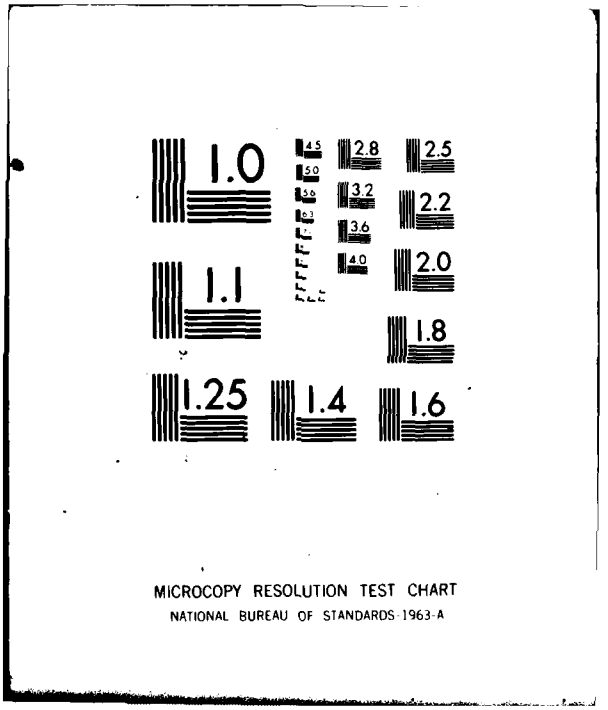
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Report Number

DISINFECTION OF VIRUSES IN WATER BY OZONE

Final Comprehensive Report

H.I. Shuval and E. Katzenelson

December 1979

Edited by - Naomi Guttman-Bass

Supported by:

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Fort Detrick, Frederick, Maryland 21701

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Environmental Health Laboratory

Hebrew University

Jerusalem, Israel

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SUMMARY : DISINFECTION OF VIRUSES IN WATER BY OZONE

A. Measurement of the Inactivation Kinetics of Poliovirus by Ozone in a Fast Flow Mixer

Inactivation kinetics of poliovirus 1 in ozone demand-free water was investigated utilizing a fast flow mixing appartus. Ozonated water and a solution of ozone demand-free water, containing a known quantity of poliovirus 1, were introduced simultaneously into a mixing chamber, both at a constant rate. This mixture was then passed through a narrow tube of known length and diameter into a neutralizing solution. By altering the rate of introduction and/or tube length, different contact periods between ozone and virus could be determined with an accuracy of 0.01 seconds.

Inactivation of the poliovirus occurred in two steps. During the first step, lasting 0.2-0.1 seconds, 95-99% of the virus was inactivated, depending on the ozone concentration (which ranged from 0.1-2.0 mg/liter). The second step apparently continued for several minutes, during which the remainder of the virus was inactivated. An obvious dose response relationship was demonstrated during the first step of the inactivation curve. The pH of the water slightly affected the viral inactivation rate, but these small differences seem to have no practical value.

B. Studies on the Kinetics of Virus Inactivation in Jerusalem Tap Water and in Renovated Wastewater in the Fast Flow Reactor

The first part of this study was carried out with Jerusalem tap water which has a low ozone demand. A single sample of Jerusalem tap water of 20 liters was taken and stored at $4^{o}C$ for the entire series of experiments. The Jerusalem tap water was free of residual chlorine and tests showed

that the ozone demand was about .1 ppm in the period of the reaction up to 1 second. Inactivation of poliovirus in tapwater occurred in two steps. In the first stage which lasted 0.1-0.4 seconds, 99.9-99.99% of the virus was inactivated, and in the second step which lasted for several seconds the remainder of the virus was inactivated. A dose response relationship between O_3 concentration and rate of virus inactivation was demonstrated.

In the second part of this study utilizing wastewater effluent from the Dan Region Water Reclamation Project,

a single 20 liter sample of finished reclaimed water was taken. The water was clarified through a "Balston" filter with a pore size of 8 microns and stored at $4^{o}C$ for the entire series of experiments.

For the inactivation experiments carried out with this renovated wastewater from the Dan Region Project (DOC - 20 ppm C) no measurable O_3 residual concentration was noted although virus inactivation occurred. Inactivation took place in two stages, similar to those for the tapwater. The first step lasted 0.24-0.5 seconds and resulted in the inactivation of only 50-90% of the viruses. No clear dose response relationship was found.

C. Mass Transfer and Reaction Kinetics in The Ozone/Tap Water System

The mass transfer and the reaction kinetics of ozone in tap water were examined. The contacting system was a well-mixed reactor with perpendicular jets. The ozone residual range was the lowest detectable range. A simple mathematical model was proposed to describe the mechanism of the ozone in the reactor and was checked over the range of water flow

rates from 2.170 to 3.080 liter/min, gas flow rates from 0.667 to 1.333 liter/min and different temperature conditions. The proposed model describes quite accurately the mass-transfer behaviour in the reactor and determines the operational parameters which control the reactor operation. There is a linear relationship between the ozone feed rate and ozone residual. Zero order kinetics best describe the autodecomposition of ozone in plain tap water. The mass transfer coefficient is about 1.14 min^{-1}, not using agitation causes a large decrease in $K_L a$ - 1 0.250 min^{-1} and the magnitude of the reactor rate constant is between 0.05 and 0.13 mg/min liter.

D. Inactivation Rate of Poliovirus in Tap Water by Ozone in a Continuous Reactor System

The inactivation rate of poliovirus I in tap water with ozone has been determined in a continuous well-mixed reactor with perpendicular jets. Water flow rates from 1.7 to 3.1 liter/min and ozone flow rates from 0.3 to 1 mg/min were employed.

The reactor examined proved to be very effective in producing inactivation (99%) within one minute for an ozone dose of less than 0.4 mg/liter.

E. Mechanism of Virus Inactivation by Ozone

Results have shown that only those amino acids containing sulfur groups react kinetically in a similar manner to poliovirus type 1. Cysteine and methionine reacted completely within less than 25 milliseconds while the oxidation of cysteine required almost one second. Unsaturated amino acids such as histidine and tryptophan reacted even more slowly, requiring between 30 seconds to 2 minutes, depending on pH. The saturated amino

[redacted] almost unattacked even [redacted] found to increase the rate of [redacted] protein ribonuclease and on [redacted] that the ozone interaction with [redacted] and not the methionine group. [redacted] possible that inactivation of the [redacted] bridging unit of the protein coat

Acknowledgements

This report is the result of a team effort by many members of the staff of the Environmental Health Laboratory.

The following list indicates the major participants in the various parts of the work:

Section A: E.Katzenelson, G. Koerner, N. Biedermann, M. Peleg, H.I. Shuval

Section B: E. Katzenelson, G. Koerner
Section C: S. Shaefer, G. Esterson and H.I. Shuval
Section D: M. Peleg

Section E: D. Meidar, M. Peleg, T. Greenwald.

In addition, we would like to thank Prof. T. Helfgott, formerly of University of Connecticutt, who participated in the initial phases of the work described in Section C, and was responsible for the design of the ozone contact reactor.

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A. Measurement of the Inactivation Kinetics of Poliovirus by Ozone in a Fast Flow Mixer

Introduction

In an earlier publication[1] we reported on a 2-stage inactivation curve of poliovirus 1 by ozone (O_3). During the first stage, which lasted less than 10 seconds, approximately 99% of the viruses were inactivated, while the remainder of the viruses were killed during the second stage, which continued for several minutes. However, a dose response relationship between O_3 concentrations and viral inactivation rate could not be demonstrated. This is not in accordance with the assumption that disinfection is of a first order reaction.[2] It should be pointed out that for technical reasons, eight seconds had lapsed before the first sample could be withdrawn, during which time interval the majority of the viruses underwent inactivation. It was hypothesized that during these eight seconds a dose response relationship did indeed exist; however, it could not be demonstrated because of the rapid inactivation rate.

The present study was undertaken to elucidate the kinetics of O_3 action on poliovirus 1 during very short periods of time, e.g., 0.2-1.0 seconds, and to determine a possible dose response relationship. The effect of pH on the viral inactivation rate was also investigated.

Materials and Methods

Virus: Poliovirus 1 (Brunhilde) was grown in BGM cells[3] and concentrated by phase separation.[4] The concentrate was centrifuged

for 30 min. at 12,000 x g to remove cell debris. The supernatant was centrifuged for 2 hr. at 100,000 x g and the pellet resuspended in 0.005 M phosphate buffer, pH 7.2. The partially purified virus concentrate (2×10^9 PFU/ml) was stored in glass vials (0.5 ml each) at $-70^{\circ}C$. The virus stock was diluted 1,000-fold in 0.02 M phosphate buffer before each experiment; the pH of the buffer was adjusted according to the requirements of the experiment. The same virus stock was used throughout.

Ozone: O_3 was generated from oxygen by means of an ozonizer (Fisher Laboratories, Ozone Generator Model 501) and bubbled through a phosphate buffer, 2×10^{-4} M, pH 5.0, for several minutes. The concentrated O_3 solution was diluted to the desired O_3 concentration for each experiment. Determination of O_3 concentration was performed according to the method described by Schechter.[5]

Ozone demand-free water: All buffers were prepared from triple-distilled water. Ozone demand-free water was obtained by adding alkaline potassium permanganate to the water during the third distillation process.

Viral inactivation kinetics experiments: A fast flow mixer[6] was used, with the following modifications. The contents of two syringes, one containing O_3 and the other diluted virus suspension are injected simultaneously into a mixing chamber, from which the mixture passes through a narrow tube (diameter 1.07 mm) into a quenching solution (0.002 M tetra-sodium pyrophosphate and 0.0004 M sodium sulphite). The movement of the plungers is electronically controlled. Contact time of ozone and virus can be varied by changing the injection rate and the length or diameter of the narrow tube.

Before the start of an inactivation kinetics experiment, O_3 and virus solutions were prepared and kept at 4^oC; the same solutions were used throughout a given experiment. Samples were taken at contact times ranging from 0.2 to 1.0 sec. O_3 concentration was determined before and after each experiment. Only those experiments in which O_3 reduction was 5% or less were incorporated into the results. In each experiment, the calculations were performed with reference to a control virus sample not containing ozone which was processed in parallel.

Calibration of the fast flow mixer was performed according to Barman and Gutfreunds[7] using alkaline hydrolysis of p-nitrophenol acetate to p-nitrophenol. The mixer was calibrated for contact times between 0.2 and 1.0 sec., with an accuracy of 0.01 sec.

Results

Inactivation of poliovirus 1 by ozone: O_3 concentrations ranged between 0.06 and 2.5 mg/l. Figure 1 depicts the viral inactivation kinetics with 0.06, 0.19, 0.4 and 1.24 mg O_3/ml, at pH 7.2. Here, too, the characteristic 2-stage inactivation curve is apparent.[7] However, in the current experiments it was possible to measure the duration of the inactivation period of the first stage, and a direct correlation between this period and the O_3 concentration could be shown: the higher the O_3 concentrations, the shorter the duration of the first stage. In fact, at O_3 concentrations of 0.06 and 0.16 mg/l, the 2-stage inactivation curve does not even appear in Figure 1. This is probably due to the very short sampling period (one second or less).

Dose response relationship between poliovirus-1 inactivation rate and O_3 concentration: The results in Figure 1 suggest a probable dose response relationship between poliovirus 1 inactivation rate and O_3 concentration. To verify this relationship, additional inactivation experiments with

various O_3 concentrations at pH 7.2 were carried out. The time required for the inactivation of 95% of the virus was calculated for each experiment, and the end results were plotted on a log-log scale as a function of time versus concentration (Figure 2). The results demonstrated an obvious dose response relationship with a coefficient of correlation r = -0.804.

The effect of pH on poliovirus-1 inactivation: In addition to pH 7.2, the following pH's were tested with respect to poliovirus-1 inactivation: 3.0, 5.0, 9.0, and 10.0. The characteristic 2-stage inactivation curve appeared at all the above pH's. The results of 95% viral inactivation were calculated and plotted as previously described (Figure 3). An obvious dose response relationship is evident for all the pH's tested, as well as slight differences in the rates of viral inactivation for the different pH's. The coefficien of correlation for the curves of the pH's are as follows: pH 3.0: r = -0.9504; pH 5.0: r = 0.9067; pH 9.0: r = -0.7392; pH 10.0: r = 0.9442.

Discussion

The main objective of the present study was to determine whether a dose response relationship exists between O_3 concentrations and the inactivation of poliovirus 1. During the course of a previous study there were indications that such a relationship could be demonstrated, provided that very short reaction times were applied. For this purpose a fast flow mixer, enabling reaction times of 0.2-1.0 sec. with an accuracy of 0.01 sec. was used in the current experiments.

The kinetics curve obtained in the present study is similar to that shown in our previous study.[1] Here, as opposed to the previous study, the first stage could actually be measured. In addition, an O_3 concentration-

viral inactivation rate (dose response) relationship was apparent (Figs. 1 and 2).

From the first part of the slope of the inactivation kinetics curve (Fig. 1), it is observed that the reaction is of a first order with respect to the virus, since the O_3 concentration is in excess and constant throughout the reaction. The first order equation is therefore

$$\ln\left(\frac{V}{V_o} \times 100\right) = kt \qquad (1)$$

where V = virus concentration (pfu/ml) at time t (sec),

V_o = virus concentration (pfu/ml) at time zero,

k = pseudo first-order specific rate constant (sec^{-1}) and depends on the O_3 concentration in any given experiment.

If k is defined in terms of the O_3 concentration, then equation (1) becomes

$$\ln\left(\frac{V}{V_o} \times 100\right) = K\,(O_3)^n t \qquad (2)$$

where K - the overall chemical reaction rate constant $[(ppm)^{-n}sec^{-1}]$

O_3 = ozone concentration (ppm),

n = exponent of the ozone concentration (n = 1 if reaction is of the first order with respect to the ozone, etc.).

