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A study of chlorine sterilizing compounds: 1, Relationship between pH and oxidation potentials

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A STUDY OF CHLORINE STERILIZING COMPOUNDS

1. Relationship between pH and Oxidation Potentials

by

Arthur Paul Hellwig

A Thesis Submitted to the Graduate Faculty
for the Degree of

DOCTOR OF PHILOSOPHY

Major Subject
Food and Sanitary Chemistry

Approved:

Signature was redacted for privacy.

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Signature was redacted for privacy.

Dean of Graduate College

Iowa State College

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I INTRODUCTION

During the spring of 1931 the author developed a simple tablet test suitable for use by the layman in evaluating the strength of chlorine sterilizing compounds. Such a test was deemed essential as certain state laws require various food industries to maintain a definite chlorine strength in various phases of processing.

It was observed during the experimental work on the proposed test that those compounds classed as organic active chlorine compounds liberated much less iodine from potassium iodide than did the inorganic hypochlorites. However, if the solutions were acidified with acetic acid as in the Bunsen method, the same quantity of iodine was liberated in both types. The amount of iodine liberated from a solution of potassium iodide was observed to be dependent in some manner upon the reaction (pH) of the menstrum.

As the ability of a chlorine solution to liberate iodine from potassium iodide or to produce a color with ortho-tolidine is dependent upon the oxidizing power of the substance, it was realized that the oxidizing power of the various compounds was not of the same magnitude.

The foregoing observations were responsible for the author's desire to determine first, the definite manner in which oxidation potential varies with the reaction (pH) and second, the apparent difference in oxidizing power between the organic chlorine compounds and the inorganic type.

The disinfecting action of active chlorine compounds is one of the principal uses of these agents. It has been suggested in the literature that the antiseptic action of active chlorine compounds is due to their oxidizing action.

The bacteriological phases of these studies have been considered by Mr. D. B. Charlton of the Department of Bacteriology of Iowa State College. Certain of these data are used in this dissertation for the purpose of studying the relationship between the chemical properties of chlorine compounds and the ability of these compounds to kill microorganisms. Proper reference to this work has been made throughout this thesis.

II HISTORICAL

Inorganic chemistry contains many examples which indicate that the oxidizing or reducing power of a given reagent is not a constant, but that this property is greatly influenced by the nature of the solvent. Ephraim (10) states that chlorine is a very much stronger oxidizing agent in a solution of potassium chloride than when it is dissolved in alkali. He gives the following potentials as measured with reference to the normal hydrogen electrode:

chlorine in potassium hydroxide = +0.626 volts
chlorine in potassium chloride = +1.106 volts.

These values would tend to illustrate the fact that the oxidizing power is a relative property.

Rideal (29) has shown that the effect of acidity on the oxidizing power of hypochlorite solutions is particularly marked. He suggested that the hypochlorous acid (HOCl) is the effective oxidizing agent, the oxidizing power of the salts and of the anion (OCl^-) being but feeble.

Crossley (5) found that the hypochlorites displayed peculiar advantages in cotton bleaching since over a narrow range of hydrogen ion concentrations near the neutral point, hypochlorite solutions are changed from a system in which the oxidizing agent is wholly hypochlorite ion to that in which oxidation is promoted wholly by the undissociated hypochlorous acid. He further states that by slight changes in alkalinity or acidity a range of solutions may be obtained with different oxidizing properties.

Kauffmann (17) has made certain observations with respect to the rate of attack on cellulose by hypochlorites at various pH values and neutral salt effects.

The oxidation potential with the chlorine electrode has been measured by Remington and Trimble (27). They observed that the oxidizing power of hypochlorous acid (HOCl) and sodium hypochlorite (NaOCl) in solution, as measured indirectly, varies with acidity; that is, with increase in hydrogen ion concentration.

Rius y Miro (31) has measured the potential with the chlorine electrode, relative to the normal calomel electrode, from pH 12.68 to pH 1.76. He observed that between pH 3.1 and 9.5 the potential is a linear function of the pH. The slope of the line was calculated to be -0.0705.

Rettie (28) observed in a qualitative manner that the reaction (acid or basic) of hypochlorite solutions markedly affected their germicidal properties. He and his co-workers observed that if boric acid were added to a solution of bleaching powder to reduce the alkalinity, the germicidal efficiency was markedly increased.

Holwerda (15) stated that for disinfection of drinking water by means of hypochlorites or chlorine, there was only the HOCl molecule and the OCl^- ion to consider.

He also concluded that the ratio $\frac{(HClO)}{(OCl^-)}$ was a function of the pH. He observed that from disinfection experiments an increasing disinfection was parallel with an increase in the (HClO) concentration.

Mallmann and Schalm (22) have also observed that increasing pH values above the neutral point caused a decreased germicidal action of chlorine.

The first attempt to correlate germicidal properties of chlorine and their oxidation potentials was made by Rideal and Evans (30). They stated that since the germicidal properties of the substance were in part due to its oxidizing power, it seemed probable that these also would be affected by the hydrogen ion concentration of the water.

Leech (19) in an extensive review of certain organic chlorine compounds raised the interesting question as to what effect the reaction (pH) of the solvent would have on the oxidation potential. He measured the oxidation potential of Chloramine-T and showed that upon acidification the oxidation potential was considerably augmented. In this manner he showed that Chloramine-T was a much stronger oxidizing agent in acid than in alkaline solutions. He stated that the antiseptic action of this type of compound was most probably due to the oxidizing power of the positively charged chlorine in the molecule. In conclusion from the above experiments he stated that it would not be surprising to find that the hydrogen ion concentration (C_H), the oxidation potential, and the bactericidal coefficients would be in direct ratio, and that Chloramine-T was weakly antiseptic in alkaline solutions.

Schmelkes (35) in a recent publication has shown the relationship of pH to oxidation potentials for hypochlorous acid, chloramine, and azochloramide. The same author in another publication (36) has stated that the oxidation potential and bactericidal properties of chlorine were found to run parallel. He stated that any changes which diminish bactericidal velocity also diminish the oxidation potential and vice versa.

The present investigation was initiated to develop in a definite quantitative manner the relationship of pH and oxidation potentials of such compounds as Chloramine-T, calcium hypochlorite, and sodium hypochlorite. The investigation will include a study of the effects of oxidation potentials upon the germicidal velocity of the foregoing solutions. Other observations recorded throughout this work must be considered as developments resulting from experience gained in carrying out the experimental procedure.

There are at present two terms used to express the strength of chlorine compounds. The terms "active" and "available" have been used synonymously by various authors. However, other authors have used the term "active" in a very different sense from the term "available". It is the author's desire to define these terms and, as a result, prevent any confusion throughout this work.

The difference in the use of the terms "active" and "available" is a matter of calculation as they are both determined by the same method. In the calculation for "available" chlorine the assumption is made that each chlorine atom has a valence change of one. In the calculation for "active" chlorine each atom of chlorine has a valence change of two. Thus the active chlorine content is always just one half of the available chlorine.

Leech (19) stated that the term "available" chlorine is probably a hang-over from the archaic methods of determining the strength of hypochlorites by measuring the amount of gaseous chlorine evolved under appropriate treatment with hydrochloric acid in the presence of chlorides. This method may be illustrated by the following equation:

$$HOCl^{+} + H(Cl)^{-} \xrightarrow{(NaCl)} Cl_2 + HOH$$

In this equation two chlorine atoms are involved: chlorine with a positive valence of one and with a negative valence of one. Each of these two molecules loses one valence and becomes a neutral chlorine molecule.

Phelps* (25) states that hypochlorous acid in the

---

* Phelps further states that the oxidizing power of hypochlorous acid (or the available chlorine) is, therefore, two hydrogen equivalents per molecule, which is twice the total chlorine content. This fact makes the term "available" chlorine a misnomer.

presence of a reducing agent is decomposed according to the following equation:

$$HOCl \longrightarrow HCl + O$$

The above equation is, however, not in keeping with many of the reactions of hypochlorous acid. For example,

$$HOCl + 2HI \longrightarrow I_2 + HCl + HOH$$

In this equation the oxygen is not evolved and the oxidizing action is dependent upon the change in valence of the chlorine from a positive valence of one in the hypochlorous acid to a valence of negative one in the hydrochloric acid. Thus the total valence change per molecule of chlorine is two.

The above reaction in which the chlorine has a valence change of two per atom is the basis for the term "active". "Active chlorine" then represents the positive chlorine ($Cl+$) or that chlorine which may act as an oxidizing agent. The term "active chlorine" then signifies the actual amount of reactive material and, as a result, represents a more fundamental value than the term "available chlorine".

The term "active chlorine" (Cl^+) will be used to express all concentrations throughout this dissertation and to represent the positively charged chlorine (Cl^+). In the event that the concentration should be desired in terms of "available chlorine" the mathematical value of the active chlorine must be multiplied by two.

III EXPERIMENTAL

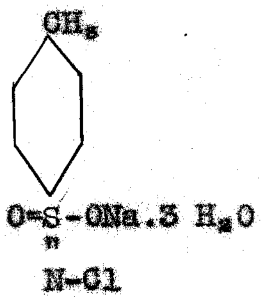
A. ANALYSIS OF THE COMPOUNDS USED AND A GENERAL DISCUSSION OF THE CHEMISTRY OF THESE COMPOUNDS

The two compounds chosen were <u>para</u>-toluene sodium-sulphonchloramide (Chloramine-T) and calcium hypochlorite. Data will be presented also for sodium hypochlorite. Chloramine-T was used as a representative of a large class of organic chloramines while the calcium hypochlorite represents the class of inorganic hypochlorites.

Chloramine-T is a member of that general class of compounds known as chloramines. The simplest member of this group is chloramine (NH_2Cl) first prepared by Raschig (26). These compounds are all characterized by the fact that they contain the (:NCl) radical. This radical, (:NCl), may be attached to such residues as benzene, substituted benzene, aromatic compounds containing the (:NCl) group separated from the nucleus by the (SO_2Na) group, naphthalene, substituted naphthalenes, dicyclic derivatives, amino acids as well as certain aliphatic compounds. The preparation of a great number of these compounds may be located in the extensive research of Chattaway (4). His method of preparation consisted of treating the corresponding amide with a solution of sodium or calcium hypochlorite. They all readily followed definite reactions which distinguish the nitrogen-halogen linkage from the carbon-halogen linkage. They liberated (Cl-)

when treated with hydrochloric acid, iodine from hydroiodic acid, and set sulphur free when treated with hydrogen sulphide. The halogen in this class of compounds may be replaced by hydrogen and the corresponding amide recovered.

The compound toluene para-sulphondichloramide was first obtained by Kastle, Keiser and Bradley (16), and it remained for Chattaway to first prepare the para-toluene sodium sulphonchloramide ($CH_3.C_6H_4.SO_2Na:NCl.3\ H_2O$). The structural formula for Chloramine-T has never been definitely proven although Chattaway (4) stated that there are reasons for believing that the salts are derivatives of the sulphonisochloramide (RSO (OH):NCl) and have a constitution represented by the general formula RSO(OM):NCl. The following may then be considered as expressing the structural formula of Chloramine-T.



The Chloramine-T used throughout this work was prepared by the Research Laboratory of the Eastman Kodak Company.

The compounds as well as the solutions prepared were analyzed for the per cent (active) chlorine by the Bunsen method following the general procedures as given by

Mahin (21). A weighed sample was introduced into a glass-stoppered Erlenmeyer flask. One hundred cubic centimeters of conductivity water was added followed by two grams of potassium iodide. Then 25 c.c. of a ten per cent acetic acid solution was added and the solution allowed to stand for five minutes. The sample was titrated with standard sodium thiosulphate using starch solution as an indicator. The starch solution had been previously prepared following the method of Scott (37).

The following Table I gives the per cent of active chlorine of the various lots of Chloramine-T used.

Table I

Analysis of Chloramine-T
for per cent (active) chlorine

Lot No.	per cent by analysis	per cent theoretical	per cent purity
I	12.27	12.58	97.53
II	12.29	12.58	97.69
III	12.15	12.58	96.58
IV	12.19	12.58	96.90

Calcium hypochlorite is a member of the general class of compounds known as inorganic hypochlorites. The calcium and sodium salts are the only salts of the hypo-acids which are known in the solid state. The empirical formula for calcium hypochlorite may be expressed as $Ca(OCl)_2$. Schafer (34) and Hantzsch (14) have to some extent cleared up the constitution of the hypochlorites as well as the free hypochlorous acid. Schafer (34) has given to the hypochlorites the structural formula $(ClO)M$ in which M signifies the metal.

The calcium hypochlorite used throughout this work carries the trade name of Perchloron. The following analysis and description of the method of preparation of this compound were furnished the author by W. A. Hadfield (12).

Chemical Composition of the Calcium Hypochlorite.

Calcium Hypochlorite	73.05
Calcium Chlorate	0.55
Calcium Chloride	7.48
Calcium Carbonate	2.53
Calcium Hydrate	13.74
Oxides (Fe, Al, and Si)	0.57
Moisture	2.15
Total	100.07

The calcium hypochlorite is produced by chlorinating a concentrated mixture of water and calcium hydrate. The solution is concentrated to the extent that, as the hypochlorite forms, it separates out of solution. The reaction is facilitated by an increased pressure and temperature.

The analysis of the calcium hypochlorite used in this work showed 37.133 per cent active chlorine.