For 95% inactivation, equation (2) becomes

$$\ln 5 = K\,(O_3)^n t_{95}$$

or

$$[O_3]^n t_{95} = 1.61/K \qquad (3)$$

which is a variation of the equation known as Watson's law.[8]

Evaluation of n and K is usually accomplished by the logarithmic form of equation (3):

$$n \log(O_3) + \log t_{95} = \log \frac{1.61}{K} \qquad (4)$$

Plots of log (O_3) versus log t_{95} are shown in Fig. 2 and the values for n and K at different pH's as shown in Fig. 3 are compiled in Table 1.

The units of K depend on the value of n and are therefore $(ppm)^{-n}$ sec^{-1}. A value of n equal to unity seems natural since this would make the over-all reaction second order (first order with respect to the virus and first order with respect to ozone). The deviation from unity may be explained by experimental aberration. Deviations of a similar magnitude for aqueous disinfectants have previously been observed. However, for ozone it is possible that more than one species is responsible for the disinfection process (i.e., O_3, OH, etc.)[9] and this may give rise to a complicated kinetics curve which cannot be described simply as in equation (2). Ozone species may vary with pH and other chemical factors in the water.

The pH of the water may bear an effect on the virus by causing viral clumping. It has been demonstrated[10] that viruses have a tendency to clump at pH's below 6.0. At pH 7.0 and above, the virus clumps disaggregate very rapidly. It is obvious that such aggregation of viruses in the water would affect the efficiency of any disinfection agent. It is reasonable that our results are a combination of the effect exerted by the pH on both the O_3 and the virus. However, the differences in the inactivation kinetics at the various pH values are so small as not to be of practical significance.

In conclusion, it can be stated that these experiments carried out in a fast flow mixer, with contact times varying from 2,0 to 1.0 seconds indicate that (1) a clear dose-response relationship between O_3 concentration and virus inactivation rate can be demonstrated; (2) pH variations between 3 to 10 do not, apparently, lead to meaningful differences in virus inactivation rates by ozone; (3) the kinetic reaction is of the first order in respect to the virus; (4) certain anomalies in relation to virus inactivation by ozone may be associated with different ozone species that may develop under varying substrate conditions and for virus clumping and disaggregation under certain conditions.

Table 1: Reaction Rate Constant (K) and O_3 Concentration Exponent (n) at Various pH Values

pH	n	$K(ppm)^{-n}(sec)^{-1}$
3.0	0.6	8.4
5.0	0.7	9.5
7.2	0.68	6.5
9.0	0.73	10.5
10.0	0.88	9.7

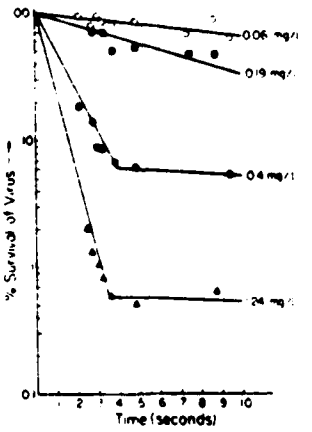


FIG. 1. *Inactivation kinetics of poliovirus type 1 with various concentrations of ozone in a fast-flow mixer.*

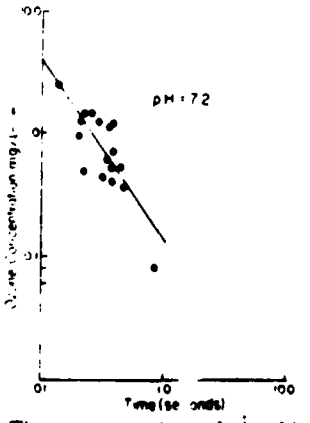


FIG. 2. *Time-concentration relationship for 95% inactivation of poliovirus type 1 by ozone (log-log scale).*

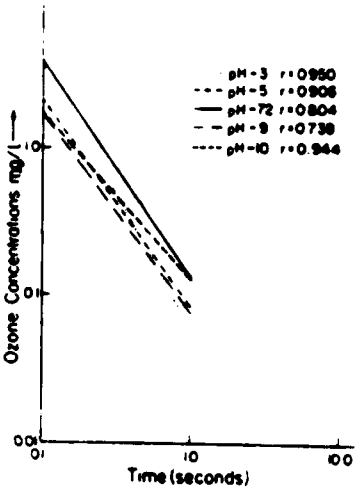


FIG. 3. *Effect of pH on time-concentration relationship for 95% inactivation of poliovirus type 1 by ozone (log-log scale). r, Correlation coefficient.*

B. Studies on the Kinetics of Virus Inactivation in Jerusalem Tap Water and in Wastewater Effluent from the Dan Region Water Reclamation Plant in the Fast Flow Reactor

Introduction

It is well known that O_3 reacts extremely rapidly and causes inactivation of bacteria and viruses[1] in water. In an earlier publication[11] we reported the kinetics of poliovirus inactivation in triple distilled water for various pH conditions, for which a two step inactivation curve was demonstrated.

The present study was undertaken to investigate the kinetics of O_3 action on poliovirus in both Jerusalem tap water and renovated wastewater from the Dan Region Project and to elucidate the effect of both the inorganics and organics which are present in the water on the O_3 inactivation process.

Materials and Methods

Virus: Poliovirus-1 (Brunhilde) was grown in BGM cells[3] and concentrated by a phase separation method.[5] The concentrate was centrifuged for 30 min. at 12,000 x G to remove the cell debris. The supernatant was centrifuged for 2 hours at 100,000 x G and the pellet resuspended in 0.005M phosphate buffer pH 7.2. The partially purified virus concentrate ($2x10^9$ PFU/ml) was stored in glass vials, 0.5 ml each, at -70^oC. The virus stock was diluted 1,000-fold in tap water or in renovated wastewater before each experiemnt. The same virus stock was used throughout.

Ozone: Ozone was generated from oxygen by means of an Ozoniser (Fisher Laboratories, Ozone Generator Model 501) and bubbled through ozone demand-free tap water or renovated wastewater in 2×10^{-4} M pH 5.0

phosphate buffer, for several minutes. The concentrated ozone solution was diluted to the desired ozone concentration in the same water. Determination of O_3 concentration was performed according to the method described by Schechter.[5]

Ozone demand-free Jerusalem tap water: Ozone was bubbled through 4 liters of Jerusalem tap water for several hours. It was left overnight at room temperature to allow all the ozone to evaporate. The water was kept at 4^oC throughout the experiments.

Ozone demand-free water: O_3 demand-free water was obtained by adding alkaline potassium permanganate to the water during the distillation process.

Jerusalem tap water: 20 liters of Jerusalem tap water were used. Several drops of HCl were added to lower the pH to 6.6 and the water was stored in 1 liter bottles, in the dark at 4^oC. Samples of 100 ml were used for each experiment. The quality of the tap water is shown in Table No. 2.

Renovated wastewater: The water for the above experiments was obtained from the Dan Region Reclamation Scheme. The raw municipal sewage received the following treatment steps: biological oxidation followed by chemical treatment by the high-lime magnesium process, and then free ammonia stripping, natural recarbonization, and additional purification carried out in the polishing ponds. The quality of the renovated water is shown in Table No. 3.

The effluent was filtrated successively with Balston filters of type grade c, grade b, and grade a. The pH remained constant at 10.

The water was divided into ½ liter glass bottles and stored at -20°C. The pH after thawing was 10.3.

Fast Flow Mixer: A Fast Flow Mixer[6] was used with the same modification previously described.[11] Contact times ranged between 0.1-1.0 seconds with an accuracy of 0.01 seconds which was obtainable with the above apparatus. In the present experiments the two syringes were filled, first with O_3 which was dissolved either in tap water or in O_3 demand-free tap water and in the renovated water experiments, with triple distilled water which also was O_3 demand free. The second syringe contained the virus, in the proper solution. The two syringes were injected simultaneously into a mixing chamber, from which the mixture passed through a narrow tube (diameter 1.07 mm) into a quenching solution (0.002 M tetra-sodium phrophosphate and 0.0004 M sodium sulphite).

Viral inactivation kinetics experiments:[12] The virus and O_3 solutions were kept at 4°C. The same solution was used throughout a given experiment. Samples were taken at contact times ranging from 0.1 to 1.0 seconds. The O_3 concentration was determined before and after each experiment. Only those experiments in which the O_3 reduction of the feed solution was less than 5% were incorporated into the results. For each experiment, the calculations were performed with reference to a control virus sample not containing ozone which was processed in parallel.

A. Jerusalem tap water experiment:

1. Ozone was dissolved in Jerusalem tap water (O_3 demand-free) and prepared as described above. The virus was also diluted in the tap water to 10^6 PFU/ml. The mixing of these two solutions in a volume to volume ratio in the inactivation experiments gave a final pH of 7.7.

From Table No. 4 it can be observed that the O_3 demand of the Jerusalem tap water is approximately 0.2 mg/l for the various contact

times. There is also no difference in residual O_3 when the virus is present.

Virus inactivation studies were not carried out in tap water with O_3 concentration below 0.18 mg O_3/liter since no ozone residue remained.

Due to the extremely high rate of inactivation, the highest O_3 concentration used was 0.68 mg O_3/liter. When using higher O_3 concentrations all the virus was inactivated even before the earliest sampling time (0.135 seconds).

2. Dose response relationship between poliovirus-1 inactivation in Jerusalem tap water and O_3 concentration: From the results shown in Figure 4, it can be assumed that a dose response relationship does exist between poliovirus-1 inactivation rate and the residual O_3 concentration. To verify this relationship additional inactivation experiments with various residual O_3 concentrations were carried out. The time required for the inactivation of 95% of the virus was calculated for each experiment and the results plotted on a log-log scale as a function of time versus concentration.

As is obvious from Figure No. 5 (full line), a dose response relationship exists between the residual O_3 concentration and the virus rate inactivation with a co-efficient of correlation r = -0.9251.

In Figure No. 5, the dotted line represents results of similar experiments performed previously,[11] which used triple distilled ozone demand-free water at pH conditions of 10.0.

B. Renovated wastewater experiments:

Because renovated wastewater has a high O_3 demand, the O_3 solution for the inactivation experiments was prepared in O_3 demand-free water, while the virus solution was prepared in the renovated wastewater.

Mixing of the two solutions in the fast flow apparatus produced a solution with a final pH of 10.1. The virus titer remained constant throughout the experiments.

From Table No. 5 and Figure 6, it can be observed that for a 2.2 mg O_3/l solution no significant O_3 residue remains even for the shortest sampling time. In the inactivation experiments, O_3 concentrations ranging from 0.15 to 1.2 mg/liter were used. More than thirty inactivation experiments were performed of which three typical results are plotted in Figure No. 7.

Clearly there is always a two-phase inactivation curve. However, the dose-response relationship between the ozone dose and the inactivation rate in the first phase is less pronounced than that shown for tap water in Figure 4.

It must be noted that in these experiments, no residual O_3 was detected (see Table 5) and the O_3 doses in Figure 7 are for the initial feed solution and not the residual mixture, as in the tap water experiments.

Discussion

In comparing the results of the inactivation of viruses by O_3 in tap water and triple distilled water we see that the inactivation process is quicker in the tap water. From Figure No. 5 it is observed that in the O_3 dose range, 0.1 to 0.7 mg/l, the virus inactivation in tap water is 2.5 to 4.0 times quicker than in triple distilled water. This result was somewhat unexpected.

However, Hoigne[12] has shown that carbonate and bicarbonate ions present in water scavenger the hydroxyl radical (OH^-) formed during dissociation of O_3 in water. Further, Peleg[9] as well as Hoigne[12] have shown that OH^- radicals react much quicker with organics in water than the O_3 molecule.

Preliminary results of Peleg and Koerner[13] have shown that in O_3 demand-free water containing carbonate and bicarbonate mixture (0.05 M), the virus inactivation rate by O_3 is similar to those obtained in pure distilled water. The above findings indicate that inactivation of the virus occurs not by OH^- radical as for organics[12] but probably by the O_3 molecule. A possible reason for the increased inactivation in tap water has been demonstrated in previous experiments[14] in which certain metal ions had a catalytic effect on the ozonization rate.

It is difficult to compare the inactivation rate of the virus in renovated wastewater with that of tap water and distilled water because in the former the O_3 concentration noted in Table No. 5 represents the O_3 introduced and not the O_3 in the mixture. However, it appears that the results for the renovated wastewater more closely resemble those obtained for distilled water than for tap water. It is interesting to note that inactivation occurs even when no measurable residual O_3 is present in the inactivation solution. This may be explained by the limited sensitivity of the analytical technique for O_3 (lower limit 0.1 mg/l).

The concentration of ozone in the wastewater may be below the level detectable by the technique. This may give rise to an inactivation effect as observed in Figure No. 7.

In order to provide a basis for comparison of the findings of this study and similar studies using chlorine as a disinfectant, Fig. No. 8 is presented. We have super-imposed our log-log time-concentration data onto the curves reported by Scarpino *et al.*[15] for virus and *E. coli* inactivation by HOCl. This required some adjustment of our data so that parallel lines could be presented. Without discussing the

validity of such an adjustment we feel that Fig. 8 provides at least a qualitative illustration of the extremely rapid rate of virus inactivation provided by ozone even in effluent as compared to HOCl.

It should be noted that the curve for effluent is based on ozone dose applied, not residual, since no residual ozone could be detected at the end of the contact periods of 1 second and under. Nevertheless, the position of the curve is considerably to the left of the HOCl and water curve reported by Scarpino[15] indicating more rapid inactivation. This provides clear support for the high level of efficacy of ozone as a virucidal agent in effluent even though no ozone residual can be detected. The lack of a detectable residual makes the control of such a disinfection process difficult, however. This is one of the main problems that must be overcome in the rational application of ozone as a disinfectant step in treatment of effluent or surface waters heavily contaminated with organics.