The chloramines ($:NCl$) and the hypochlorites

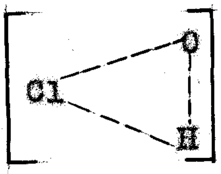
(OCl)M have one point in common which has not been emphasized to the fullest extent. They both possess the positively charged chlorine atom (Cl+) and are, as a result, oxidizing agents in the proper medium.

Both types of active chlorine compounds hydrolyze in water with the formation of hypochlorous acid (HOCl). This fact can easily be illustrated in the case of inorganic hypochlorites as the acid may be distilled from an acidified solution of the salts. The following convincing evidence by Leech (19) shows that chloramines contain the positively charged chlorine (1). The formula and the method of preparation (the replacing of a hydrogen atom of an amide group by chlorine) strongly suggest that the chlorine is positively charged (2). On hydrolysis they yield hypochlorous acid (HOCl) (3). They are active oxidizing agents. Leech (19) states that we may consider that the antiseptic action of these compounds is most probably due to the oxidizing action of the hypochlorous acid. In the inorganic hypochlorites the positive chlorine (Cl+) is linked to a hydroxyl group (OH) while in the chloramines the positive chlorine (Cl+) is linked to a trivalent nitrogen atom ($N\equiv$).

If one accepts the statement that hypochlorous acid (HOCl) is a resultant of the hydrolysis of compounds containing a positive chlorine (Cl+), a discussion of some of the fundamental properties is important.

Schafer (34) was able to show that the ultra-violet absorption spectra of free hypochlorous acid are similar to that of the esters, but entirely different from that of the salts. Thus the formula of the acid is of form (I) in which the H atom is closely attached to the other atoms, while that of the salts is of type II.

I



II $[ClO]$ M

He states that the differing structures of the free acid and the salts account for their unlike chemical behavior.

The free acid (HOCl) may be obtained by distilling under a vacuum a saturated solution of mercuric oxide into which gaseous chlorine has been introduced. Ephraim (11) stated that the reaction of chlorine with mercuric oxide is typical of its action with other oxides and thus with H_2O.

$$Cl_2 + HOH = HOCl + HCl$$

The oxidation potential of hypochlorous acid as measured against the normal hydrogen electrode is expressed in the following equation as given by Ephraim:

$$Cl^- + HOH + 2 \longrightarrow H' + HClO + 1.49 \text{ volts}$$

Steiglitz and Senior (40) in a study of the molecular rearrangement of syn-bis triaryl-methyl-hydrazines stated that their conclusion may well be extended to include hypochlorous acid (HOCl), hypobromous acid (HOBr), and their derivatives. These compounds have a common characteristic, an atom with an unstable positive charge and a tendency to convert this atom into the common stable negative variety.

B. TECHNIQUE AND APPARATUS

The development of the glass electrode has made the study of this problem possible; the glass electrode offers one of the few, if not the only, method of determining the hydrogen-ion concentration of an oxidizing solution. Lewis and Kukolich (20) have employed a colorimetric method of determining the pH of solutions containing hypochlorites. However, the concentration of chlorine must be very small. The hydrogen electrode, the quinhydrone and metallic electrodes also fail under oxidizing conditions.

Ross, Mitchell, and Yorsten (32) have used the glass electrode for determining the changes in cuprammonium viscosity due to bleaching at various pH values. Voightman and Rowland (42) have written an extensive review of the application of the glass electrode in controlling the pH of the pulp bleaching processes. Davis (9) in a paper on the factors influencing the stability of hypochlorite solutions determined their respective pH's with the glass electrode. Recently Britton and Dodd (2) have measured the dissociation constant of hypochlorous acid by means of the glass electrode. The foregoing references would tend to illustrate the reliability of measuring the pH values of hypochlorites by means of the glass electrode.

The glass electrode set-up as illustrated in Plate I has been constructed following the general set-up of Goodhue, Schwarte, and Fulmer (13).

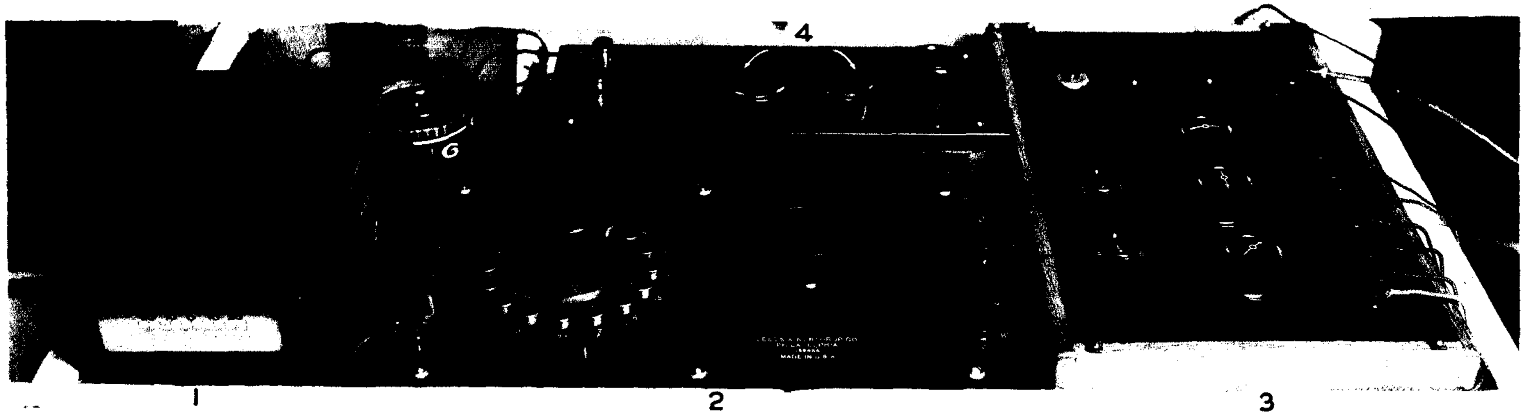
A detailed discussion of the vacuum tube amplifier general set-up, and methods of calculating the pH values may be found in the publication of Goodhue, Schwarte, and Fulmer (13).

The general set-up as illustrated in Plate I was also used for determining the value of the oxidation reduction potentials. This set-up has one very definite advantage, namely, due to the vacuum tube amplifier the amount of current drawn from the platinum electrode is so very small that it has no tendency to become polarized. This fact eliminated any necessity of agitating the solution.

Plate II shows the cell assemblage. This entire assemblage was maintained in a constant temperature box. The temperature was kept at 25° C. ± 0.5° C. Number 1 on Plate II shows a KCl-agar bridge. The bridges were constructed by filling a bent tube with a solution of three per cent agar in a saturated solution of potassium chloride. The bridges were carefully inspected after cooling to make certain that they contained no air bubbles, or that the potassium chloride had not crystallized. The tips of the bridge were broken off at the beginning of each run so that a fresh surface would be exposed. When not in use these bridges were

PLATE 1

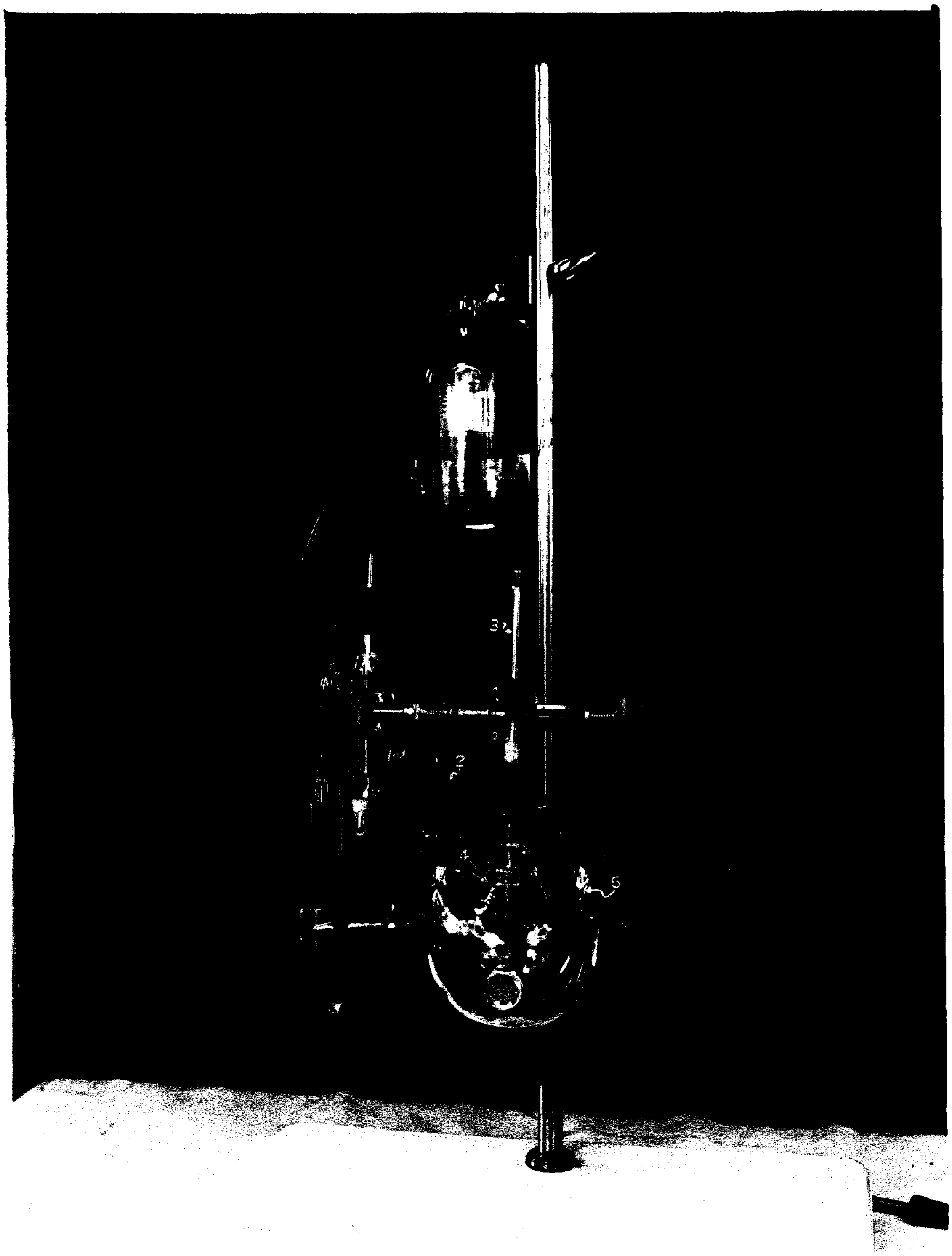
pH AND OXIDATION-REDUCTION SET UP



1. ENCLOSED LAMP AND SCALE GALVANOMETER
   LEEDS & NORTHRUP
2. POTENTIOMETER (STUDENT TYPE)
   LEEDS & NORTHRUP
3. VACUUM TUBE AMPLIFIER
4. VARIABLE RESISTANCE BOX

PLATE 2

pH AND OXIDATION - REDUCTION CELL ASSEMBLAGE



1. KCL-AGAR BRIDGE
2. NITROGEN OUTLET
3. GLASS ELECTRODE
4. NITROGEN INLET
5. PLATNIUM GAUZE ELECTRODE

kept in a saturated solution of potassium chloride. Number 2 is an opening made of a piece of capillary tubing placed through the stopper to serve as an outlet for the nitrogen; number 3, the glass electrode; number 4, an auxillary neck sealed on to a 200 c.c. three-neck pyrex Woulff flask. This opening served as a nitrogen inlet. Number 5 is a platinum gauze electrode. The platinum gauze electrodes were of cylindrical shape with a diameter of 0.5 cm. and a height of 1 cm. and the mesh was 52 to the inch. The wire had a diameter of 0.01 mm. The platinum gauze electrodes were cleaned in the following manner. They were heated in a bath of nitric acid for fifteen to twenty minutes, the nitric acid never being permitted to reach the boiling point, washed in distilled water, wiped dry with a lintless cloth, and heated to white heat in the flame of a burner. After the electrodes had cooled sufficiently, they were washed with the solution whose potential was to be determined.

It should be emphasized that the ability to obtain oxidation-reduction potential values which could be reproduced depended to a great extent upon the type of electrodes used. In the preliminary work the author used platinum electrodes constructed of coiled wire or flags. These proved unsatisfactory since the values obtained could not be reproduced. It is the author's belief that in using the gauze electrodes such a large surface was exposed that equiligrium was obtained in a shorter period of time which reduced the detrimental effects on the electrode.

The manner in which the platinum electrode comes to equilibrium is of utmost importance. In the Chloramine-T solutions equilibrium was obtained usually within fifteen minutes. The solutions whose pH values were above seven required a longer time to reach equilibrium, and the potentials were more difficult to reproduce. Those solutions whose pH values were below seven usually reached equilibrium quite readily. The values of the oxidation potentials of the inorganic hypochlorites were more difficult to reproduce due to their higher pH values, and were therefore less reliable.

The cell assemblage was developed after considerable experimental work and was found to have the following advantages:

1. The pH or oxidation-reduction potential could be determined without disturbing the solution.

2. In the cases where considerable time was involved in obtaining a constant oxidation-reduction potential value, the pH could be redetermined to ascertain that it had remained constant.