Table 2: Quality of Jerusalem Tap Water

Parameter	Units
Turbidity	J.U. 1.2
Conductivity	Mhoms/cw 10^{-6} 780
TDS	mg/l 575
Hardness as $CaCO_3$	mg/l 320
Alkalinity as $CaCO_3$	mg/l 265
Bicarbonates	mg/l 320
Chlorides as Cl^-	mg/l 130
Nitrates as N	mg/l 5.1
Sulphates as $SO_4^=$	mg/l 34.6
Calcium	mg/l 74.0
Magnesium	mg/l 37.5
Sodium	mg/l 110
Potassium	mg/l 4.2
T.O.C.	mg/l 0.7

Table 3: Tertiary Effluent Quality

(Based on Pilot Plant Results from Period of Dec. 1975-April 1976)

Parameter	Units	Concentrations
pH	--	10
Suspended solids, 105°C	mg/l	13
BOD	mg/l	3
COD	mg/l	63
Alkalinity, total, as $CaCO_3$	mg/l	122
Hardness, as $CaCO_3$	mg/l	173
Calcium, as Ca	mg/l	60
Magnesium, as Mg	mg/l	1.5
Phosphorus, as P	mg/l	1.0
Kjeldhal Nitrogen, total, as N	mg/l	13
Ammonia, as N	mg/l	10
Dissolved solids, total, 105°C	mg/l	660
Electrical conductivity	micromhos/cm	700
Chloride, as Cl	mg/l	253
Cyanide, as CN	mg/l	0.0025
Fluoride, as F	mg/l	0.15
Sulfate, as SO_4	mg/l	36.6
Aluminum, as AL	microgram/l	40
Arsenic, as As	microgram/l	<30
Boron, as B*	mg/l	0.29
Chromium, as Cr	microgram/l	5.5
Copper, as Cu	microgram/l	8
Iron, as Fe	microgram/l	50
Lead, as Pb	microgram/l	<20
Lithium, as Li	microgram/l	5.5
Manganese, as Mn	microgram/l	4
Nickel, as Ni	microgram/l	40
Potassium, as K	mg/l	19.8
Zince, as Zn	microgram/l	37

Table 4: Ozone Demand of Jerusalem Tap Water

Reaction time (sec)	Solutions	mg/l O_3 demand	Reaction time (sec)	Solutions	mg/l O_3 demand	Reaction time (sec)	Solutions	mg/l O_3 demand	Reaction time (sec)	Solutions	mg/l O_3 demand
	Tap water	0.15		Tap water	0.12		Tap water	0.18		Tap water	0.17
0.135			0.15			0.16			0.18		
	Tap water + Virus	0.15		Tap water + Virus	0.15		Tap water + Virus	0.17		Tap water + Virus	0.16
	Tap water	0.21		Tap water	0.11		Tap water	0.13		Tap water	0.17
0.24			0.27			0.3			0.32		
	Tap water + Virus	0.19		Tap water + Virus	0.15		Tap water + Virus	0.14		Tap water + Virus	0.17
	Tap water	0.19		Tap water	0.16		Tap water	0.21		Tap water	0.19
0.36			0.48			0.73			0.86		
	Tap water + Virus	0.20		Tap water + Virus	0.19		Tap water + Virus	0.2		Tap water + Virus	0.21
				DDW + Virus O_3 free demand	0						
	Tap water	0.18									
0.94			0.94								
	Tap water + Virus	0.20		DDW O_3 free demand	0						

Table 5: Ozone Demand of Effluent: 2.2 mg/liter O_3 was introduced to virus solution in renovated wastewater for various times

O_3 residual mg/l	Contact time in seconds
0.0	0.86
0.0	0.73
0.03	0.48
0.05	0.36
0.02	0.3
0.05	0.24
0.02	0.18
0.1	0.15
0.15	0.135

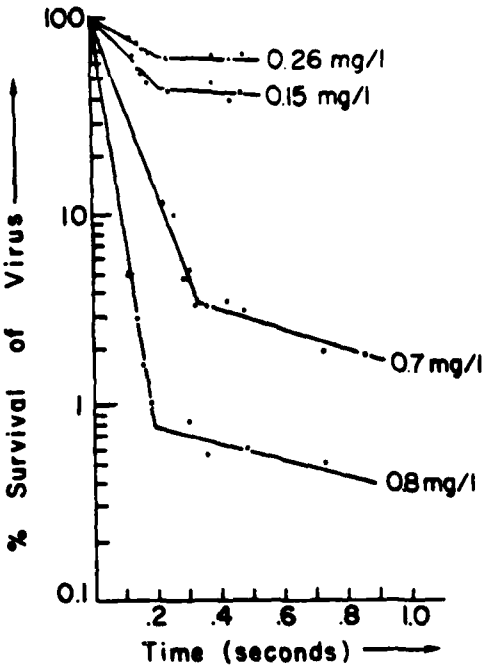


Fig. 4. Inactivation kinetics of Poliovirus in tap-water with various concentrations of ozone residual.

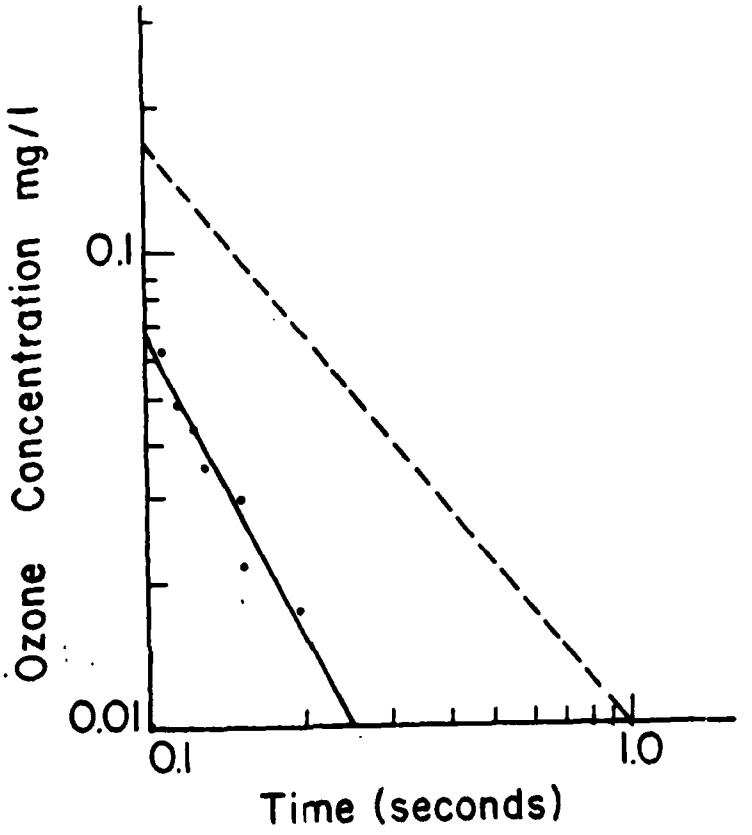


Fig. 5. Time - Concentration relationship for 95% kill of Poliovirus in tap-water (full line) and in ozone demand free distilled water (broken line).

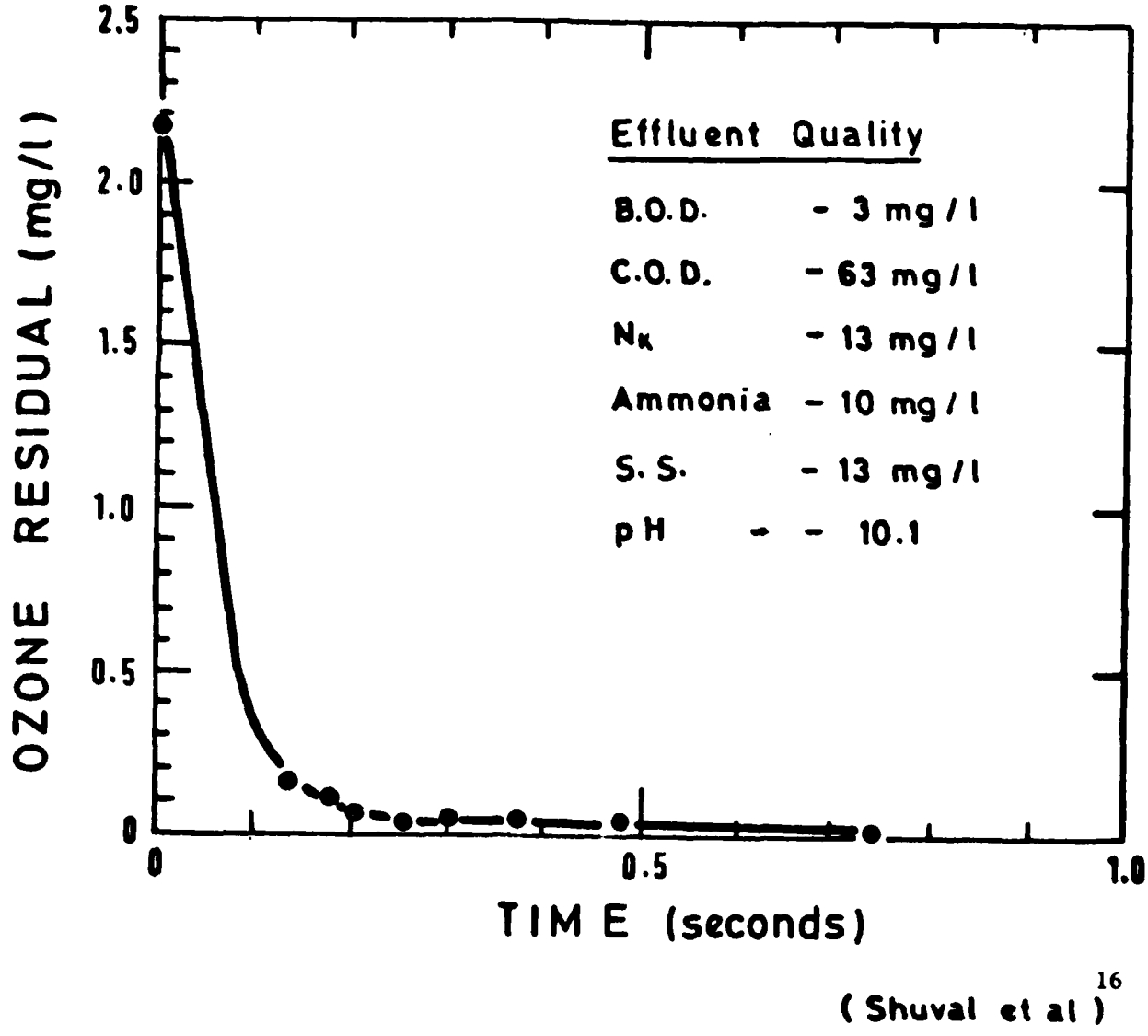


Fig. 6: Rapid loss of ozone residual with time in Dan project tertiary effluent with a single ozone dose of 2.2 mg/l.

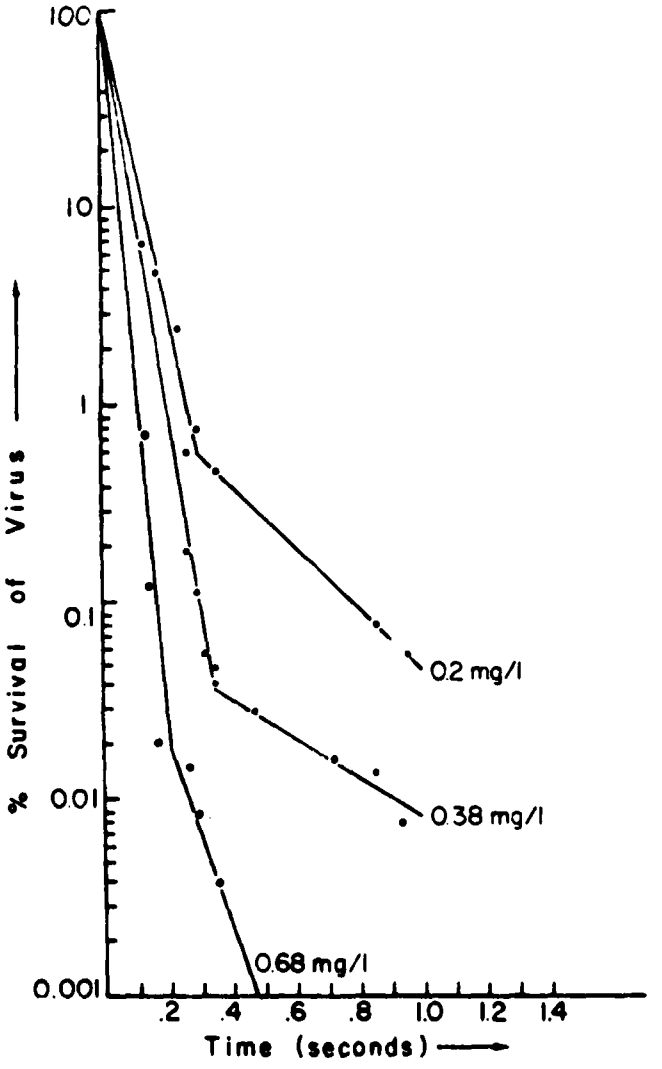


Fig. 7. Inactivation kinetics of Poliovirus in Dan Region tertiary wastewater effluent with various applied concentrations of ozone.