3. It permitted a positive pressure of nitrogen to be maintained, thus eliminating the detrimental effects of carbon dioxide.[*]

[*] Remington and Trimble (27) have shown that slightly alkaline solutions of hypochlorites are very readily decomposed by the action of carbon dioxide of the air, with a marked increase in oxidizing power.

The nitrogen gas used to maintain an inert atmosphere was carefully purified and dried to remove traces of oxygen and carbon dioxide.

The solutions were prepared in 1000 c.c. or 500 c.c. volumetric flasks.which had been previously cleaned and dried. The air in the flasks was displaced by allowing nitrogen to sweep through the flask prior to introducing the weighed sample of the salt. Conductivity water, prepared by redistilling a good grade of distilled water to which had been added alkaline permanganate, was used in preparing the solutions.

In order to obtain a series of solutions of a given concentration with varying pH and oxidation-reduction potential values it was necessary to add either acid or base depending upon the original pH of the solutions. For this purpose a standardized acid (HCl) or base (NaOH) was employed.

The oxidation potentials and pH values presented throughout this work represent an average of at least three determinations. Each determination was carried out on a freshly prepared solution. No set of determinations was considered satisfactory if the oxidation potentials varied more than ± .002 volts.

The oxidation potential values as presented are stated with reference to the saturated calomel half cell. The tables include the oxidation potential as referred to the normal hydrogen electrode at zero.

The oxidation potentials indicated throughout this work are not absolute values, but may be considered comparable.

C. A STUDY OF THE INFLUENCE OF pH ON THE OXIDATION-REDUCTION POTENTIAL

1. Chloramine-T (para-toluene sodium sulphochloramide).

Leech (19) has measured the oxidation potential of Chloramine-T. He states that when one gram of Chloramine-T was added to 100 c.c. of a solution having a pH of 9, the voltage reading (using the platinum electrode connected with the positive pole of the galvanometer) was 0.12 volts. One gram of Chloramine-T dissolved in 100 c.c. of water, having a pH of 7 (the calomel cell was connected to the positive pole of the galvanometer), gave a voltage reading of 0.4. However, acidifying the solution increased the voltage very materially. The above values illustrate in somewhat of a qualitative manner the effect of pH on the oxidation potential of Chloramine-T.

The data as presented in Table III and illustrated graphically in Figure 1 represent in a qualitative manner the effect of pH on the oxidation potential of Chloramine-T at 50 p.p.m. (active). The temperature was maintained at 25° C. ± .05°. *

---

* The oxidation potential and pH values recorded in this work were determined at 25° C. ± .05°. Any deviation from this temperature is noted.

Table II

Oxidation Potential and pH Values of

Chloramine-T at 50 p.p.m. (active).

pH	Observed Oxidation Potential in volts	Oxidation Potential in volts (N.Hydrogen Electrode = 0)
9.56	0.3636	0.6094
8.28	0.5318	0.7776
6.87	0.7218	0.9676
6.56	0.7620	1.0078
5.80	0.8660	1.1118
5.36	0.8985	1.1443
5.01	0.9185	1.1643
4.50	0.9435	1.1693

It may be observed from Table II and Figure 1 that between the pH values of 9.56 and 5.80 the oxidation potential is a linear function of the pH. The slope of the line is (-7.50). Between the pH values of 5.80 and 4.50 a new relationship has been established. This change of slope may be accounted for by the fact that at a pH of 5.80 the <u>para</u>-toluene sulphonamide becomes insoluble and as a result separates out of solution. As the amide separates out, the solution becomes decidedly milky in appearance. The reaction may be stated:

$$RSO.ONa{:}NCl + HOH + HCl \longrightarrow RSO_2.NH_2 + HOCl + NaCl$$

The data presented in Table III and shown graphically

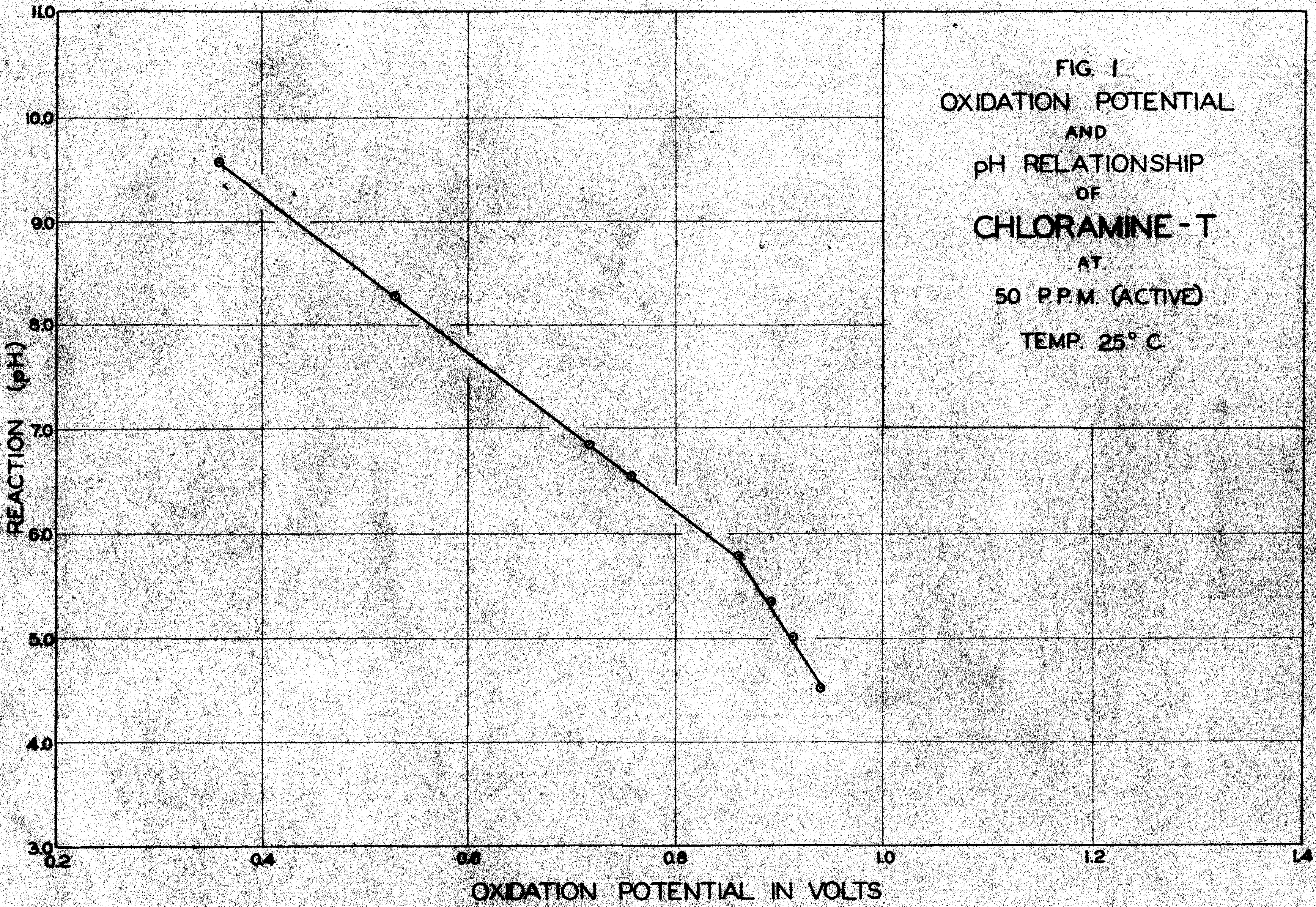


FIG. 1

OXIDATION POTENTIAL AND pH RELATIONSHIP OF CHLORAMINE-T AT 50 P.P.M. (ACTIVE)

TEMP. 25° C

in Figure 2 represent the pH and oxidation values for Chloramine-T at 500 p.p.m. (active).

Table III

Oxidation Potential and pH Values of Chloramine-T at 500 p.p.m. (active)

cubic centimeters of 0.1N NaOH or HCl added per one liter	pH	Observed oxidation potential in volts	Oxidation potential in volts (N.Hydrogen electrode)
1.06 NaOH	9.23	0.4261	0.6719
	8.64	0.5020	0.7478
0.66 HCl	6.99	0.7280	0.9738
1.09 HCl	6.74	0.7620	1.0078
2.18 HCl	6.40	0.8035	1.0493
3.27 HCl	6.20	0.8275	1.0733
3.36 HCl	6.02	0.8525	1.0983

At this concentration the oxidation potential is a linear function of the pH between the values of 9.23 and 6.02*. The slope of the line is (-7.48).

The values as presented in Table IV and Figure 3 indicate the pH and oxidation potential for Chloramine-T at 1000 p.p.m. (active).

* The pH values denoted throughout this work between which the linear relationship holds may not be the limiting values. For reasons to be stated later the author was primarily interested in the range of pH values between 9 and 6 in the case of Chloramine-T.

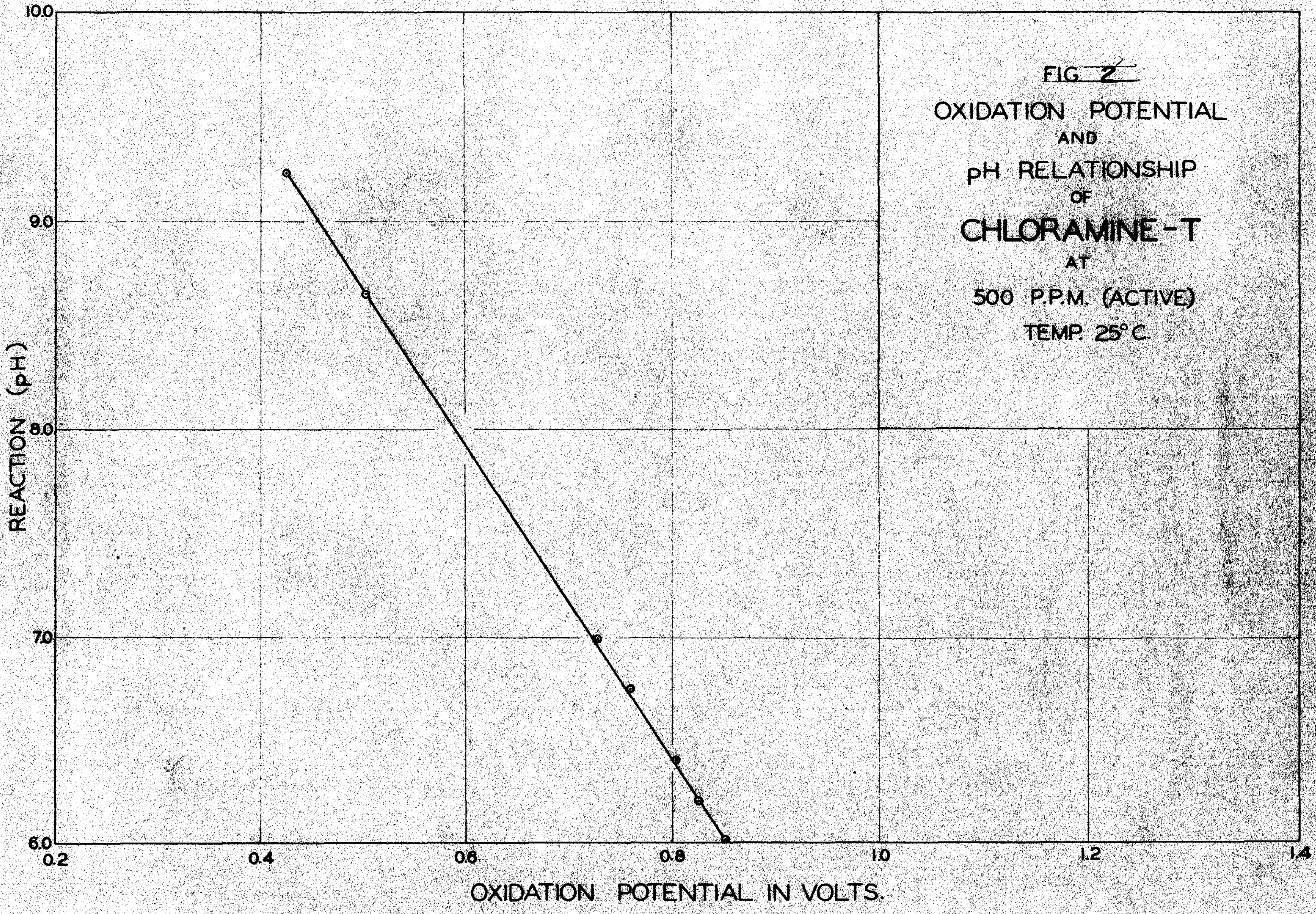


FIG 2

OXIDATION POTENTIAL
AND
pH RELATIONSHIP
OF
CHLORAMINE-T
AT
500 P.P.M. (ACTIVE)
TEMP. 25° C.

Table IV

Oxidation Potential and pH Values of Chloramine-T at 1000 p.p.m. (active)

cubic centimeters of 0.1 N NaOH or HCl added per liter	pH	Observed oxidation potential in volts	Oxidation potential in volts (N. hydrogen electrode).
0.21 NaOH	9.43	0.4085	0.6543
	8.78	0.4855	0.7313
0.87 HCl	7.51	0.6630	0.9088
1.31 HCl	7.17	0.7090	0.9548
2.20 HCl	6.74	0.7694	1.0152
4.36 HCl	6.38	0.8186	1.0644
6.55 HCl	6.20	0.8476	1.0934
10.91 HCl	6.00	0.8690	1.1148

The oxidation potential is observed to be a linear function of the pH between the values 9.43 and 6.00. The slope of the line has been calculated to be -7.34.