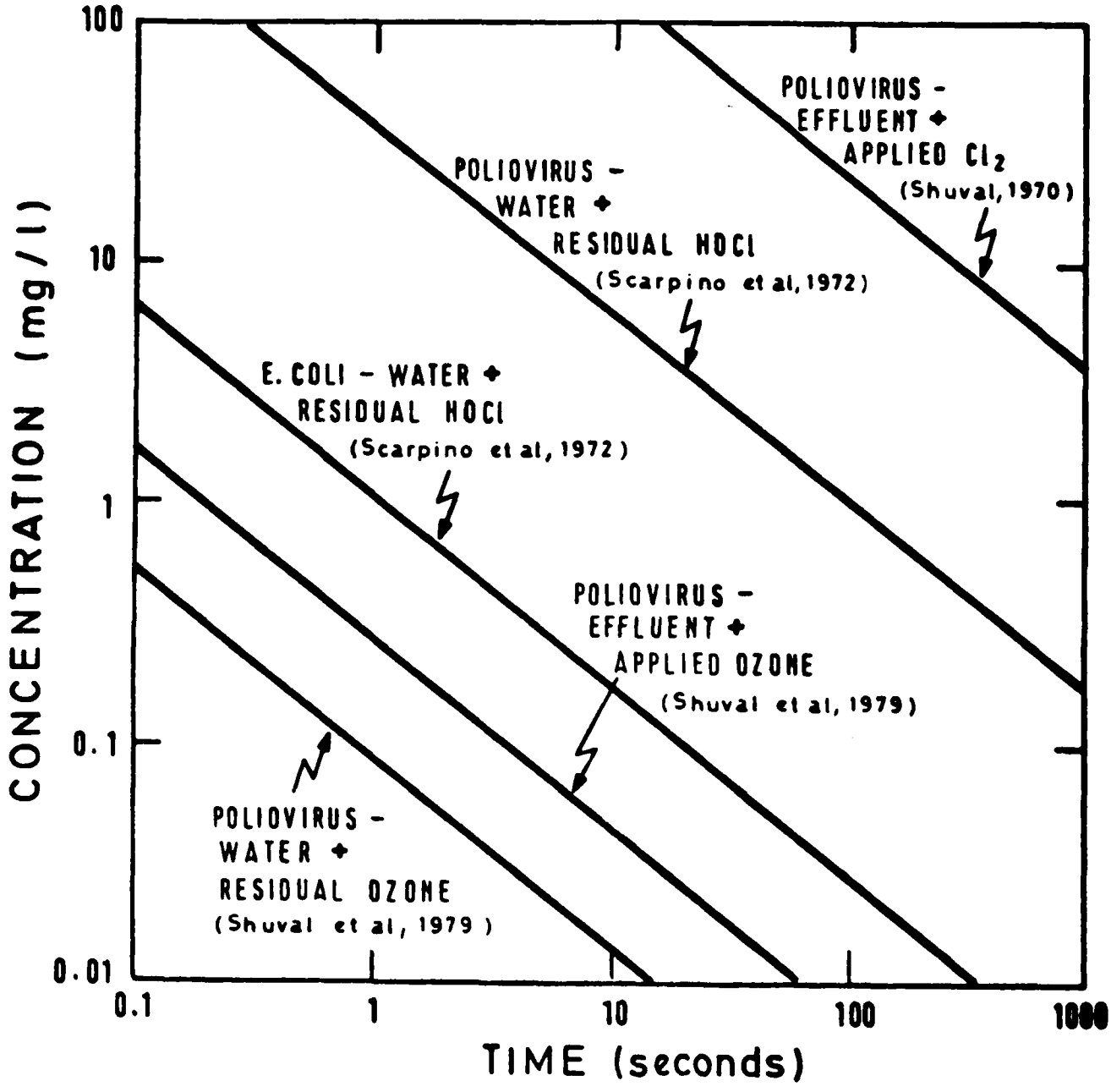


Fig. 8: Concentration-time relationship for 99% inactivation of poliovirus I and E. Coli with ozone and chlorine in demand-free water and effluent. (Data adjusted for purposes of comparison).

C. Mass Transfer and Reaction Kinetics in the Ozone/Tap Water System

Introduction

The increasing demand for clean, high quality water has accelerated the use of ozone in water and wastewater treatment. The high oxidation potential of ozone has made it a very attractive alternative to chlorination. Ozone has an excellent capacity for inactivation of viruses and bacteria in water. It is also capable of reduction of dissolved organic substances, destruction of cyanides and pesticides and precipitation of iron and manganese.

The design of a contacting system for ozone and the solution to be treated play important roles in achieving efficient utilization of ozone. However, an examination of the literature makes quite clear that not enough data are available concerning the mass-transfer coefficient of ozone in water in different contact systems. There is also insufficient differentation between the parameters for mass-transfer, contact time and reaction rate.

Through knowledge of the above parameters, and using appropriate mathematical models, design of the system may be carried out. Detailed evaluation of gas liquid contacting devices is necessary so that ozone utilization can be optimized and operating cost estimated.

This paper reports on research to measure the mass transfer coefficient and kinetic parameters of a well-mixed, perpendicular-jet contacting system with continuous tap water treatment using ozone. The low-ozone-residual range which is important in virus deactivation was investigated over a range of operating parameters. A simple mathematical model was proposed and validated experimentally.

Nomenclature

$\bar{a}$	Intercept from the linear regression of g_{in} vs. C_L
$\bar{b}$	Slope from the linear regression of g_{in} vs. C_L
C_L	Ozone concentration in the liquid phase ML^{-3}
C*	Ozone concentration in equilibrium with the bulk concentration in the gas phase ML^{-3}
G	Gas flow rate L^3T^{-1}
H	Henry's constant
K	Reaction rate constant $MT^{-1}L^{-3}$
K_Ga	Overall coefficient in the gas phase T^{-1}
K_La	Overall coefficient in the liquid phase T^{-1}
N_A	Rate transfer of ozone $MT^{-1}L^{-3}$
n	Order of the reaction
P_A	Partial pressure of ozone in the gas phase $ML^{-1}T^{-2}$
P_A*	Partial pressure of ozone in equilibrium with bulk liquid Concentration in the liquid phase $ML^{-1}T^{-2}$
V	Reactor volume L^3

Theory

The two-resistance theory[17] applies to the process of mass-transfer of ozone from the gas to the water. Using an overall mass transfer coefficient, based on an overall driving force, the ozone mass transfer rate is

$$N_A = K_G a \ (P_{AG} - P_A^*)$$

or in terms of the liquid phase concentration driving force,

$$N_A = K_L a \ (C^* - C_2) \qquad (1)$$

For dilute ozone concentration we can assume the validity of Henry's law:

$$Y_A = HX_A^* \text{ or } P_{AG} = HC_A^* \qquad (2)$$

The reactor is considered to behave as an ideal stirred reactor.

An ozone mass balance may be made on both the liquid and the gaseous phases of stead state conditions. Average water and air densities at 20°C may be used. Changes of the water and air densities with the temperatures are neglected.

Ozone is known to be a very strong oxidant and is subject to self-decomposition.[18, 19] This autodecomposition reaction may be taken into account by assuming simple kinetics of n^{th} order for the ozone. The final form of the mathematical model then becomes:

$$K_L aV \ ((g_{in} - QC_L - KVC_L^n) \ \frac{1231}{HG} - C_L) - KVC_L^n = QC_L \qquad (3)$$

The unknowns in this model are $K_L a$, K and n. Accordingly, we have a non-linear parameter estimation problem. The above parameters were estimated over a range of ozone rates, water and air flow rates, temperature and mixing conditions, in a continuous stirred-tank reactor.

Experimental Method

The experimental system is shown in Fig. 9. Ozone was produced by a Fischer ozonator which was supplied with clean air from a gas cylinder. The ozonator voltage set by a variac was used to control the amount of ozone in the gas stream.

Fig. 10 shows the glass reactor. Its volume is 2.775 liters. An agitator, baffles and port samples were located as indicated in the figure. The ozone gas and the water were injected at the bottom of the reactor through nozzles perpendicular to each other. In this manner the impingement of the two feed streams created small bubbles and high turbulence at the bottom of the reactor.

The mixer was operated in the high region of agitation (700 rpm).

The material used for construction of the different parts was resistant to ozone: the reactor was built of glass with teflon stopcocks; the gas pipe was a 0.5 cm ID tygon tube; the mixer was made of stainless steel and teflon with a teflon seal.

For a given parameter set, the ozone rate was varied and the ozone residual measured. A complete calibration of the produced ozone vs. the applied voltage was done before each run. Several series of runs were performed changing on operating parameter in each run. The parameters checked were: three different water flow rates - 2.170, 2.625, 3.080 liter/min; three different gas flow rates - 0.667, 1.000, 1.333 liter/min and different temperature and mixing conditions.

The amount of ozone produced by the ozonator was determined by absorption of the ozone in a wash bottle containing a neutral solution of 2% potassium iodide, and performing standard titration with sodium thiosulfate.

The determination of the ozone residual in the water was done by utilizing the spectrophotometric method developed by Schecter.[5]

Since it was desired to investigate the low ozone residual range for which it is known that virus inactivation occurs,[20, 21] low ozone generation rates were necessary and were obtained by applying low voltage to the ozonator.

Results and Discussion

The relationship between g_{in} and C_L: The results obtained from the measurements taken during the runs with changes in the water rate Q and the air rate G, are shown as plots of ozone rate g_{in} vs. ozone residual C_L in Figs. 11, 12 and 13.

Comparison of the graphs of the ozone residual as a function of the ozone feed applied, reveals a linear relationship between g_{in} and C_L. A linear regression program shows a correlation coefficient higher than 0.996 for all the runs, using 7 pairs for each run. The linear relation may be written:

$$g_{in} = \bar{a} + \bar{b}\, C_L \tag{4}$$

Writing model equation (3) in this form:

$$g_{in} = \left(\frac{HKG}{1231K_La} + KV\right) C_L^n + \left(Q + \frac{HG}{1231} + \frac{QHG}{1231VK_La}\right) C_L \tag{5}$$

Comparing this equation to the linear equation obtained from the experimental results suggests that the only simple kinetic order which can describe the results and the linearity is zero order (n=0). Thus, by setting n=0, the above equation describes the relationship between g_{in} and C_L and specifies the parameters which are included in the linear expression $g_{in} = \bar{a} + \bar{b}\, C_L$.

Thus,

$$\bar{a} = \left(\frac{HG}{1231 K_L a} + V\right) K$$

$$\bar{b} = \left(Q + \frac{HG}{1231} + \frac{QHG}{1231\ K_L a\ V}\right)$$

There is a very good agreement between the obtained graphs of g_{in} vs. C_L and the equation of the proposed model with zero order kinetics.

The mass transfer coefficient K_L a:: The obtained values of $K_L a$ vary between 0.80 and 1.00 min^{-1} for the lower water flow rate and between 1.15 and 1.50 min^{-1} for the higher water flow rate. The calculated results of $K_L a$, using different values for the controlled parameters are summarized in Table 6.

The value of $K_L a$ was calculated by two methods:

(1) Using the equation of the mathematical model in difference form:

$$K_L a = \frac{HGQ(C_i - C_j)/V}{1231\ (g_i - g_j) - (HG + 1231Q)(C_i - C_j)}$$

(2) Using the results of the linear regression calculation of g_{in} vs. C_L and evaluating $K_L a$ from the slope of the function.

$$K_L a = \frac{HGQ/1231\ V}{\bar{b} - \left(Q + \frac{HG}{1231}\right)}$$

$\bar{b}$ = slope obtained from the linear regression.

The results of the calculation by these two methods, as outlined in Table 6 are quite similar.

The resulting values of $K_L a$ show that it reaches higher values with higher water flow rates (3.080 liter/min), which implies creation of smaller bubbles with a larger interfacial area.

The average value of $K_L a$ is 1.14 min^{-1} which is very similar to the value 1.08 min^{-1} obtained by Prengle et al[18] using a stirred reactor with diffusers for the gas feed. The value of $K_L a$ in the stirred reactor is about 300% larger than the mass transfer coefficient found by Hill et al[22]-- 0.350 min^{-1} in a gas sparged reactor without mechanical mixing.

A noteworthy phenomenon is the 400% difference in $K_L a$ between "with" and "without" turbine action ($K_L a$ of "without" turbine action was calculated by the same equation as for "with" agitation since preliminary tests showed that the system behaves more like a stirred reactor than a plug flow reactor). The agitation serves mainly to reduce the liquid film resistance and it is a very important parameter in this type of mass-transfer.

The rate of decomposition and the values of K: The linearity of g_{in} vs. C_L discussed previously suggests that the zero order kinetics best describe the mechanism of the ozone reaction in the reactor. In such a mechanism ozone is decomposed at a fixed rate determined by the value of K.

K is calculated by two methods similar to those used to calculate $K_L a$:

(1) Using the calculated value of $K_L a$ in the model equation:

$$K = \frac{K_L a \left(\frac{1231 g_i}{HG} - C_{Li}\right)}{1 + K_L a V \frac{1231}{HG}} - QC_{Li}$$

(2) Using the intercept obtained from the linear regression calculation and the obtained value of $K_L a$.

$$K = \frac{\bar{a}}{\frac{HG}{1231\ K_L a} + V}$$

$\bar{a}$ = the intercept from the linear regression of g_{in} vs. C_L.

Both methods give similar results, as outlined in Table 7.

The mechanism of the zero-order decomposition of the ozone in the reactor is not known, but the phenomenon has been previously observed. A similar phenomenon in diffusers is indicated by Perrich et al.[23] Several parameters--UV, catalytic effects and rate of agitation are reproted[18, 24] to have an effect on the rate of decomposition.

The results for K show that it reaches its highest values at the highest water flow rate that was tested. The influence on the water flow rate, UV and catalytic effects may imply that the decomposition is an interphase phenomenon. In this reactor, decomposition is enhanced by the turbulence created by the water feed stream.

The magnitude of the calculated K is between 0.05 and 0.13 mg/min-liter, which indicates a self-decomposition rate of ozone between 0.160 and 0.360 mgO_3/min. This decomposition rate is up to 85% of the applied ozone in the gas feed. These high amounts of decomposition clearly explain the phenomenon of an undetectable ozone residual in the water when feeding ozone at a rate of 0.420 mgO_3/min or less in the inlet gas.

Other kinetic orders of decomposition reported in the literature[22,25] suggest a possible parallel reaction model:

$$\frac{dC_L}{d+} = K + k'C_L^{\ n}$$

where the effect of $k'C_L^n$ is significant only at higher ozone residuals than those measured in the runs performed.

Parameters affecting ozone transfer: The parameters of the water flow rate (Q), gas flow rate (G) and temperature (which appears in Henry's constant) are included in the mathematical model so that their effect on the transfer of ozone and on the residual concentration of the ozone in the water can be predicted:

$$g_{in} = \left(\frac{HG}{1231\ K_L a + V}\right) K + \left(Q + \frac{HG}{1231} + \frac{QHG}{1231 K_L av}\right) C_L \qquad (5)$$

As can be seen from equation (5), using higher water flow rates or higher gas flow rates causes a lower ozone residual. This effect was seen in the experimental results (Figs. 11, 12, 13).