The observed values for the pH and oxidation potential of Chloramine-T at 2000 p.p.m. (active) are tabulated in Table V and shown graphically in Figure 4.

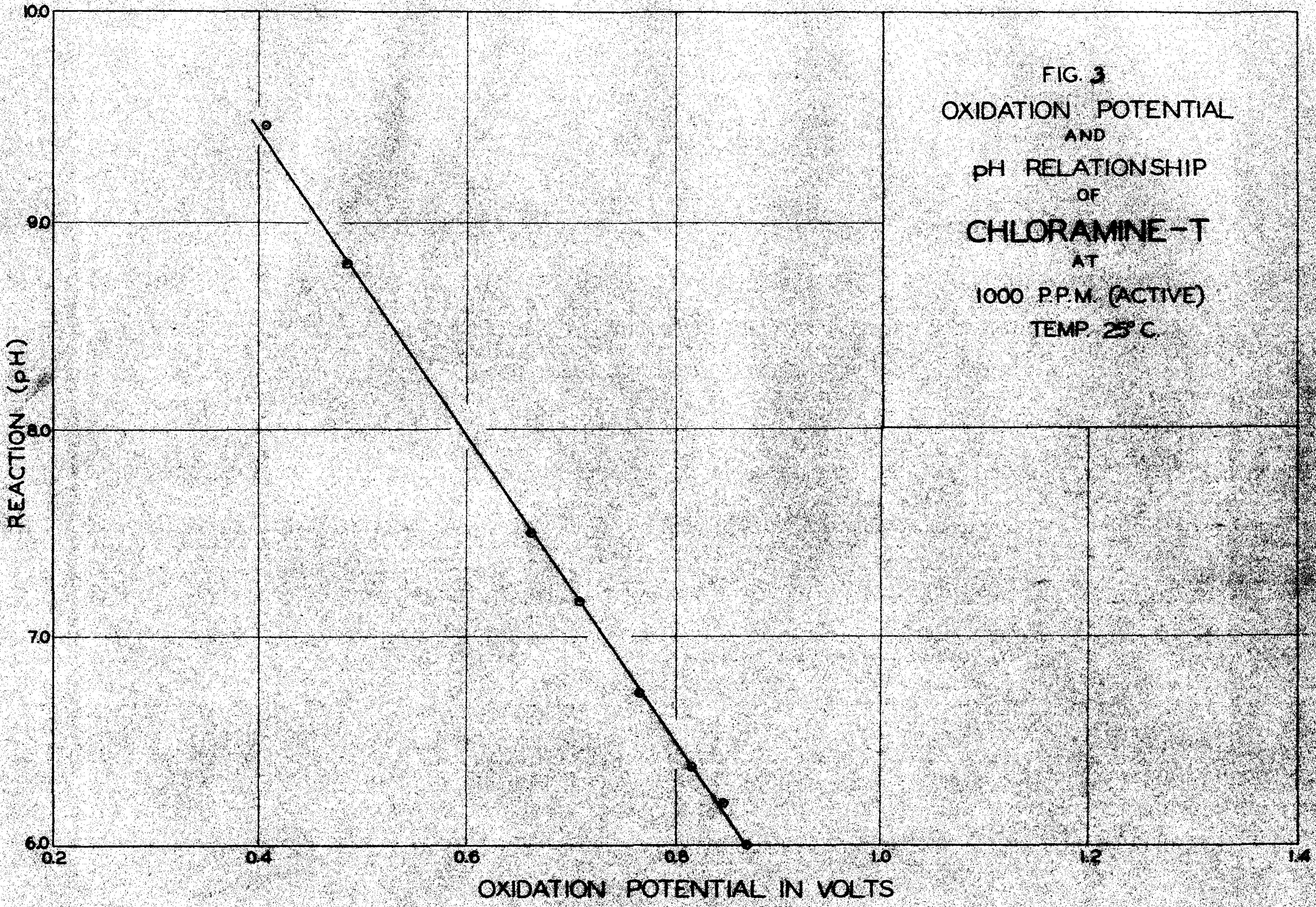


FIG. 3
OXIDATION POTENTIAL
AND
pH RELATIONSHIP
OF
CHLORAMINE-T
AT
1000 P.P.M. (ACTIVE)
TEMP. 25° C.

Table V

Oxidation Potential and pH Values of Chloramine-T at 2000 p.p.m. (active)

cubic centimeters of 0.1 N HCl added per liter	pH	Observed oxidation potential in volts	Oxidation Potential in volts (N. Hydrogen Electrode)
	8.80	0.4878	0.7336
2.18	7.33	0.6840	0.9298
4.36	6.80	0.7570	1.0028
10.91	6.18	0.8345	1.0803
17.46	6.05	0.8567	1.1025

The oxidation potential is observed to be a linear function of the pH between the values 8.80 and 6.05. The slope is calculated as -7.38.

It may be observed from Figures 1, 2, 3, and 4 that the oxidation potential in all concentrations is a linear function of the pH between certain limits. The average slope is expressed by the value -7.425. This slope then signifies that for each change of one pH unit there is an approximate change of 0.14 volts in the oxidation potential.

A study of Figures 1, 2, 3, and 4 brings out the fact that the oxidation potential is apparently independent of the concentration. In every oxidation reduction reaction two forces come into play, namely, an intensity factor and a capacity factor. The oxidation potential would be considered

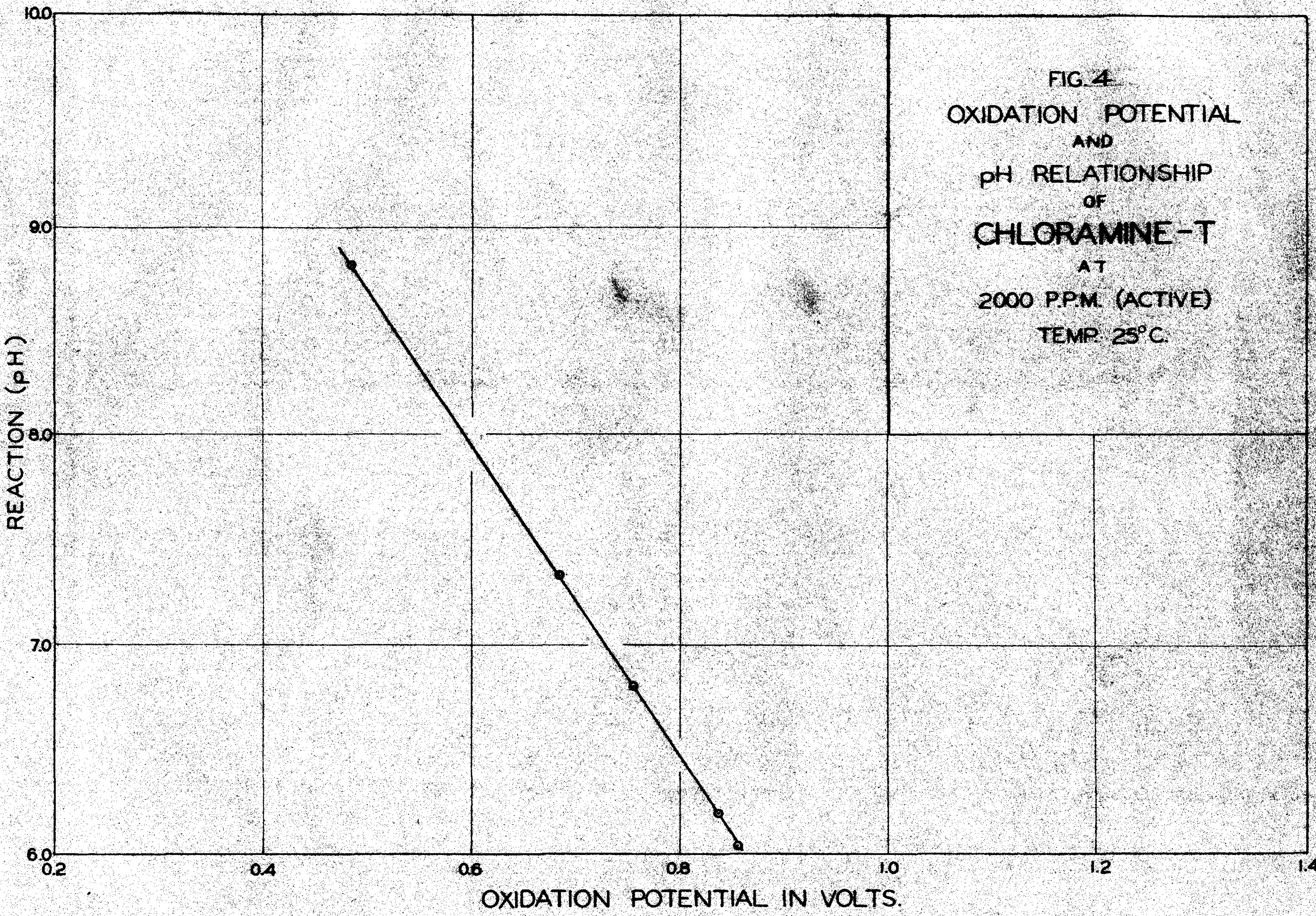


FIG. 4
OXIDATION POTENTIAL
AND
pH RELATIONSHIP
OF
CHLORAMINE-T
AT
2000 P.P.M. (ACTIVE)
TEMP. 25°C.

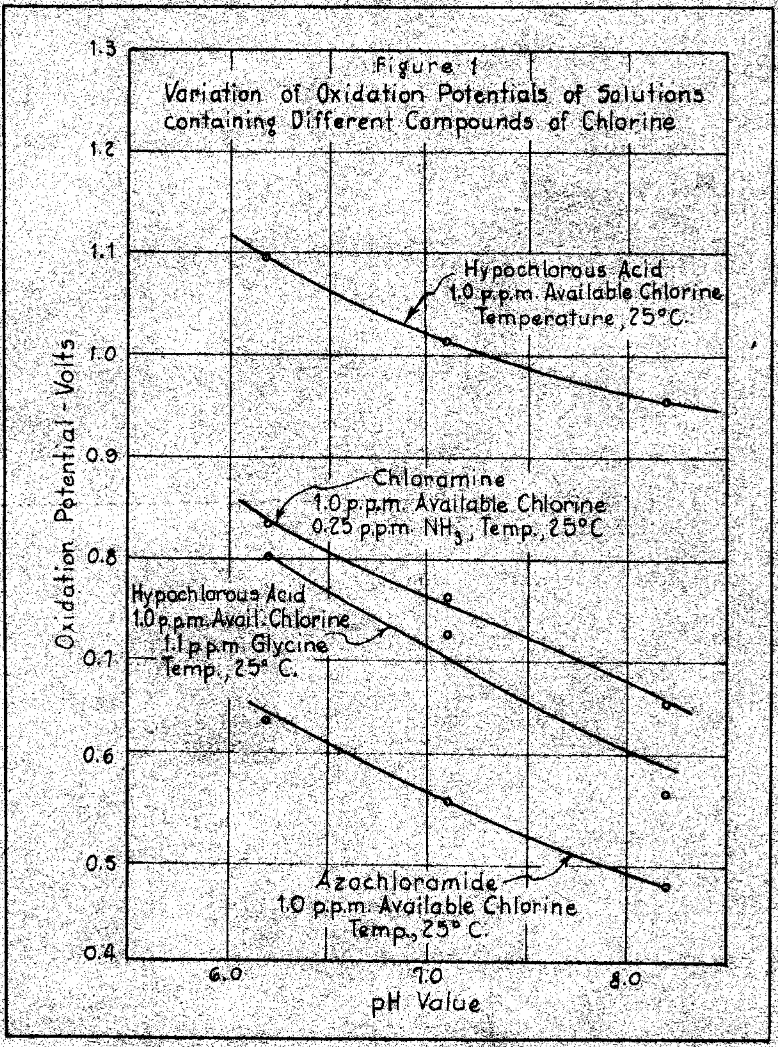
as the measure of the intensity factor as the concentration has little effect upon the values of oxidation potential. Since oxidation is a process in which a substance loses electrons, and reduction is a process in which a substance gains electrons, the oxidation potential may be considered as a measure of the intensity of the oxidant to lose electrons. With this consideration the oxidation potential signifies a tendency (intensity) and does not indicate the amount (capacity) of a given substance it can reduce.

In Figure 5 is presented a graph taken from a recent publication of Schmelkes (35). It shows the pH and oxidation potential relationship for hypochlorous acid ($HOCl$), chloramine (NH_2Cl), chlorinated glycine, and azochloramide. Due to the fact that no table of values is included nor any standard indicated for expressing oxidation potential values, comparison is difficult. The slopes as presented do not definitely show a linear relationship.

2. Chloramine-T in 0.5 M Sodium Chloride.

As previously stated it has been observed that chlorine is a much better oxidizing agent in a solution of potassium chloride than it is in a solution of potassium hydroxide. Ruis y Miro (31) has given the effects of various concentrations of sodium chloride, lithium chloride and potassium chloride on the oxidation potential of the chlorine electrode.

FIG 5



(After F.C.Schmelkes - J.A.W.A. Vol.25 - 657 - 1933)

King and Smith (18) have shown that chloramine (NH_2Cl) in water purification is as effective a germicide in the presence of 500 p.p.m. of salt as it is without. However, larger additions of salt (2000 p.p.m.) decrease the germicidal efficiency.

The author has determined the pH, oxidation potential relationship for Chloramine-T at 1000 p.p.m. (active) in the presence of 0.5 molar sodium chloride.

The experimental values are given in Table VI. Figure 6 shows these values graphically.

Table VI

Oxidation Potential and pH Values of

Chloramine-T at 1000 p.p.m. (active)

pH	Observed Oxidation Potential in volts	Oxidation Potential in volts (N. Hydrogen Electrode)
7.92	0.5506	0.7964
6.32	0.7700	1.0158
6.07	0.8025	1.0483

It may be observed by referring to Tables 5 and 7 that the oxidation potential has been decreased in the presence of sodium chloride; that is, a solution of the same concentration, at the same pH and temperature has a lower oxidation potential in 0.5 molar sodium chloride.

However, if the same amount of standard HCl were added which normally would produce a pH of 6 with Chloramine-T

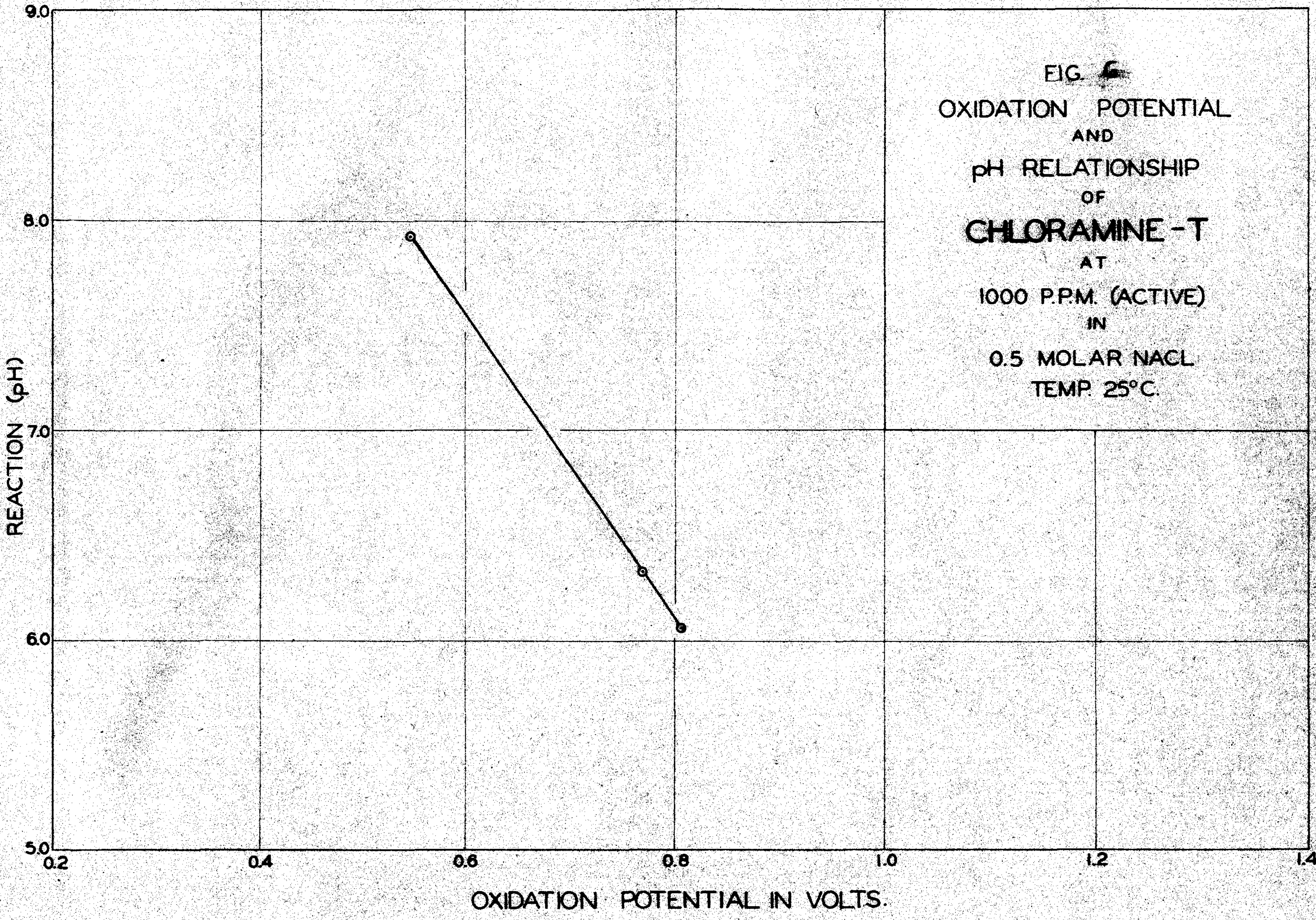


FIG. 6
OXIDATION POTENTIAL
AND
pH RELATIONSHIP
OF
CHLORAMINE - T
AT
1000 P.P.M. (ACTIVE)
IN
0.5 MOLAR NACL
TEMP. 25°C.

at 1000 p.p.m. (active) in the 0.5 molar sodium chloride solution, the pH would be 5.27; that is, by adding sodium chloride a lower pH is obtained and, as a result, a higher oxidation potential.

This concept of the effect of sodium chloride may be augmented by the results of Remington and Trimble (27). They state as follows.

> "The results of our work with buffer solutions are given in Tables III and IV and shown graphically in Figure 4*. The concentration of HOCl in these buffered solutions was in every case 0.00102 grams per cubic centimeter.

Table III

Variation of Potential with Ph with

Sorenson's Phosphate Mixture

pH	Cell Potential	Oxidation Potential	pH	Cell Potential	Oxidation Potential
5.29	0.934	1.272	7.16	0.820	1.158
5.59	0.912	1.250	7.73	0.798	1.135
6.24	0.873	1.211	8.04	0.790	1.127

Table IV

Variation of Potential with pH with Auerbach

and Pick's Carbonate Mixture

pH	Cell Potential	Oxidation Potential	pH	Cell Potential	Oxidation Potential
8.35	0.777	1.115	10.10	0.623	0.961
8.90	0.723	1.061	10.65	0.584	0.922
9.15	0.695	1.033	11.59	0.546	0.884

We are at a loss to explain adequately the discontinuity of the curves as found with phosphate and with carbonate buffer mixtures. Certainly the small quantity of hypochlorous acid added could not shift the hydrogen ion concentration of the buffer mixtures in such manner as to give these discrepancies. We believe that the reason for this failure to give a continuous curve lies in some intrinsic difference in the action of phosphate and carbonate mixtures; that is, in a specific 'salt effect'."

The above work has been included as it illustrates that the oxidation potential is influenced by the presence of the other salts. Therefore, the oxidation potential should be measured in a water solution rather than in a solution of buffers.

This point may be of practical value in that the addition of various salts may increase or decrease the oxidation potential of active chlorine compounds, and thus alter their efficiency as bleaching agents or as sterilizing media.

3. Calcium Hypochlorite.

The following oxidation potential and pH relationships are those obtained by a study of the inorganic type of hypochlorites.

In Table VII are stated the experimental values and these values for calcium hypochlorite at 10 p.p.m. (active) are shown graphically in Figure 7.

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* Their Figure 4 has not been included as the values expressed in the tables are significant.

Table VII

Oxidation Potential and pH Values of Calcium Hypochlorite at 10 p.p.m. (active)

cubic centimeters of 0.1 N HCl added per liter	pH	Observed Oxidation Potential in volts	Oxidation Potential in volts (N. Hydrogen Electrode)
	9.81	0.6340	0.8798
1.09	8.21	0.7645	1.0103
2.18	7.24	0.8310	1.0768
2.84	6.51	0.8855	1.1313
3.27	6.46	0.8908	1.1366

Between the pH values of 9.81 and 6.46 the linear relationship* of the oxidation potential and pH apparently holds. The slope of the line is calculated to be -13.85.

The values for 50 p.p.m. (active) may be observed from Table VII and Figure 8.

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* In Tables VIII and IV are included the pH and oxidation potential of the solutions as made from the salt without the addition of acid. These values were not included in the respective figures as they deviate from the linear function.

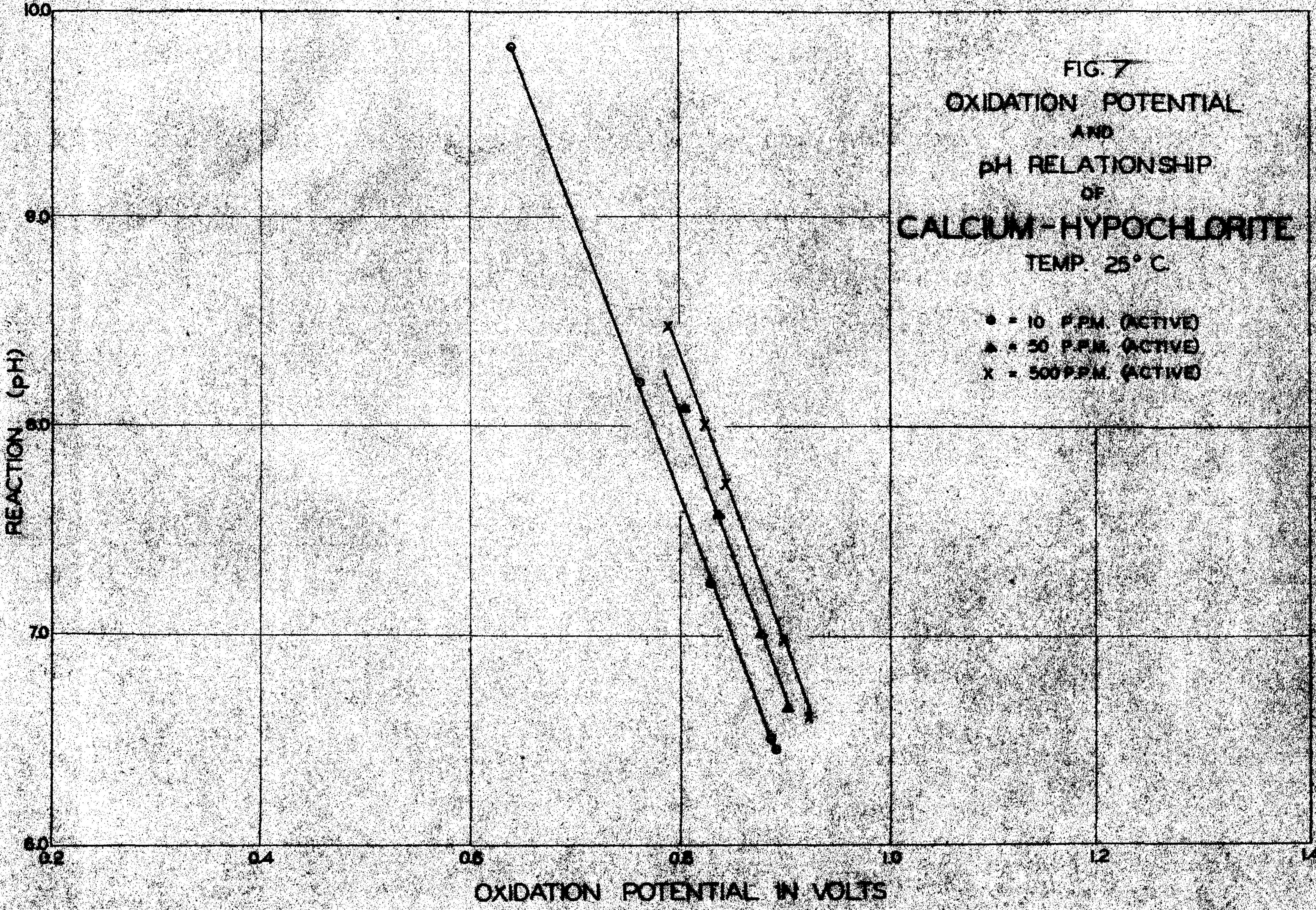

FIG. 7
OXIDATION POTENTIAL
AND
pH RELATIONSHIP
OF
CALCIUM - HYPOCHLORITE
TEMP. 25° C

Table VIII

Oxidation Potential and pH Values of Calcium Hypochlorite at 50 p.p.m. (active)

cubic centimeters of 0.1 N HCl added per liter	pH	Observed oxidation potential in volts	Oxidation Potential in volts (N. Hydrogen Electrode = 0)
	10.45	0.6170	0.8628
6.55	8.10	0.8050	1.0508
9.82	7.57	0.8350	1.0808
14.18	7.00	0.8775	1.1233
16.37	6.65	0.9000	1.1458

The linear relationship* is shown to hold between the pH values of 8.10 and 6.65. The slope of the line is -15.17.

Table IX shows the experimental values for calcium hypochlorite at 500 p.p.m. (active). The values are shown graphically in Figure 7.

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* The pH values signifying the linear relationship may not be the limiting values. The pH values were brought into the range in which they could be compared with Chloramine-T.