The relationship between Henry's constant and the temperature[26] explains the strong dependence of ozone transfer on the temperature. As illustrated in Fig. 14, a decrease of $5^{o}C$ causes a large increase in the ozone residual of 20% to 100%.

The changes in the residual, due to changes in the above parameters are predictable if their influence on $K_L a$ is known.

Accordingly, the proposed model is adequately confirmed by the experimental results.

Summary

The mass transfer coefficient of ozone in tap water is about 1.14 min^{-1} and reaches a higher value at a higher water flow rate. Not using agitation causes a large decrease in $K_L a$ - to 0.250 min^{-1}. Zero order

kinetics best describes the autodecomposition of ozone in plain tap water. There is a linear relationship between the ozone feed rate and ozone residual. Water flow rate (Q), gas flow rate (G) and temperature determine the slope and the intercept of the linear function. The proposed model describes quite accurately the mass transfer behavior in the reactor and determines the operational parameters which control the reactor operation. Accordingly, the design of an ozone contacting system may be based on this model and the specific requirements of the treatment.

Table 6: Calculated values of $K_L a$ suing different values for the controlled parameters

	Q=2.170 $\frac{\text{lit } H_2O}{\text{min}}$		Q=2.625 $\frac{\text{lit } H_2O}{\text{min}}$		Q=3.080 $\frac{\text{lit } H_2O}{\text{min}}$		
	Method 1	Method 2	Method 1	Method 2	Method 1	Method 2	
H = 3188	0.780*	0.900	0.870	0.960	0.770	0.800	
H = 4125			0.860	0.900	1.370	1.400	G = 0.667 $\frac{\text{lit air}}{\text{min}}$
H = 3584	1.000	0.960	0.920	0.950	1.150	1.160	
H = 2270			1.290	1.300			
H - 3672	without turbine	0.330	0.210				
H = 3187	0.800	0.850	1.340	1.730	1.130	1.160	
H = 4125	1.040	1.070	1.560	1.620	1.360	1.560	G = 1.000 $\frac{\text{lit air}}{\text{min}}$
H = 2210	0.890	0.880					
H - 3584	0.880	0.870	0.890	0.890	1.540	1.540	
H = 3056			0.990	1.080			
H = 4328			1.040	1.050	1.480	1.490	G = 1.333 $\frac{\text{lit air}}{\text{min}}$
H = 3672			1.530	1.560			

* The units of $K_L a = \text{min}^{-1}$

Table 7: Calculated values of K using different values for the controlled parameters

	Q=2.70 $\frac{\text{lit } H_2O}{\text{min}}$		Q=2.625 $\frac{\text{lit } H_2O}{\text{min}}$		Q=3.080 $\frac{\text{lit } H_2O}{\text{min}}$		
	Method 1	Method 2	Method 1	Method 2	Method 1	Method 2	
H = 3188	0.080*	0.100	0.090	0.100	0.090	0.090	
H = 4125			0.090	0.090	0.140	0.130	
H - 3584	0.100	0.110	0.080	0.080	0.130	0.130	G = 0.667 $\frac{\text{lit air}}{\text{min}}$
H = 2270			0.080	0.080			
H = 3672	without turbine		0.060	0.060			
H = 3188	0.060	0.070	0.080	0.100	0.120	0.120	
H - 4125	0.110	0.120	0.110	0.110	0.130	0.140	G = 1.000 $\frac{\text{lit air}}{\text{min}}$
H = 3320	0.070	0.070					
H - 3584	0.100	0.100	0.090	0.080	0.140	0.140	
H - 3056			0.080	0.090			
H - 4328			0.060	0.050	0.110	0.120	G = 1.333 $\frac{\text{lit air}}{\text{min}}$
H = 3672			0.090	0.090			

*The units of K = mg min^{-1} lit^{-1}

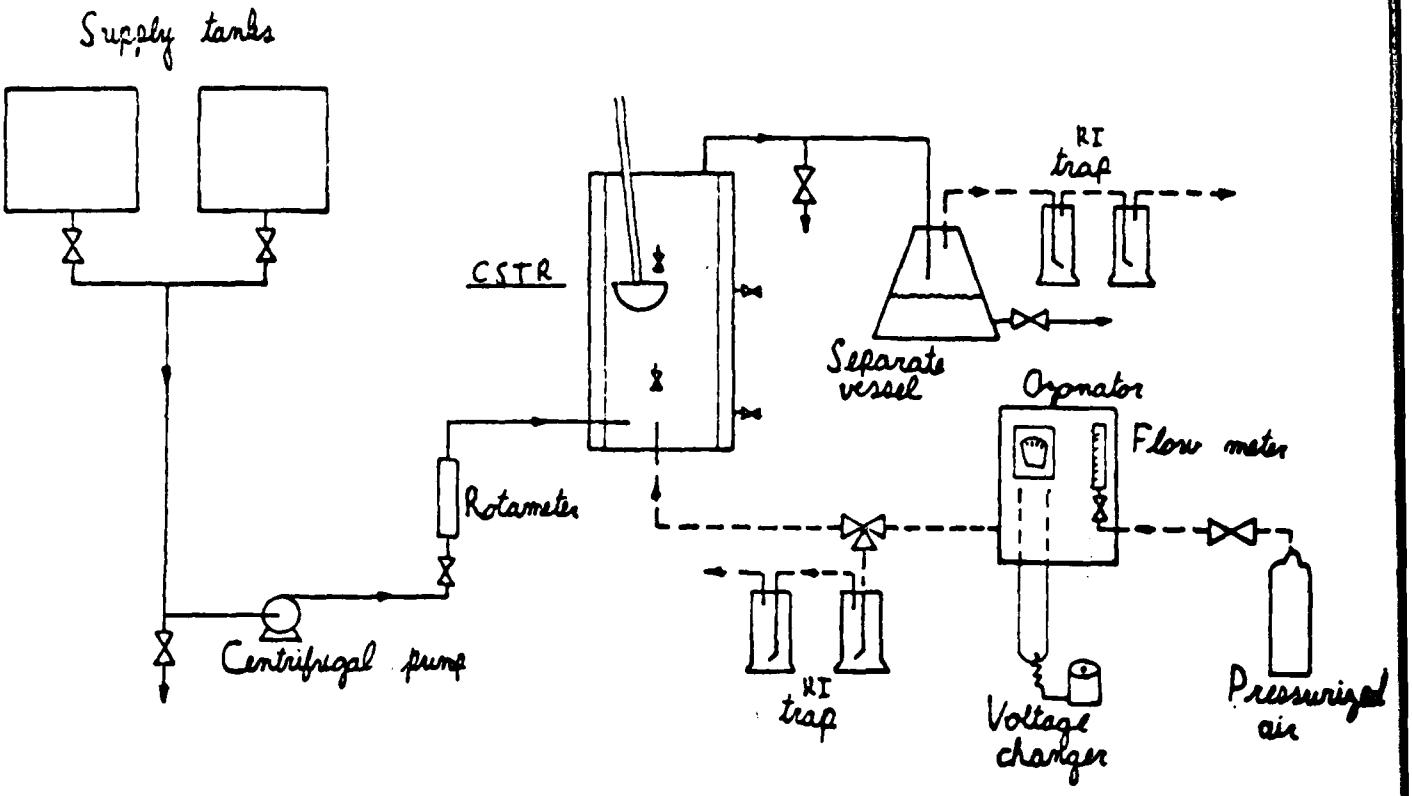


Fig. 9. The experimental ozonation system

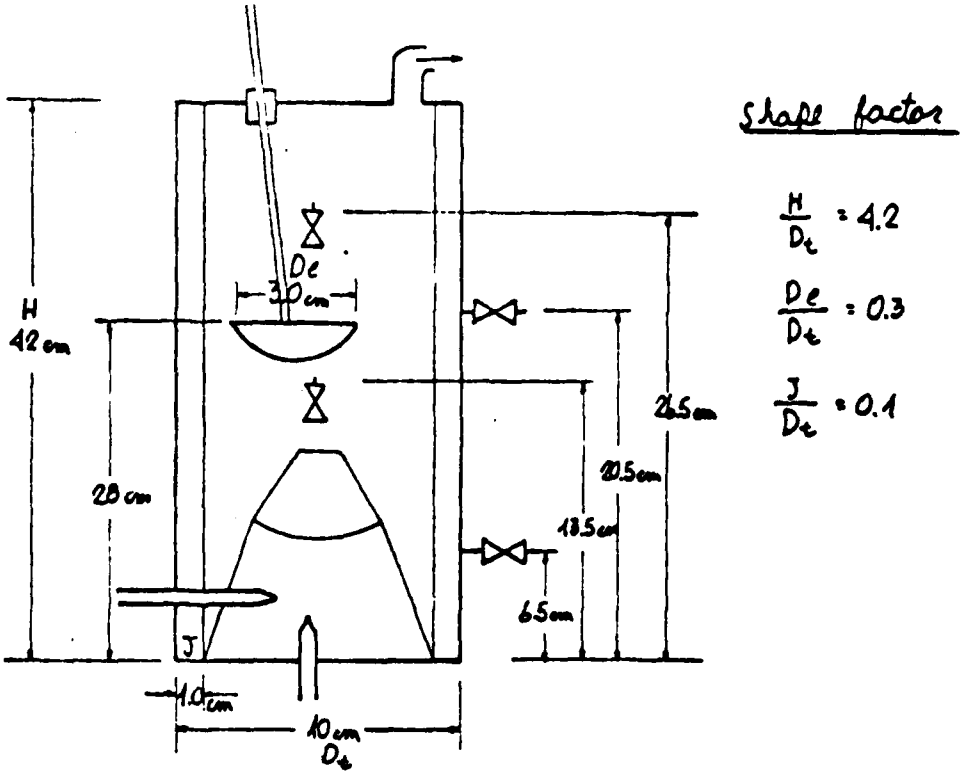


Fig. 10: The glass reactor

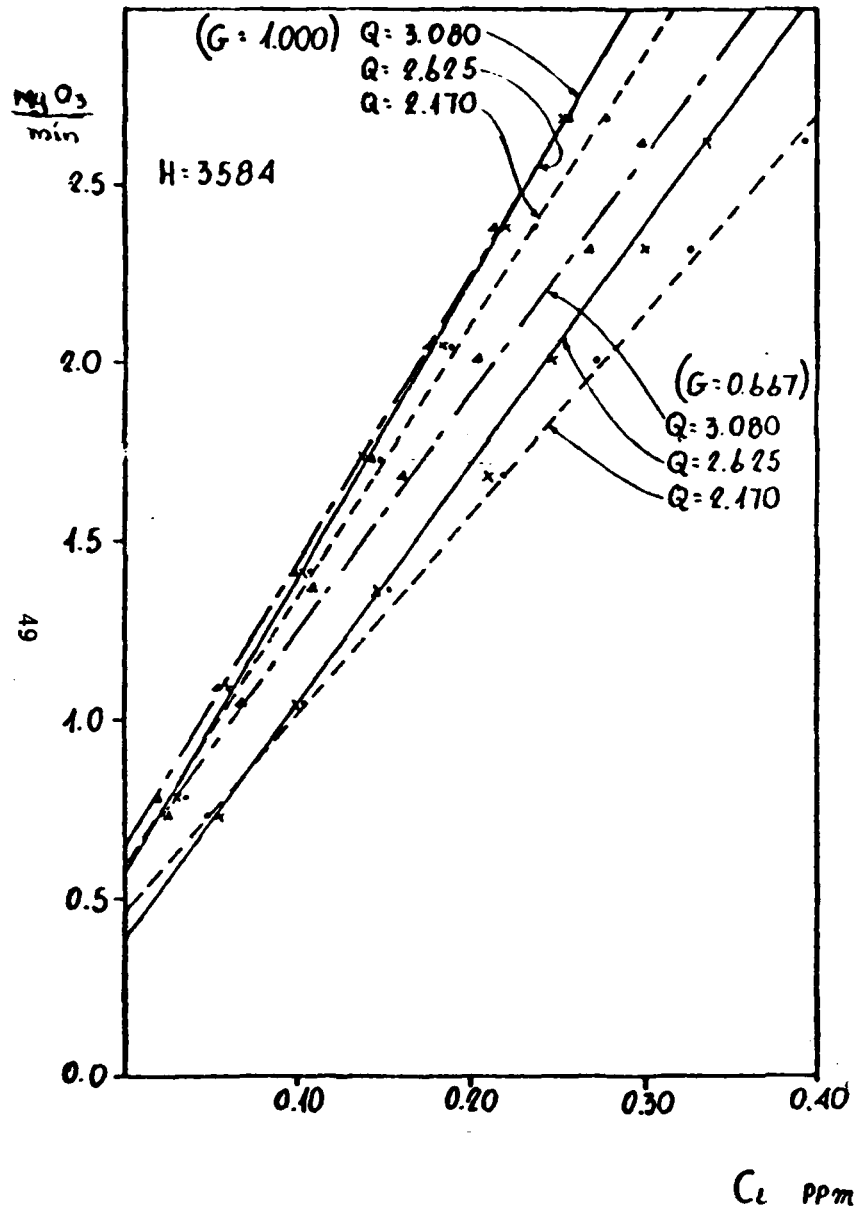


Fig. 11. Relationship between ozone feed and ozone residual using different rates of gas and water flow.