Table IX

Oxidation Potential and pH Values of Calcium Hypochlorite at 500 p.p.m. (active)

cubic centimeters of 0.1 N HCl added per liter	pH	Observed Oxidation potential in volts	Oxidation Potential in volts (N. Hydrogen Electrode = 0)
	11.20	0.6065	0.8521
13.09	10.96	0.6165	0.8623
54.55	8.48	0.7910	1.0368
65.46	6.06	0.8200	1.0658
87.28	7.72	0.8430	1.0888
141.83	6.99	0.8968	1.1426
163.65	6.59	0.9220	1.1678

The linear relationship is shown to hold between the pH values of 8.48 and 6.59 with a slope of -14.08.

The curves as shown in Figure 7 illustrate the fact that the linear relationship between pH and oxidation potential holds for calcium hypochlorite between the limits as stated. The average slope is calculated as -14.37. The slope then signifies that for each change of one pH unit there is an approximate change of 0.70 volts in the oxidation potential.

It has been shown in Figures 1, 2, 3, 4, and 6 for Chloramine-T that the oxidation potential varied but little with the concentration. However, in the curve for calcium hypochlorite, Figure 7, a gradual change is noted in proceeding from 10 p.p.m. (active) to 500 p.p.m. (active). This fact might be explained as being due to the relatively large amounts of acid which must be added to neutralize the excess calcium hydroxide which is in suspension. If one refers back to the analysis of calcium hypochlorite as stated in section "A", it will be obvious that the percentage of inert material is large. This inert material may also have an effect on the oxidation potential as the concentration is increased.

4. Sodium Hypochlorite.

The influence on the oxidation potential of sodium hypochlorite by the addition of hydrochloric acid has been noted by Remington and Trimble (27). They state that the oxidizing power of hypochlorites in solution as measured indirectly by potentiometric methods increases with acidity, that is, with increase in hydrogen ion concentration.

The data in Table X which are expressed graphically in Figure 8 present the values for sodium hypochlorite at 50 p.p.m. (active).

Table X

Oxidation Potential and pH Values of Sodium Hypochlorite at 50 p.p.m. (active).

pH	Observed Oxidation Potential in volts	Oxidation Potential (N. Hydrogen Electrode = 0)
9.20	0.7159	0.9617
7.34	0.8336	1.0794
6.64	0.8785	1.1243
6.20	0.9099	1.1557

It is apparent from Table X and Figure 8 that the oxidation potential is a linear function of the pH between the values of 9.20 and 6.20. The slope of the line is calculated to be a -14.94. Thus, for each change of one pH unit we have a corresponding change of 0.0$\overset{?}{6}$47 volts in the oxidation potential.

It may be observed that the sodium hypochlorite at 50 p.p.m. has practically the same slope as calcium hypochlorite at the same concentration. The oxidation potential values of sodium hypochlorite are lower than for the corresponding calcium salts, however, the difference is not large.

The fact then becomes evident that the two compounds having the general formula OCl M are similar in their action in regard to oxidation potential values.

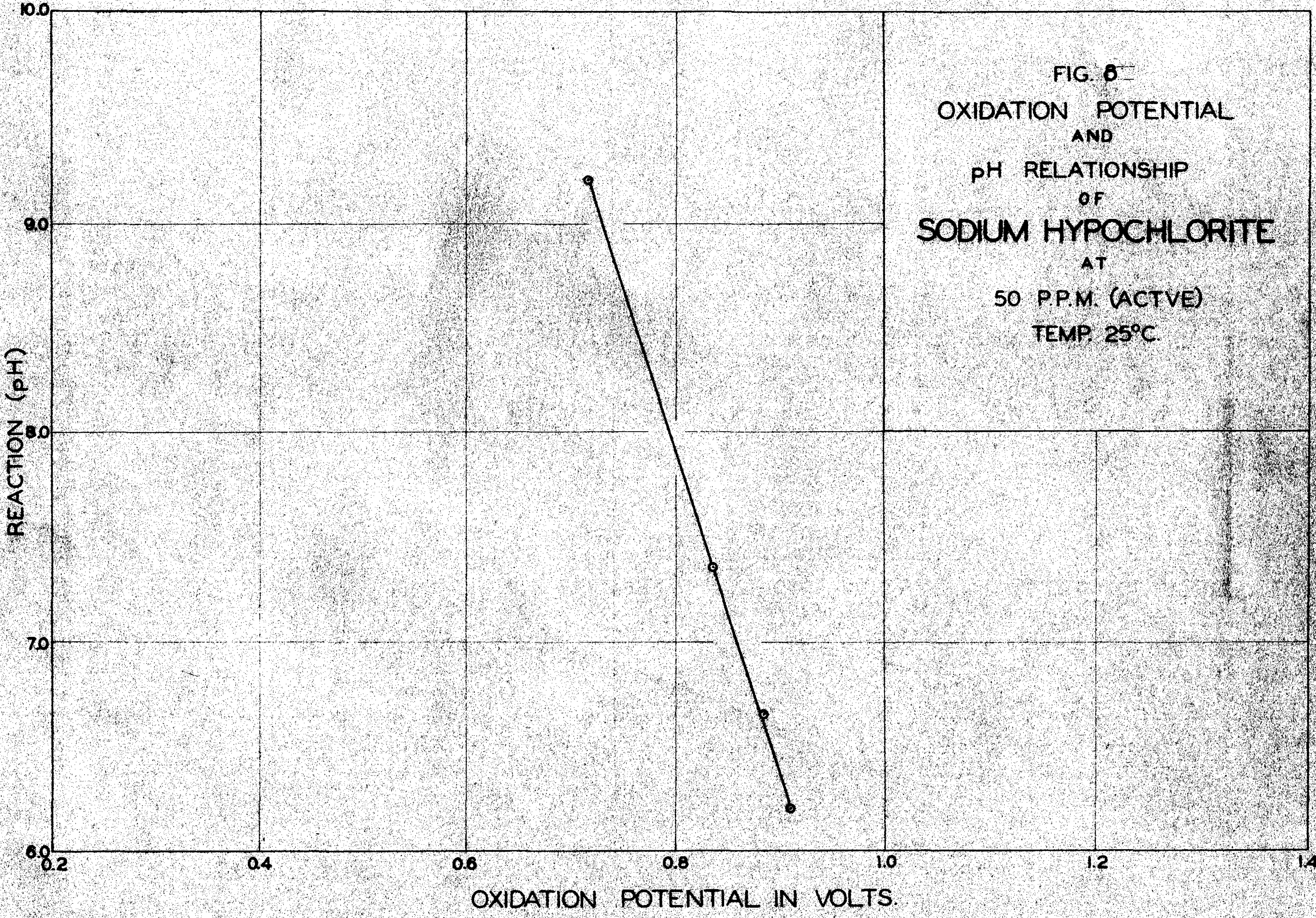

FIG. 8
OXIDATION POTENTIAL
AND
pH RELATIONSHIP
OF
SODIUM HYPOCHLORITE
AT
50 P.P.M. (ACTVE)
TEMP. 25°C.

5. *para*-Toluene Sulfonamide.

The pH oxidation-potential relationship for *para*-toluene sulfonamide* is given in Table XI. The values are shown graphically in Figure 9.

Table XI

Oxidation-Reduction and pH Values of

para-Toluene Sulfonamide at 248 mg. per liter

pH	Observed Oxidation-Reduction Potential in volts	Oxidation-Reduction Potential in volts (N.Hydrogen Electrode=0)
6.01	0.3425	0.5883
7.02	0.2666	0.5124
8.54	0.1620	0.4078
8.95	0.1272	0.3730
9.24	0.1005	0.3463
9.58	0.0825	0.3283

The linear relationship between pH and oxidation potential apparently holds between the values of 9.58 and 6.01.

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* The concentration of 248 mg. per liter was used as it represented the quantity of amide added in preparing a 50 p.p.m. solution of Chloramine-T.

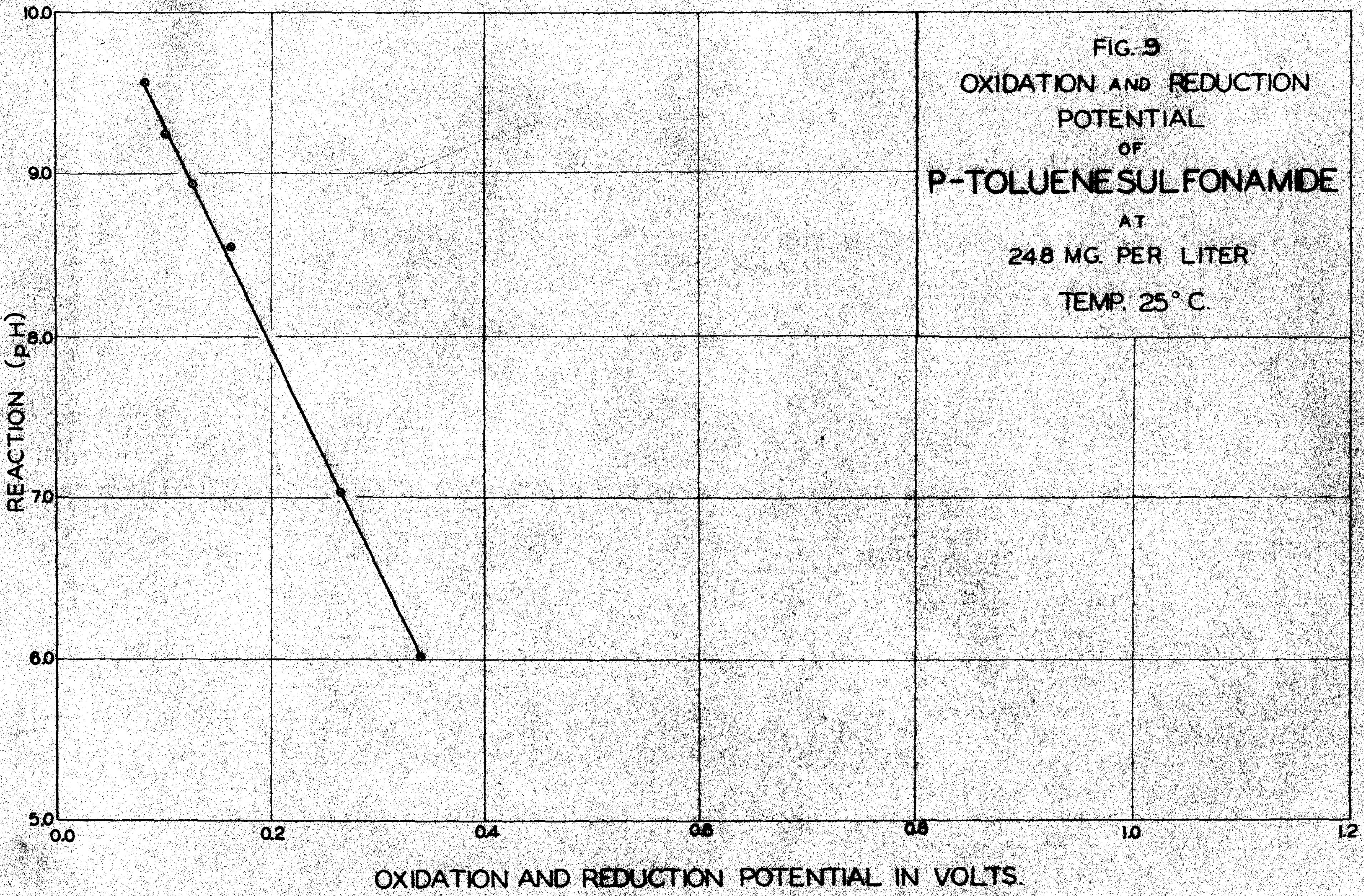

FIG. 9
OXIDATION AND REDUCTION
POTENTIAL
OF
P-TOLUENESULFONAMIDE
AT
248 MG. PER LITER
TEMP. 25° C.

The summary curves in Figure 10 show in a quantitative manner the difference in oxidation potentials of the various compounds at 50 p.p.m. (active).

Figure 10 indicates clearly that the trend of the chloramine and inorganic hypochlorite curves is different. The difference in slope is especially noticeable. The following is suggested as an explanation for the difference in slope of the two compounds.

In the case of the inorganic hypochlorites we have the positive chlorine (Cl+) linked through oxygen to a metal. Ephrain (10) has described an electromotive series of the elements indicating quantitatively their affinity for electric charges. The quantitative values expressed in volts were produced by electrodes of the various elements placed against the normal hydrogen electrode. He has arranged these elements so that the affinity for negative charges increases progressively in passing from the top to the bottom of the series. The tendency to form positive ions in solution, therefore, decreases in this same respect. He has placed potassium at the head with a potential of -2.56 volts and calcium sixth with a potential of -1.88 volts, while the series is terminated with chlorine at +1.36 volts and fluorine at +1.9 volts.