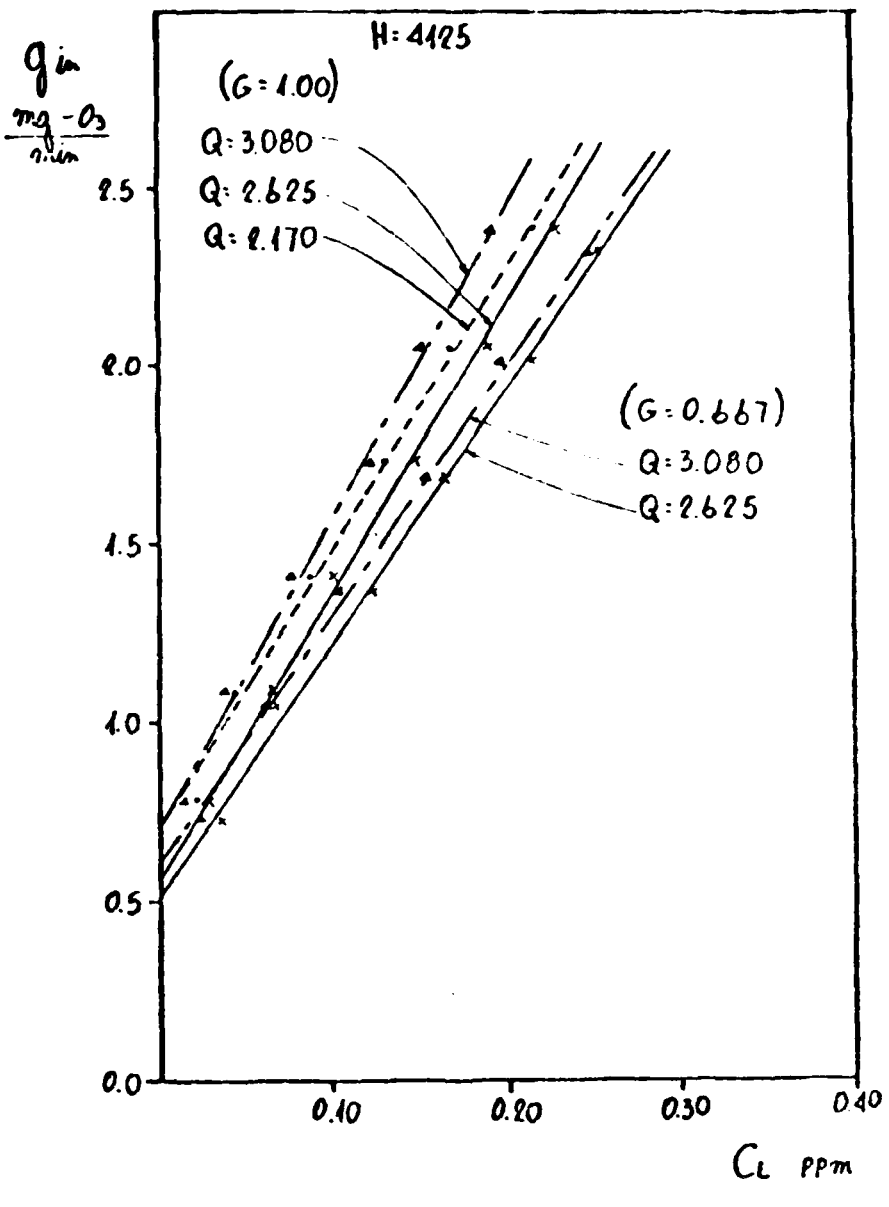


Fig. 12. Relationship between ozone feed and ozone residual using different rates of gas and water flow.

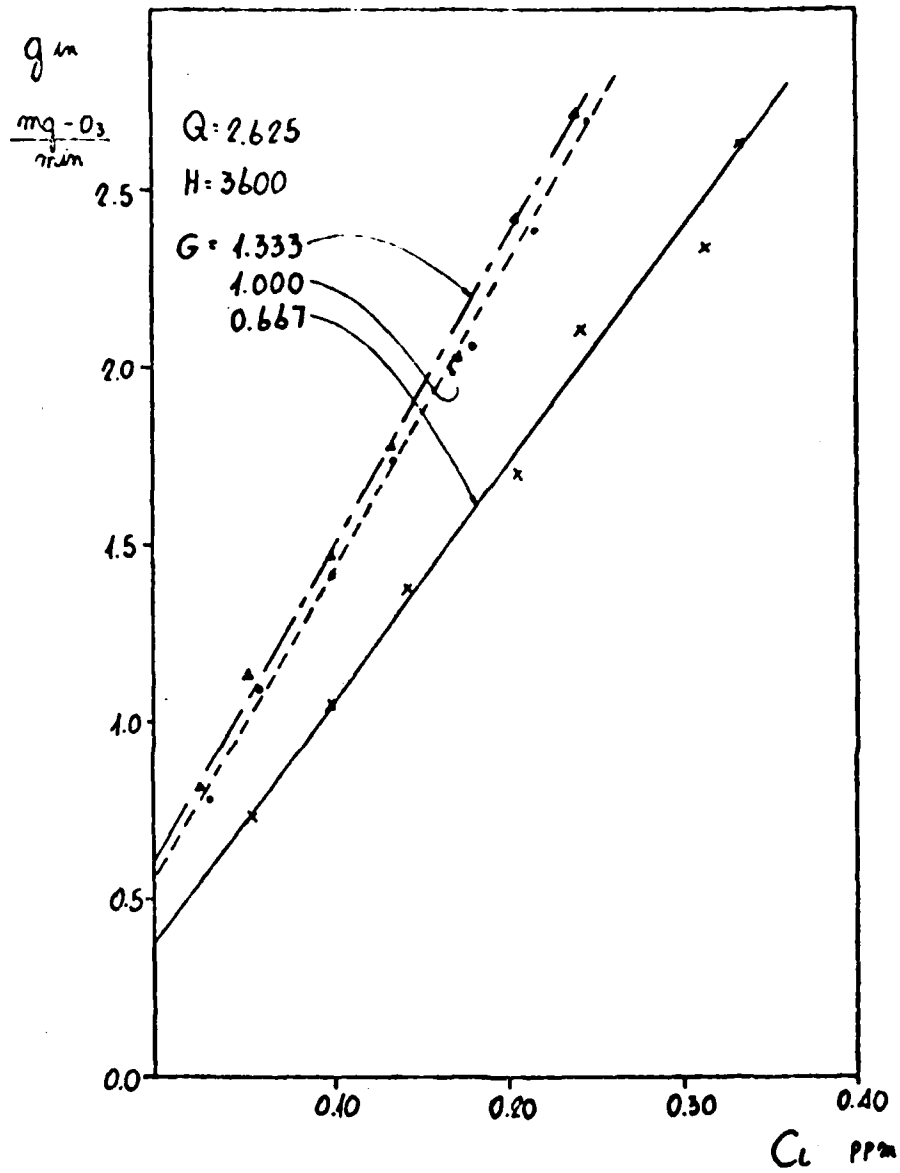


Fig 13. Relationship between ozone feed and ozone residual for three different gas flow rates.

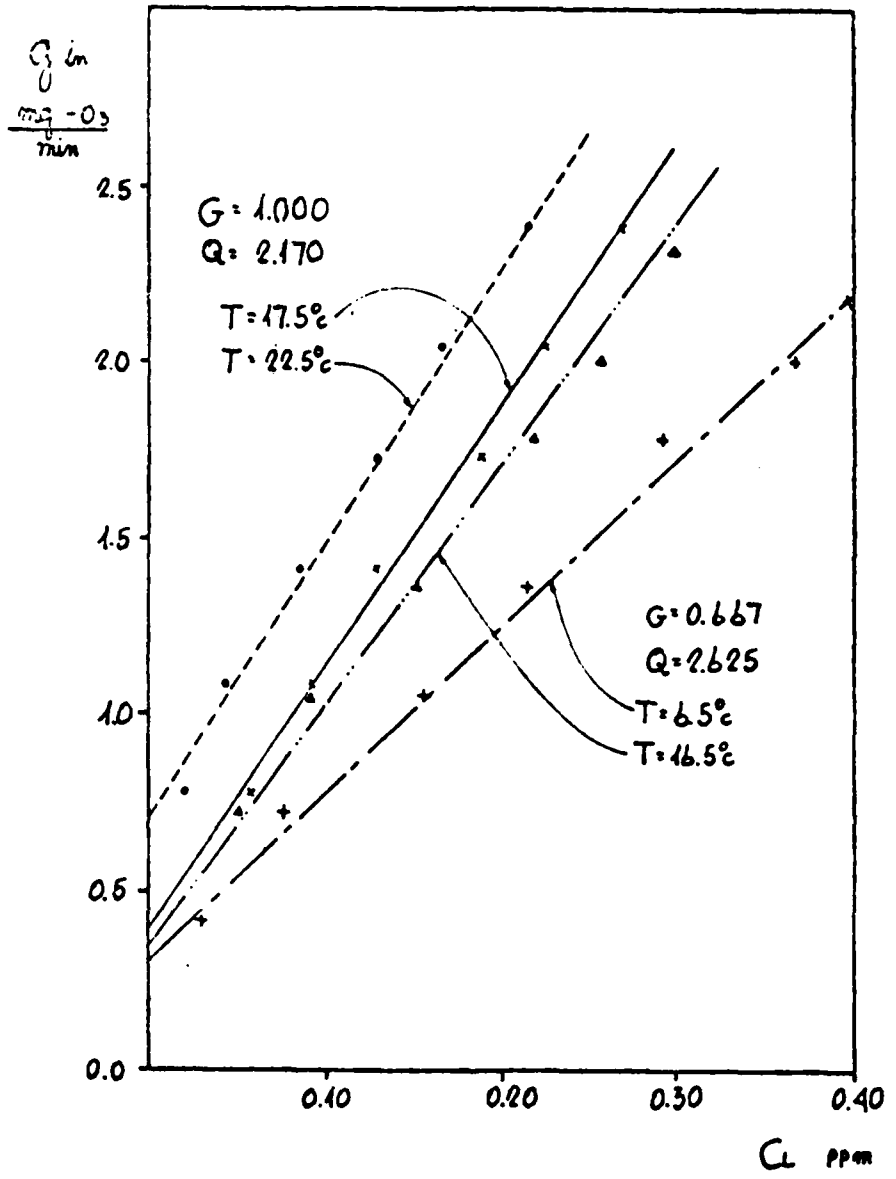


Fig. 14. Relationship between ozone feed and ozone residual at different temperatures.

D. Inactivation Rate of Poliovirus in Tap Water by Ozone in a Continuous Reactor System

Introduction

Numerous studies have been previously carried out in our laboratories on the inactivation kinetics of polioviruses by ozone in batch-type experiments. However, in order to use ozone as a disinfectant on a practical scale, a continuous flow system must be employed.

Experiments were carried out using the well-mixed, perpendicular jet contacting system described in Section C in order to determine the inactivation rate of ozone on viruses in tap water using a continuous flow system.

Nomenclature

C_L = Ozone concentration in the liquid phase (ppm)

DT = Detention time of viruses in system (sec)

g = Ozone input flow rate (mg O_3/min)

Q = Water flow rate (lit H_2O/min)

Experimental Methods

The well-mixed, perpendicular jet contacting system was identical to that described in Section C.

Viruses at a known concentration (10^6 pfu/ml) were added to the tap water in the supply tanks. Otherwise the experimental techniques were exactly as previously described (see Section A). The initial virus titer was determined for each experiment, and a control sample without addition of ozone was processed in parallel. There was no reduction in virus titer due to experimental processing in the absence of ozone.

Water flow rates of between 1.7 to 3.1 liters per minute were used with a retention time of between 50 to 100 seconds. Ozone flows of between 0.3 to 1 mg/min were injected into the system.

Results:

The results of the various experiments are tabulated in Tables 8 to 11.

Tables 8 and 9 show the effect of varying the input flow rate for two different constant ozone input rates while Tables 10 and 11 indicate the effect of varying the ozone input for constant water flow rates.

The work of Koerner and Katzenelson[11] has shown from batch tests in tap water that 95% of the polioviruses are inactivated by ozone ($C_L \leq 1$mg/liter) within less than one second. In the present experiments the time scale was relatively long, of the order of one minute.

The present study indicates that even with the small ozone concentration present in the dissolved phase (0.06 to 0.20 mg/lit) inactivation greater than 95% occurred and in some cases better than 99%.

From Tables 8 to 11 and Fig. 15, it is apparent that decreasing the water input flow rate (increased detention time) and increasing the ozone input flow rate (increased dissolved ozone concentration) increase the viral inactivation.

Conclusion

It is apparent that the present perpendicular jet contacting system is a very efficient method of viral inactivation. Almost total inactivation can be achieved within one minute for a dose of less than 0.4 mg per liter tap water (Tables 10 and 11).