Hence, it may be stated that the chlorine atom has a greater tendency for negative charges than does the calcium. In the compound, calcium hypochlorite, the electrons (negative charges) will be more closely attached to the chlorine (due

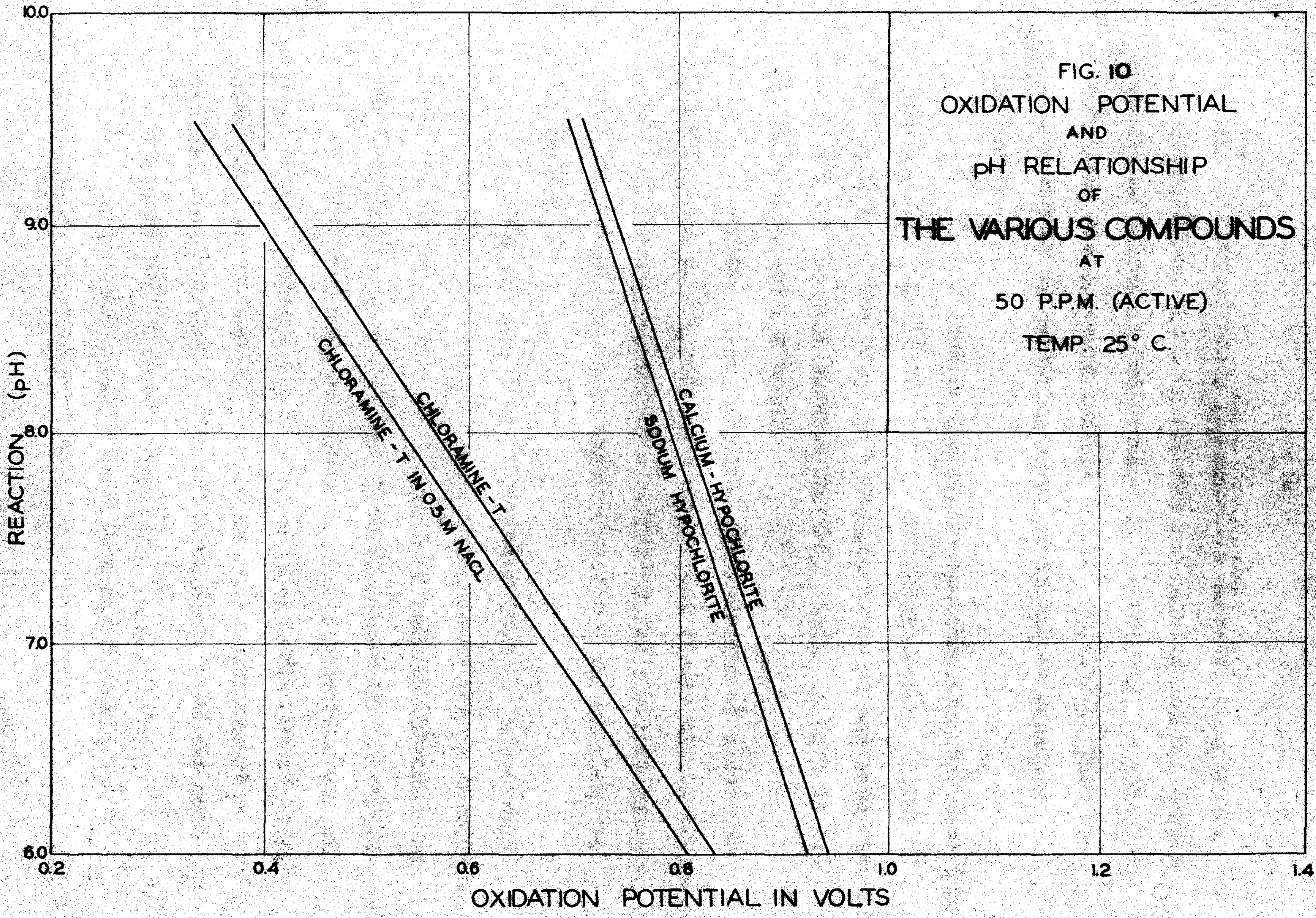

FIG. 10
OXIDATION POTENTIAL
AND
pH RELATIONSHIP
OF
THE VARIOUS COMPOUNDS
AT
50 P.P.M. (ACTIVE)
TEMP. 25° C.

to its greater affinity for negative charges) than to the calcium. Thus, the bond between the calcium and the oxygen should be materially weakened. As the $Ca(OCl)_2$ is placed in solution, the compound ionizes as $Ca^{++} + 2(OCl)^-$. This ion cannot act as an oxidizing agent as it has an unpaired electron.

In the case of the chloramine compounds the positive chlorine (Cl^+) is linked in the following manner: R:NCl. The ability of the (Cl+) to hydrolyze is dependent upon the strength of the bond between the N-Cl, which in turn is dependent upon the R:N bond.

If the value, -0.5124, (N. hydrogen at zero) for para-toluene sulfonamide at a pH of 7.02 (Table XI) is assumed to represent the negativity of affinity for positive charges, the bond between nitrogen and the positive chlorine should be strong.

From Figure 4 it is observed that the negativity (affinity for positive charges) decreases as the pH decreases. That is, as the pH decreases, the bond between nitrogen and the chlorine becomes weaker. This statement is emphasized by observing the summary Figure X. It is probable that if the pH of Chloramine-T and calcium hypochlorite could be decreased low enough the two curves would converge at a point. In other words, each solution would have the same oxidation potential at the same pH.

The importance of this fact may be noted by reference to Figure 5, Schmelkes (35). This shows that as the (R) groups proceed from the simple chloramine to the complex azochloramide, the potential becomes considerably less. If this point is explained on the basis of the negativity of the (R) group, which governs the strength of the nitrogen-chlorine linkage, the potential will be a resultant of the strength of the nitrogen-chlorine linkage. This linkage is dependent upon the negativity of the (R) group.

This is suggested as an explanation as to why the chloramines are not active oxidizing agents.

D. A METHOD OF CALCULATING THE pH or OXIDATION-REDUCTION POTENTIAL FROM THE DATA PRESENTED

The advent of the glass electrode has been a very recent development and as a result, is not to be found in a great many chemical laboratories. It was deemed essential that a method be developed for calculating the pH of a given solution when the oxidation potential is known. The oxidation potential value may be obtained with sufficient accuracy by using the usual electrometric hydrogen-ion equipment with a few minor changes.

It may be observed from the figures showing the pH and oxidation potential relationship that these values are a linear function between certain limits of pH values. Thus is furnished the opportunity of developing an equation for calculating the pH, the oxidation potential, and the slope of the curve being known.

Assuming the general equation for determining the slope of the line as given by Daniels (8).

$$y = mx + b \qquad (1)$$

"y" = the ordinate expressing pH

"x" = the abscessia expressing oxidation-reduction potential designated by "E"

"m" = the slope

Substituting the above notations into equation (1)

$$pH = mE + b$$

Equation (2) represents the general equation for calculating pH from the oxidation-reduction value and the value of the slope of the line.

In order to illustrate the usefulness of the general equation the following sample calculation is made using the data of Chloramine-T at 500 p.p.m. (active).

Take two points on the line as expressed in Figure where a pH of 8.64 = oxidation potential of 0.5020 volts and a pH of 6.02 is equal to an oxidation potential of 0.8525 volts and substituting these values into equation (1) to determine the slope of the line,

$$\begin{array}{l} 8.64 = m\,(0.5020) + b \\ \underline{6.02 = m\,(0.8525) + b} \end{array}$$

$$\begin{array}{l} b = 8.64 - 0.5020\,m \\ \underline{b = 6.02 - 0.8525\,m} \end{array}$$

$$\begin{array}{lcl} 8.64 - 0.5020\,m & = & 6.02 - 0.8525\,m \\ \underline{8.64 - 6.02} & \underline{=} & \underline{0.5020\,m - 0.8525\,m} \\ 2.62 & = & .3505\,m \\ & = & m \end{array}$$

$$-2.62 = 0.3505\ m$$

$$m = \frac{-2.62}{0.3505} = -7.48 \text{ slope}$$

Therefore,

$$8.64 = (-7.48)(0.5020) + b$$
$$b = 8.64 + (-7.48)(0.5020) = 12.395$$

The general equation to calculate the pH for Chloramine-T at 500 p.p.m. (active) is stated as follows:

$$pH = -7.48\ (E) + 12.395 \qquad (3)$$

This equation may be used to calculate the pH of any solution of Chloramine-T (500 p.p.m. active) provided the values are within the limits through which the pH and oxidation potential are a linear function. These limits may be observed from Figure 2.

The general equation (2) offers an accurate method of calculating the pH when the oxidation potential is available.

In the event that the pH of a given solution were known or could be determined the oxidation potential may be calculated. It was found convenient throughout this work to determine the oxidation potential of a solution at a given pH.

The general equation may be stated:

$$E = \frac{pH - b}{m} \qquad (4)$$

Substituting the values as taken from equation (3)

$$E = \frac{pH - 12.395}{-7.48} \qquad (5)$$

The equation represents the general equation from which the oxidation potential of any solution of Chloramine-T (500 p.p.m. active) may be calculated provided the pH values fall within the limits which produce a linear function of the pH and oxidation potential.

This equation would have practical application in such industries as laundries. In this case the chlorine is added to destroy microörganisms. However, the oxidizing power, as indicated by the oxidation potential, should not be sufficiently high to bleach colored material.

In Table 12 is given the slopes of the pH oxidation potential relationship curves for the various compounds.

Table 12

Slopes Calculated from the pH Oxidation-Potential Relationship of the Various Compounds

Compounds	Concentration p.p.m. active	Slope
Chloramine-T	50	- 7.50
Chloramine-T	500	- 7.48
Chloramine-T	1000	- 7.34
Chloramine-T	2000	- 7.41
Chloramine-T in 0.5 M NaCl	50	- 7.38
Sodium Hypochlorite	50	-14.94
Calcium Hypochlorite	10	-13.85
Calcium Hypochlorite	50	-15.17
Calcium Hypochlorite	500	-14.08

This table gives the slope values so that equations (2) and (4) may be used.

E. A COMPARISON OF THE OXIDATION-POTENTIAL WITH GERMICIDAL EFFICIENCY*

The first suggestion as to a possible connection between the germicidal properties of chlorine and the oxidation potential was made by Rideal and Evans (30). Leech (19) stated the concept that the oxidation potential and the bacterial coefficient might be in a direct ratio. Schmelkes (36) has stated that measurements were presented of the oxidation potential in solutions of hypochlorous acid, chloramine, monochloroglycine, and azochloramide. These measurements, he states, were compared with measurements of the rate of bacteria removal. The oxidation potential and bactericidal properties of chlorine were found to run parallel. He states that any changes which diminish bactericidal velocity also diminish the oxidation potential and vice versa.

The following evidence is given to test the assumption that the killing time and oxidation potential are linear functions. Table 13 gives the data showing the oxidation potential of Chloramine-T solutions at 1000 p.p.m. (active) and the time necessary to kill 99 per cent of the bacterial spores. In Figure 11 the data are presented graphically.

---

* The killing times as reported in this section are from the data of Mr. D. B. Charlton (8).

FIG. 11

RELATIONSHIP OF THE OXIDATION POTENTIALS OF CHLORAMINE-T (1000 P.P.M. ACTIVE) AND THE TIME TO KILL 99% OF BACTERIAL SPORES

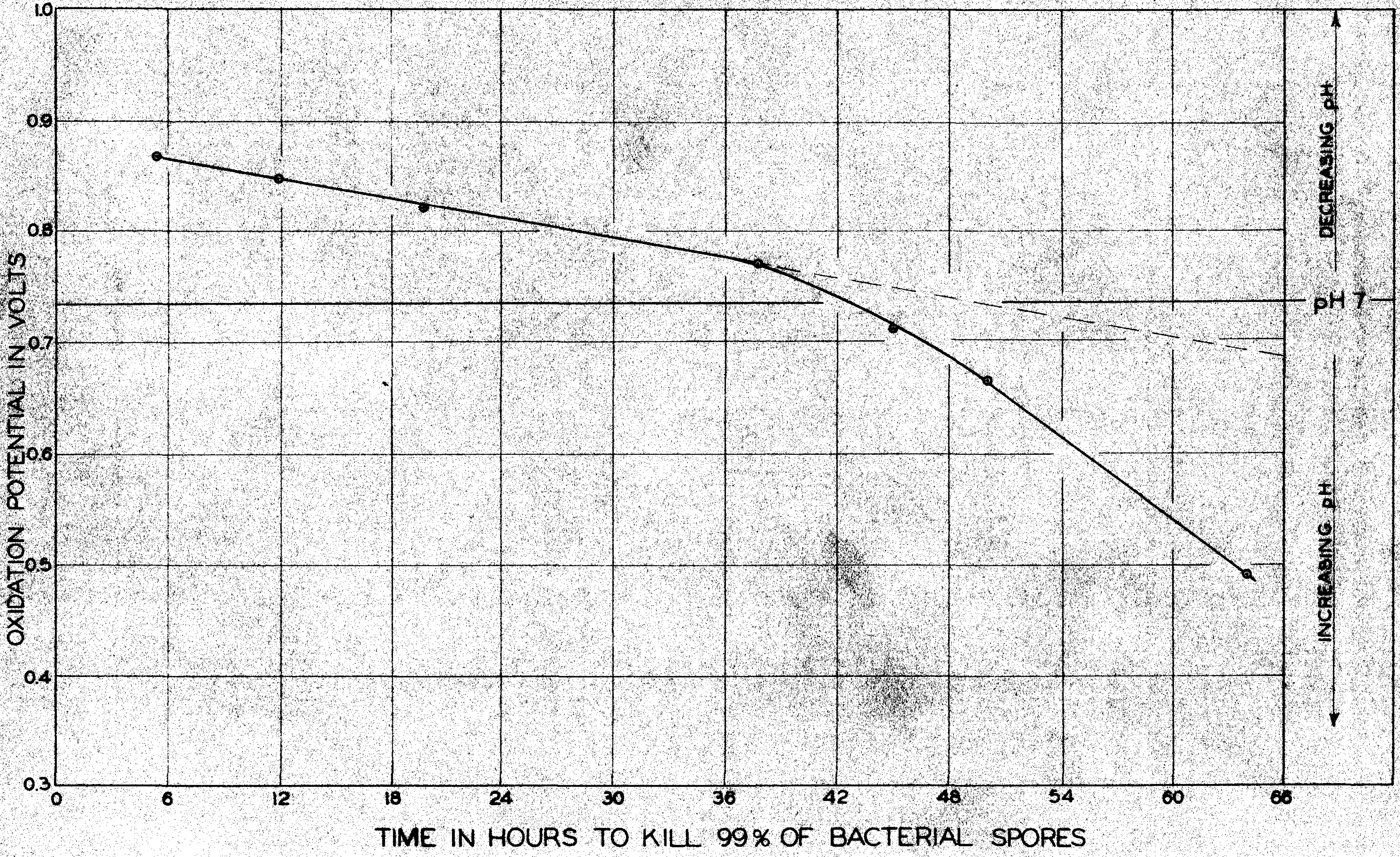

Table XIII

Influence of pH and Oxidation Potential Values
of Chloramine Solutions (1000 p.p.m. active)
upon Time Required to Kill 99 o/o Bacterial Spores

pH	Oxidation Potential in volts	Killing time in hours - 99 per cent of Spores
6.0	0.8690	5.4
6.20	0.8476	12.0
6.38	0.8186	19.8
6.74	0.7694	37.8
7.17	0.7090	45.0
7.51	0.6650	50.0
8.78	0.4855	64.0