Table 8: Effect of Water Flow Rates on Virus Survival at Constant Ozone Flow Rates

$Q(\frac{\text{lit } H_2O}{\text{min}})$	DT (sec)	C_L (ppm)	Virus Survival (%)
1.715	97	0.20	0.3
2.170	77	0.19	1.2
2.625	63	0.16	2.1
3.080	54	0.14	2.4

Table 9: Effect of Water Flow Rates on Virus Survival at Constant Ozone Flow Rates

$Q(\frac{\text{lit } H_2O}{\text{min}}$	DT (sec)	C_L (ppm)	Virus Survival (%)
1.715	97	0.12	0.4
2.170	77	0.10	1.9
2.625	63	0.08	2.9
3.080	54	0.06	3.4

Initial virus concentration in water = 8.81×10^3 pfu/ml

Ozone rate g = 0.65 mg O_3/min

Table 10: Effect of Ozone Flow Rates on Virus Survival at Constant Water Flow Rates

$g(\frac{mg\ O_3}{min})$	C_L (ppm)	Virus Survival (%)
0.31	0.01	0.18
0.50	0.04	0.02
0.63	0.07	0.00
0.85	0.12	0.00
1.01	0.19	0.00

Initial Virus concentration in water = 1.1×10^4 pfu/ml

Q = 2.625 lit H_2O/min

DT = 63 sec

Table 11: Effect of Ozone Flow Rates on Virus Survival at Constant Water Flow Rates

$g(\frac{mg\ O_3}{min})$	C_L (ppm)	Virus Survival (%)
0.29	0.04	0.11
0.51	0.07	0.05
0.67	0.09	0.02
0.83	0.14	0.00
1.01	0.16	0.00

Initial virus concentration in water = 7.8×10^4 pfu/ml

Q = 2.170 lit H_2O/min

DT = 77 sec

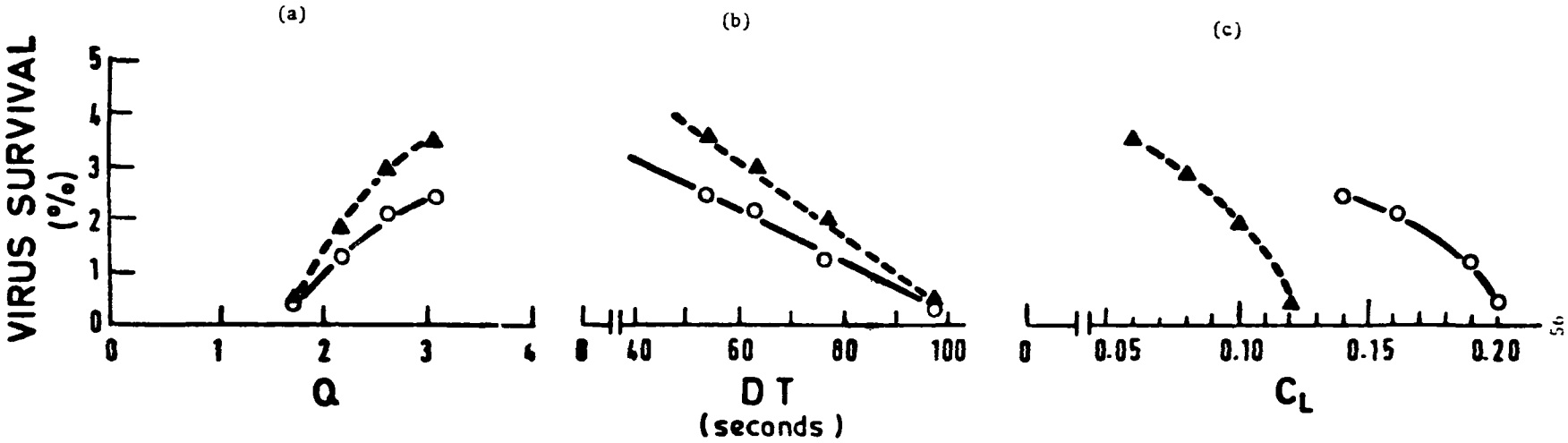


Fig. 15: Effect of water flow rate [a], detention time of viruses [b], and ozone concentration [c] on virus-survival in the continuous flow reactor.

▲: g=0.05 mg O_3/min
o: g=1.1 mg O_3/min

E. Mechanism of Virus Inactivation by Ozone

Introduction

Ozone is being increasingly considered as a disinfectant for drinking water. Studies have shown that ozone disinfects very rapidly both in drinking water[1] and treated sewage.[14] Recent studies by Katzenelson[11] have shown that in ozone-demand-free water, 95% inactivation of poliovirus by ozone occurs within less than one second (ozone dose of 0.2 ppm).

It is thought that inactivation of poliovirus occurs by chemical attack either on the protein coat or on the RNA-nucleus of the virus. In this study, the kinetics of the ozonization of amino acids were carried out in an attempt to determine possible sites of ozone attack on the protein coat. It was assumed that if a chemical attack occurs first on the protein coat, it must involve chemical interaction with the amino acids of the coat.

Experimental

Materials: All materials used were commercially available analytical grade reagents and were used with no further treatment. Water was distilled from o.01% $KMnO_4$ solution and was brought to the desired pH by adding HCl.

Solutions of ozone in water were prepared by bubbling a mixture of O_2/O_3, comging from an ozone generator into distilled water (at pH 2), shortly before carrying out the experiment, in order to maintain a high concentration of ozone. Ozone concentrations were measured by the spectrophotometric method developed in our laboratory,[5] with a slight variation. The 2% KI solutions were buffered to pH = 2.0 by a KCl/HCl

solution. Determination of the stability of a pH = 2.0 solution of KI/I_2 showed no significant error towards that of a pH = 6.8 solution. Therefore, no interference is to be expected by carrying out the determination at pH 2 instead of pH 6.8.[27, 28]

Procedures: The reactions of ozone with amino acids were carried out using two different procedures depending on the reaction rate.

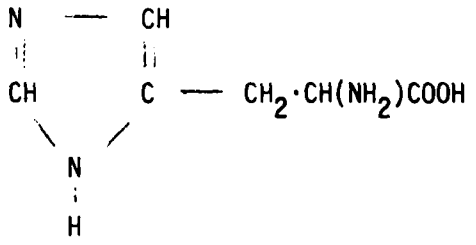
(i) Procedure A: For reaction rates slower than approximately one minute to completion: 4.5 ml portions of an ozone solution (freshly prepared) were introduced into a series of test-tubes, which had been previously washed with the ozone solution in order to minimize ozone demand. The ozone concentration was determined before and after each experiment by adding 5.5 ml of 2% KI solution at pH - 2.0. Into each tube, 0.5 ml of the amino acid solution (1.10^{-3} molar) was introduced. The reaction was quenched at predetermined intervals by adding KI solution and the O_3 concentration was measured spectrophotometrically. Amino acid content was analyzed using a Beckman amino acid analyzer (Model 120).

(ii) Procedure B: For fast reaction rate mixtures--quicker than one minute to completion--a fast-flow apparatus was used as shown in Fig. 16. This instrument provided measurements for reaction times of as low as 25 msec. Reaction time was defined as the time taken for the reactants to flow from the reaction chamber (4) through tube (5) to the quenching chamber (6). Reaction time could thus be altered by varying the linear driving force (8) on the syringes or by changing the diameter and/or length of the reaction tube (5). Reaction times were calibrated both mechanically (timing the plunger) and chemically (hydrolysis of p-nitrosophenol) (7). Syringe (1) was filled with a solution of the

amino acid under investigation and the system flushed out. Syringe (2) was then filled with the ozone solution so that tube (5) was also full of the ozone solution. Finally syringe (3) was filled with the quenching solution which was also introduced in the quenching chamber and outlet tube to the collector. The motor driving the syringe plunger was then adjusted to give the desired speed of drive and after it stabilized, the clutch was engaged and the linear driving force(8) was applied to the syringe plunger (10). The quenched samples were collected in a test-tube (7) and analyzed as previously described (Procedure A).

Results: No reaction was observed when ozone was mixed with saturated amino acids such as glycine $CH_2(NH_2)COOH$; alanine $CH_3 \cdot CH(NH_2)COOH$ and leucine $(CH_3)_2CH \cdot CH_2 \cdot CH(NH_2)COOH$ at room temperature for a period of 15 minutes.

However, once unsaturated amino acids were studied, an immediate reaction with ozone was observed as noted in Fig. 17 for histidine

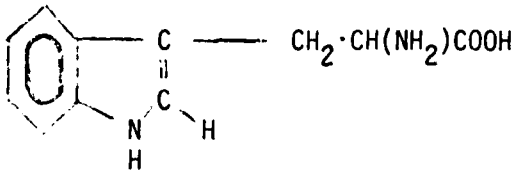


The histidine was rapidly attached (at the imidazol ring) being oxidized to at least three compounds: aspartic acid $HOOC \cdot CH_2 \cdot CH(NH_2) \cdot COOH$; serine $HO \cdot CH_2 \cdot CH(NH_2) \cdot COOH$, and ammonia. However, since the amino acids produced were saturated, they were not oxidized further on the time scale under investigation.

The effect of pH on the ozonization of histidine was also examined (Fig. 17). In the acidic range the effect of pH on the ozone

decomposition rate is expected to be minimal as compared to its effect in the basic range on the basic amino acid-histidine. The pH dependence on the histidine is shown quite clearly in Fig. 17, demonstrating that the less acidic the solution the faster the kinetics.

Table 12 shows the percent reaction after one minute of reaction time as a function of the initial concentration of histidine. The results suggest that the reaction is first order with respect to the amino acid.

Tryptophan  was found to react

with ozone at a similar rate to that of histidine (Fig. 18). However, the effect of varying pH was quite minimal.

On extending the investigation to include sulfur-containing amino acids, it was observed that the reaction rate was even quicker than for the unsaturated acids mentioned above. Studies on cysteine, $HS{\cdot}CH_2{}^{CH(NH_2)}COOH$, and methionine, $CH_3{\cdot}S{\cdot}CH_2{\cdot}CH_2{\cdot}CH(NH_2)COOH$, showed that even at the shortest available reaction times (ca. 25 msecs) complete reaction of the acids occurred. Methionine was also rapidly converted (25 msec) even when other amino acids (other than cysteine) were present in the system. This applied even when the additives were present in concentrations 20 times higher than that of the methionine.

It was possible to follow the ozonization of cysteine, $HOOC{\cdot}CH{\cdot}(NH_2){\cdot}CH_2{\cdot}S\text{-}S{\cdot}CH_2CH(NH_2){\cdot}COOH$, using the fast flow apparatus even when the complete reaction occurred within less than one second as shown in Fig. 19. Since cystic acid is the final oxidation product, it suggests that ozonization causes breakage of the S-S bond which occurs prior to oxidation of the sulfur site yielding cystic acid. The effect of pH on the

reaction kinetics is also shown in Fig. 19 and only a weak dependence is observed, the effect of pH probably being on the ozone and not on the organic substrate.

From the above results, it is obvious that only those amino acids with sulfur-containing groups were attacked kinetically in a manner similar to that of poliovirus with ozone. The above suggests that if it is a chemical attack on the virus coat which causes inactivation of the virus, it must occur by the reaction of ozone with the sulfur-containing acids. Attention was therefore centered on the reaction of ozone with sulfur-containing proteins in an attempt to evaluate the effect of the micro-environment on ozonization. In the proteins themselves, because of their secondary and tertiary sturctures, the reactivity of the sulfur groups towards chemical agents will vary greatly as compared to the free amino acids. This difference is a consequence of steric hindrance and changes in their physical-chemical conditions (i.e., change of polarity of the micro-environment). It is well known that the pH of lysine changes by ±2 in a protein as compared to the pH of a solution containing the free amino acid.

The reaction of the sulfur-containing protein, ribonuclease, with ozone was studied. It was observed that the above enzyme was completely inactivated with respect to its ability to degrade RNA by reaction with ozone within 7 seconds. The reaction was carried out at pH 7 in a 0.02 M phosphate buffer. In order to determine the place of the ozone attack on the protein, a kinetic study was performed on an "S-peptide" containing 21 amino acids. The peptide was a derivative of the enzyme with methionine in the thirteenth place. Kinetically the S-peptide reacted much slower than the enzyme itself, while the single methionine acid reacted kinetically similar to the enzyme.

Conclusions

Previous studies by Mudd, et al.,[29] have also been made on the reaction of ozone and amino acids. However, these investiagtions were carried out using a continuous flow of ozone which makes it difficult to obtain accurate kinetic data. Our results differ from those of Mudd, et al.,[29] both in the order of susceptibility of the amino acids in aqueous solution to oxidation by ozone and also in time scale (minutes as against seconds, as in the present study).

From the present study, the following conclusions can be drawn. Ozone reacts extremely rapidly (less than one second) with those amino acids containing sulfur groups. The S-S linkage in single amino acids reacts with ozone on a time scale similar to that of the virus, while C-S-H and C-S-C groupings are attacked even quicker. However, the interaction of ozone with the enzyme is apparently with the S-S link and not the methionine group. Further, the isolated methionine acid reacts differently from the acid in a peptide chain with ozone. Apparently the effect of the entire peptide chain causes steric hindrance as well as physical-chemical changes in the micro-environment and hence slows down the kinetics.

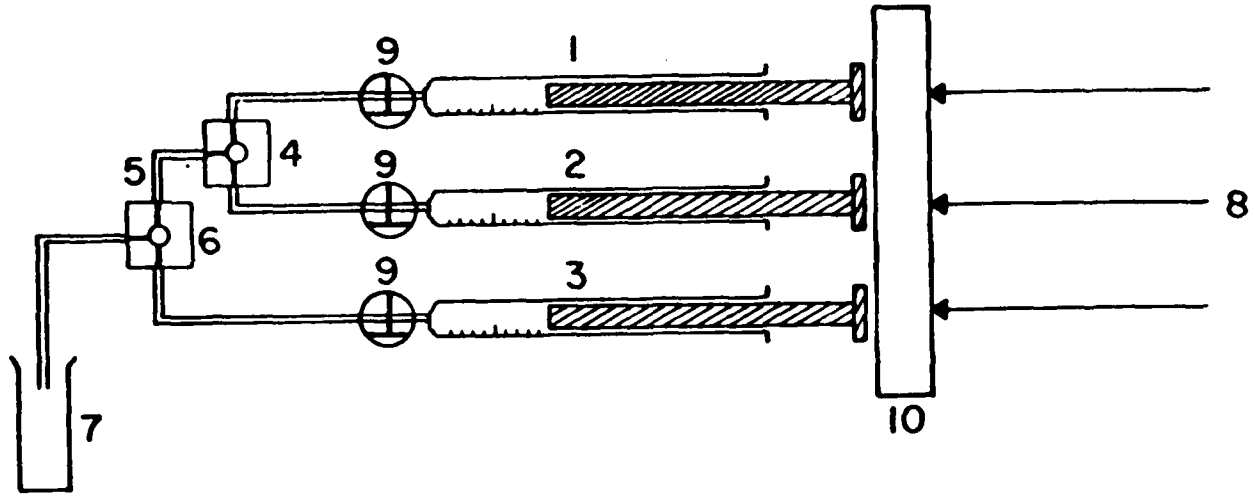
In conclusion: The S-S linkage is important as a bridging unit in the protein coat and oxidation of this bridging unit could possibly cause a break-up of the coat and hence lead to the inactivation of the virus.

Consideration, however, should also be given to the possibility that the ozone might inactivate the virus via attack on the RNA core. Preliminary results in our laboratory were carried out on purified RNA derived from yeast. Results have shown that the ozone reacts rapidly (less than 7 sec) with the RNA and breaks down the chain to single nucleotides. It should be stated that even a minor attack on RNA can completely reduce its biological activity and prevent the biosynthesis of certain proteins.

Table 12: Percent reaction as a function of the initial concentration of the amino acid (Histidine)

2.5-2.6μ moles O_3 present; pH = 3.8; T = 0°C;
reaction time = 1.0 min; reaction volume = 5.0 ml

Initial	Histidine moles reacted	Final	% Reaction	O_3 moles reacted
0.56	0.22	0.34	40%	0.48
0.91	0.41	0.50	45%	0.74
1.23	0.52	0.71	42%	0.87
1.66	0.63	1.03	38%	1.31



1 Syringe - Reaction solution
2 Syringe - Ozone solution
3 Syringe - Quenching solution
4 Reaction mixing chamber
5 Reaction tube
6 Quenching chamber
7 Collector
8 Linear driving force
9 Three-way stop-cock (for filling the syringe)
10 Syringe plunger

Fig. 16. Fast - Flow Apparatus

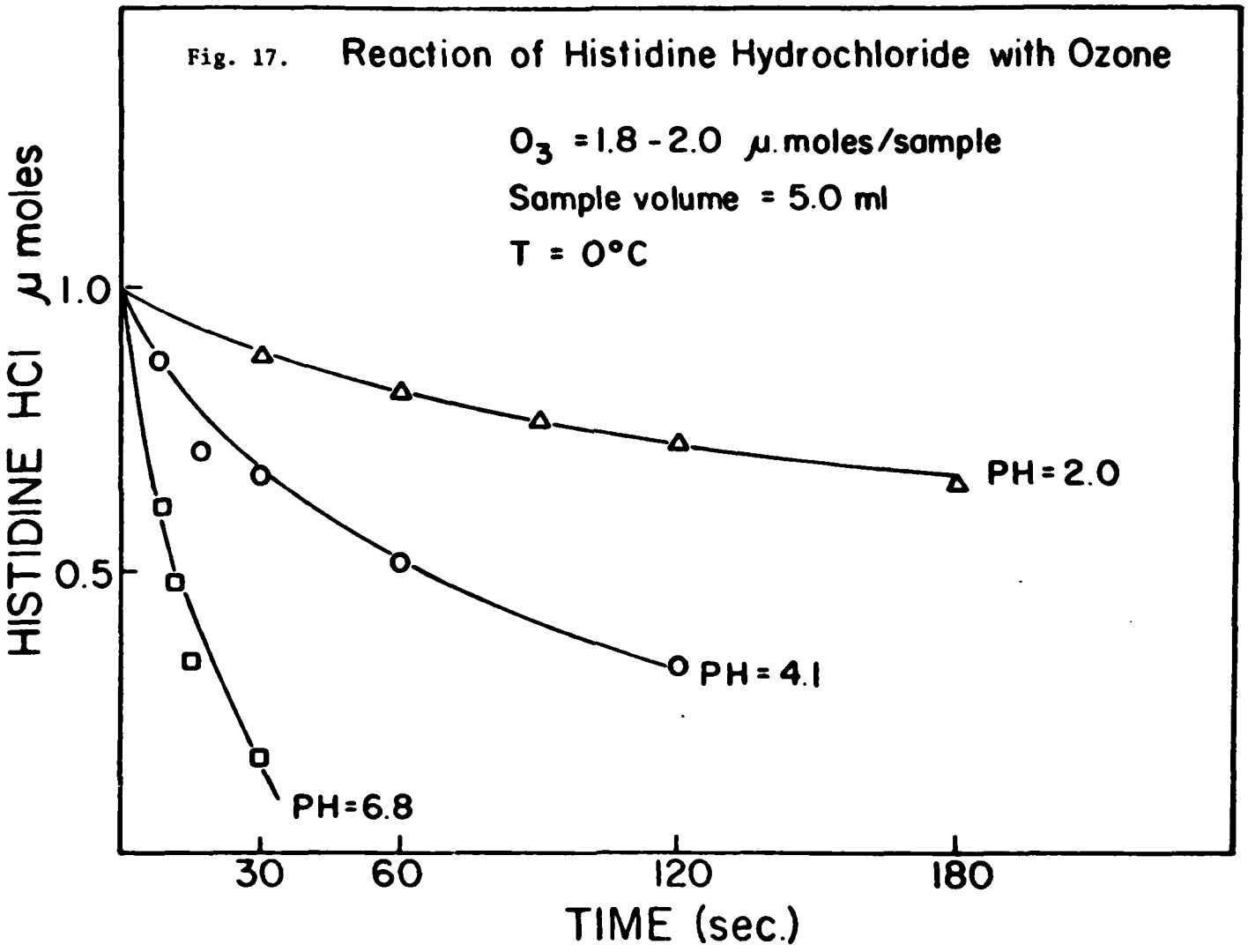


Fig. 17. Reaction of Histidine Hydrochloride with Ozone
O_3 = 1.8 - 2.0 μ. moles/sample
Sample volume = 5.0 ml
T = 0°C
HISTIDINE HCl μ moles
1.0
0.5
PH = 2.0
PH = 4.1
PH = 6.8
30
60
120
180
TIME (sec.)

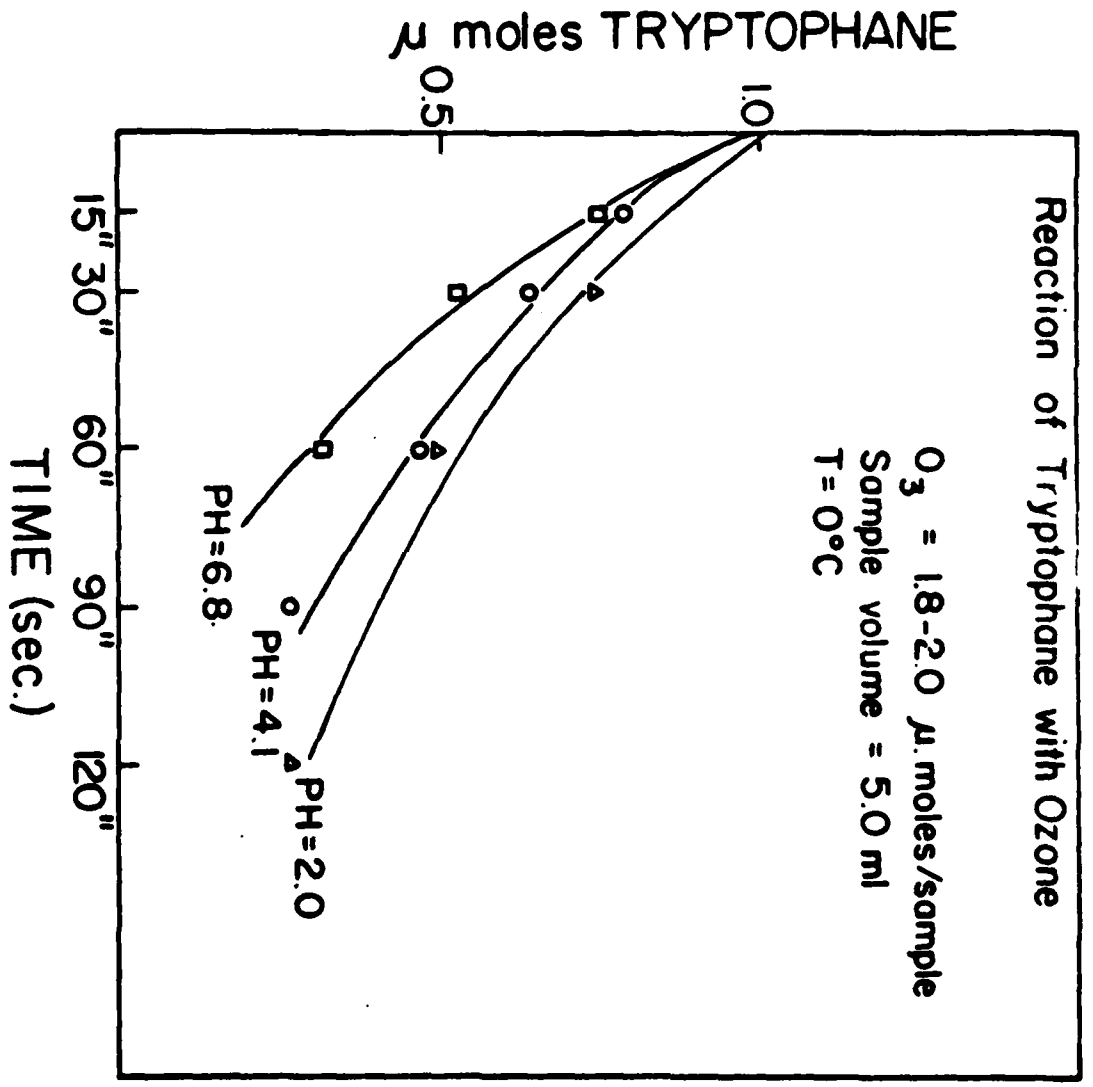
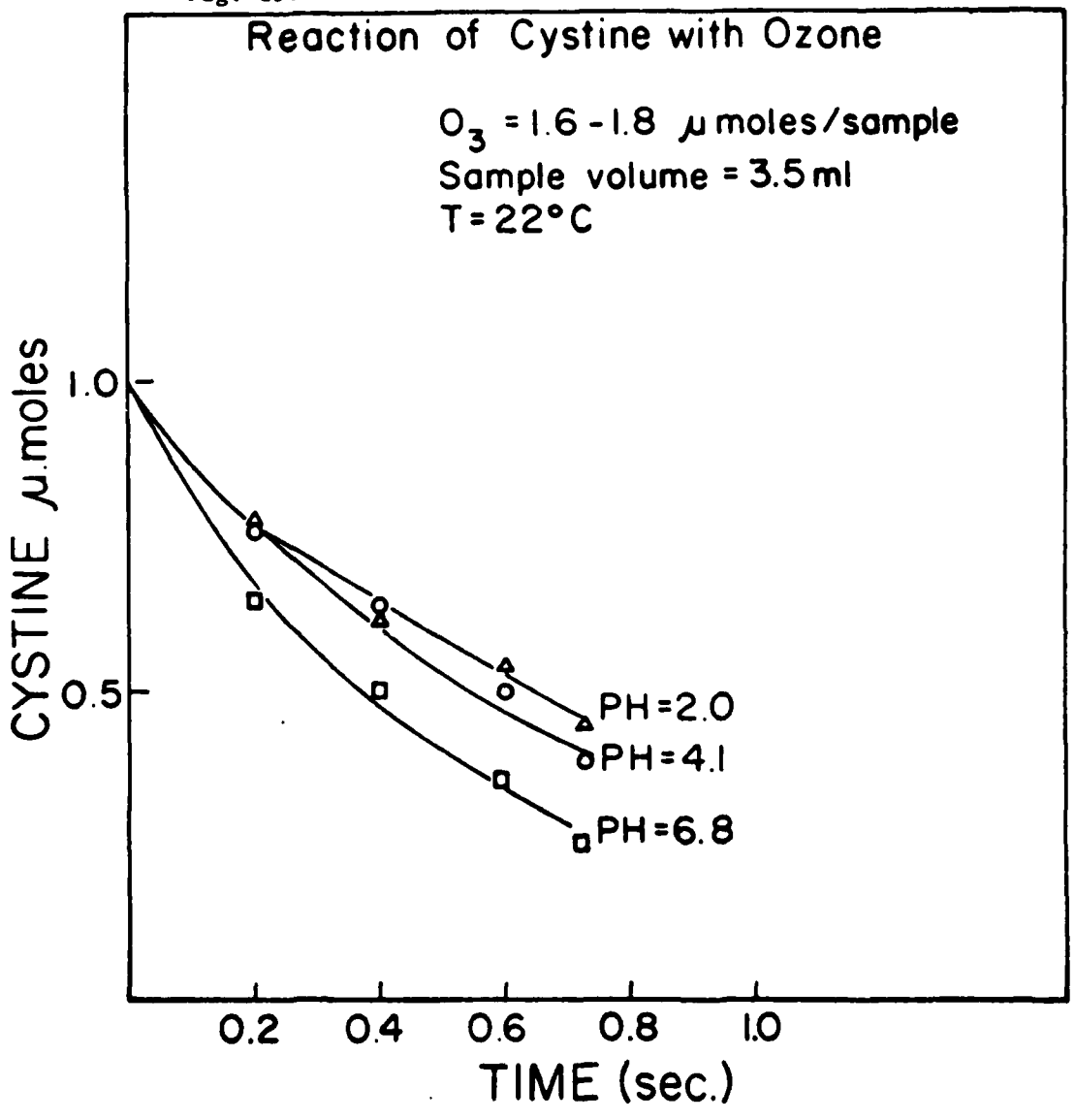

μ moles TRYPTOPHANE
0.5
1.0
15" 30" 60" 90" 120"
TIME (sec.)
Reaction of Tryptophane with Ozone
O_3 = 1.8-2.0 μ moles/sample
Sample volume = 5.0 ml
T= 0°C
PH=6.8
PH=4.1
PH=2.0

Fig. 18.

Fig. 19.


Reaction of Cystine with Ozone
O_3 = 1.6 - 1.8 μ moles/sample
Sample volume = 3.5 ml
T = 22°C
CYSTINE μ moles
1.0
0.5
PH=2.0
PH=4.1
PH=6.8
0.2
0.4
0.6
0.8
1.0
TIME (sec.)

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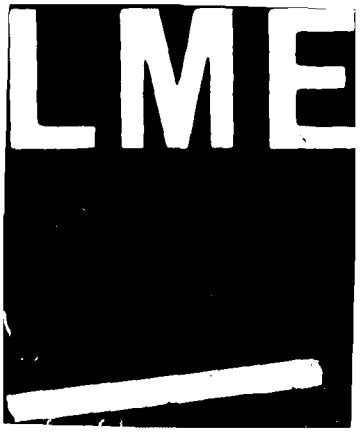
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