It is evident from Figure 11 that between the oxidation potential of 0.8690 and 0.7694 the time to kill 99 per cent of the bacterial spores is a linear function of the oxidation potential. However, as an oxidation potential of 0.7090, which is on the alkaline side of the pH range, the relationship as noted on the acid side fails to hold. It was noted in the experimental work that the pH of the solution (and thus the oxidation potential) became more acid in the relatively long time necessary to kill 99 per cent of the bacterial spores. Consequently the oxidation potential was actually higher at the end of the experiment than at the beginning. Thus the killing time was shorter than it should have been assuming the potential as it was at the beginning.

The broken line on Figure 11 shows the line on which the point should fall, assuming that the potential did not change, and as a result the killing time would be a linear function of the oxidation potential on the alkaline side of the pH range.

The cause for the apparent drop in pH (and oxidation potential) may be primarily due to the effects of carbon dioxide from the air* on the solutions which are so weakly buffered.

Two identical series of solutions of Chloramine-T at 1000 p.p.m. were prepared in order to determine the effect of air on the pH of the solutions. Table XIV shows the change in pH of various solutions with time when stored tightly stoppered in the dark (to eliminate light effects) at a temperature of approximately 25°C.

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* Mr. D. B. Charlton (3) has stated in a discussion of technique used that the solutions were mechanically rotated prior to removing samples for bacterial analysis. This would greatly facilitate carbon dioxide absorption.

Table XIV

Influence of Storage on Chloramine-T

at Various pH Values in a Closed System

Time in days	pH	Concentration	pH	Concentration	pH	Concentration	pH	Concentration
0	9.27	1010	8.53	1009	6.74	1009	6.10	1008
5	9.27		8.50		6.76		6.14	
12	9.26	1010	8.32	1009	6.76	1009	6.15	1007
34	9.27		8.23		6.86		6.18	
43	9.16	1009	8.10	1009	6.88	1009	6.18	1007

The data illustrate that the decrease in pH was evident but to a very small degree.

In Table XV is presented like data excepting that the solutions were stored in volumetric flasks closed by a short funnel loosely packed with cotton.

Table XV

Influence of Storage on Chloramine-T

at Various pH Values in an Open System

Time in days	pH	Concentration	pH	Concentration	pH	Concentration	pH	Concentration
0	9.52	1005	8.88	992	6.80	1003	6.18	1010
6	9.20		7.82		6.88		6.24	
14	8.20	1008	7.42	992	6.88	1001	6.24	
33	7.53	1007	7.27	992	6.84	1005	6.21	1010

In Table XV it is evident that the alkali solutions have decreased nearly to the neutral point. Thus, the oxidation potential would be greatly increased and the bactericidal properties entirely changed.

As has been stated previously the oxidation potential must be primarily considered as an intensity factor. It does not indicate the amount of a substance which can be reduced.

It has been shown that the oxidation potential for Chloramine-T at various concentrations is practically the same at any given pH. Table XVI gives the effect of various concentrations at the same pH and of oxidation potential on the killing time.

Table XVI

Time to Kill 99 per cent Bacterial Spores

at various Concentrations and same pH

Concentration Chloramine-T p.p.m. (active)	pH	Time to kill 99 per cent bacterial spores in hrs	Concentration Chloramine-T p.p.m. (active)	pH	Time to kill 99 per cent bacterial spores in hrs.
500	6.0	14.8	500	6.4	43.4
1000	6.0	5.4	1000	6.4	19.8
2000	6.0	2.6	2000	6.4	8.0

This table shows the significance of the oxidation potential as an intensity factor. In other words, the oxidation potential can only be considered as an index of germicidal efficiency in a series of solutions of one type and at a given concentration.

It was originally the author's concept that the two types of chlorine compounds, namely, the chloramines and the inorganic hypochlorites, might be compared bacteriologically on the basis of oxidation potential. However, in the experimental work of Mr. D. B. Charlton (3) the data showed that the spores were killed by calcium hypochlorite so quickly that even in the extreme alkaline ranges comparison is made only with difficulty. For comparison, we may take a solution of calcium hypochlorite at 500 p.p.m. (active having an oxidation potential of 0.6063 and a killing time - 99 per cent- in 64 minutes). A solution of Chloramine-T at the same concentration of 500 p.p.m. (active) and with the same oxidation potential of 0.6063 has a killing time (99 per cent) of approximately twelve hours. It is then obvious that the two solutions with the same oxidation potentials do not have the same germicidal efficiency.

In conclusion we believe that the oxidation potential can only be used as a guide to germicidal efficiency if the same compound is used at the same concentration and temperature. The only variables possible are pH and oxidation potential.

F. A PROPOSED EXPLANATION AS TO THE MANNER IN WHICH OXIDATION POTENTIAL MAY BE TAKEN AS A GUIDE TO GERMICIDAL EFFICIENCY.

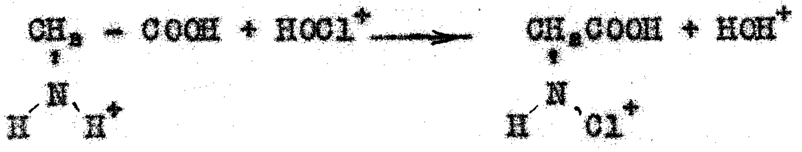
It is not intended to enter into a general discussion of the various explanations that have been proposed with regard to the action of chlorine in killing a cell. However, an explanation as to the manner in which the oxidation potential may be a guide to germicidal efficiency would be of interest. For this purpose the difference in the germicidal efficiency of the two types of chlorine compounds will be presented.

Schmelkes (35) has stated in a recent publication as follows:

> "We believe that the basis for sterilization is a chemical reaction. The cellular structure of microörganisms consists of a cell wall and an internal cell fluid. Both these materials contain protein, which in turn is composed of amino acids, which in turn contain amino groups. These amino groups will react with chlorine. Chloramines are formed by the reaction between these groups and chlorine, whereby the properties of the protein, particularly the colloidal properties, must be profoundly altered. The proteins probably coagulate and cannot discharge their normal biological functions in the life of the cell. If a sufficiently large part of the cell is affected in this manner the cell dies. If it is only affected to a small extent, it very probably is capable of sloughing off the affected part and to recover."

Other authors such as Dakin (6), Wright (44), and Chapin and Tilley (41) have observed that chlorine may replace the hydrogen atom of such compounds as amino acids, proteins, and certain organic amines.

Let us assume that the germicidal action is a resultant of replacing the hydrogen of an amino group by chlorine. Then the action of an active chlorine compound is not that of oxidation, but one of replacement. The hydrogen atom has been replaced by a chlorine and no oxidation has taken place as there has been no valence change. The following equation will illustrate this point.



$$\underset{\underset{H\;\;\;H^+}{N}}{CH_2} - COOH + HOCl^+ \longrightarrow \underset{\underset{H\;\;\;Cl^+}{N}}{CH_2}COOH + HOH^+$$

It would then be stated that the germicidal power of a chlorine compound is vested in the ability of the chlorine to replace the hydrogen atom of the amino group.

The question then follows as to what governs the ability of the positively charged chlorine to replace the hydrogen of an amine group. This tendency is measured by the oxidation potential.

It has been previously stated that the oxidation potential is effected by the nature of the (R) group to which the active chlorine is attached. The following equation will tend to illustrate this point:

$$R:NCl^+ + HOH \rightleftarrows HOCl + R:NH$$

In the case of Chloramine-T the reaction lies well to the left as the (R) group is very electro negative, especially so on the alkaline side. Thus, the reaction can only proceed to the right when the (R) group attached to the amine is more electro-negative than the (R) group of the existing chloramine. This concept has been previously stated in another manner by Dakin, Cohen, Daufresne, and Kenyon (7). They state that it appears that the aromatic sulphochloramides act as chlorinating agents only when there is an opportunity for the chlorine to leave the chloramine and attach itself to nitrogen in a second compound in which the nitrogen atom is united to less acid groups than those in the original substance.

Dakin, Cohen, Daufresne, and Kenyon (7) have measured the germicidal power of various chloramines prepared from substituted benzenes and other dicyclic derivatives. They demonstrated that these compounds did not all possess like germicidal properties. This fact may be explained on the basis of the previous discussion. The negativity of these various (R) groups no doubt are very different and as a result affect the (:NCl) bond resulting in weaker or stronger linkage and thus altering the germicidal properties.

The concept of electro-negativity which has been previously described may be applied to the inorganic hypochlorites. In the case of calcium hypochlorite, the (R) group or calcium has a great tendency to exist as a positive ion even in highly alkaline solutions. Thus the chlorine is less firmly held and may proceed to a more electro-negative group.

If then, one assumes that the oxidation potential is a resultant of the oxidation potential of the free hypochlorous acid on the one side and the force of the (R) group on the other side tending to form the chloro-derivative, the resultant potential may be taken as the tendency for the chlorine positive (Cl^+) to migrate and thus replace a hydrogen of an amine group in the cell structure.

The pH and oxidation potentials have been observed to be a linear function between certain limits, for Chloramine-T, Chloramine-T in 0.5 M sodium chloride, calcium hypochlorite, sodium hypochlorite and para-toluene-sulfonamide.

The oxidation potential values for Chloramine-T in 0.5 molar sodium chloride have been shown to be smaller than the same concentration of Chloramine-T at the same pH value. In the preparation of a series of chlorine solutions in buffers of various pH values, the potential developed will be influenced by the various buffer salts used.

The calcium hypochlorite and sodium hypochlorite curves were shown to have nearly the same slope. The values for the oxidation potential of the sodium salt were found to be less than that of the calcium salt. Reasoning by similarity of chemical properties the free hypochlorous acid at various pH values should have a similar slope.

Due to the fact that the pH and oxidation potentials are linear between certain pH values a method of calculating either value is stated knowing the opposite value and the value for the slope of the line.

In a comparison of the oxidation potential and germicidal efficiency certain limitations were found necessary. It was observed that the time to kill 99 per cent of the bacterial spores was a linear function of the oxidation potential with the same compound at a given concentration and within a certain pH range.

A mechanism has been proposed to explain the manner in which the oxidation potential may function in the destruction of a cell with active chlorine. It has been shown that if the assumption is made that chlorine kills the cells by replacing the hydrogen atom or atoms of the amine group, the process is one of replacement and does not involve oxidation.

G. INFLUENCE OF CONCENTRATION ON THE pH OF:

1. Chloramine-T.

It was observed in the early experimental work that the initial pH of the solutions as made directly from the salt was dependent upon the concentration employed. This became an important factor when the bacterial properties of the various concentrations of Chloramine-T were considered.

To observe the exact nature in which the pH of Chloramine-T changes with increasing concentration, solutions were prepared and the pH determined. These values as well as the oxidation potentials are recorded in Table XVII and shown graphically in Figure 12.

Table XVII

Change of pH with Increasing Concentration of Chloramine-T

Concentration in p.p.m. (active)	pH	Oxidation Potential in volts
25	6.69	0.7218
50	6.87	0.7218
100	7.32	
200	7.90	
400	8.54	
500	8.64	0.5020
1000	8.78	0.4855
2000	8.80	0.4878

Thus the Table XVII shows that at 25 and 50 p.p.m. (active) Chloramine-T solutions have a pH actually on the acid side. The increase in pH is observed to enlarge from 100 p.p.m. to 500 p.p.m. Then from 500 p.p.m. to 2000 p.p.m. the increase is small.

2. Calcium Hypochlorite.

The values for calcium hypochlorite are given in Table XVIII and presented graphically in Figure 12.

FIG. 12

INFLUENCE OF CONCENTRATION ON pH

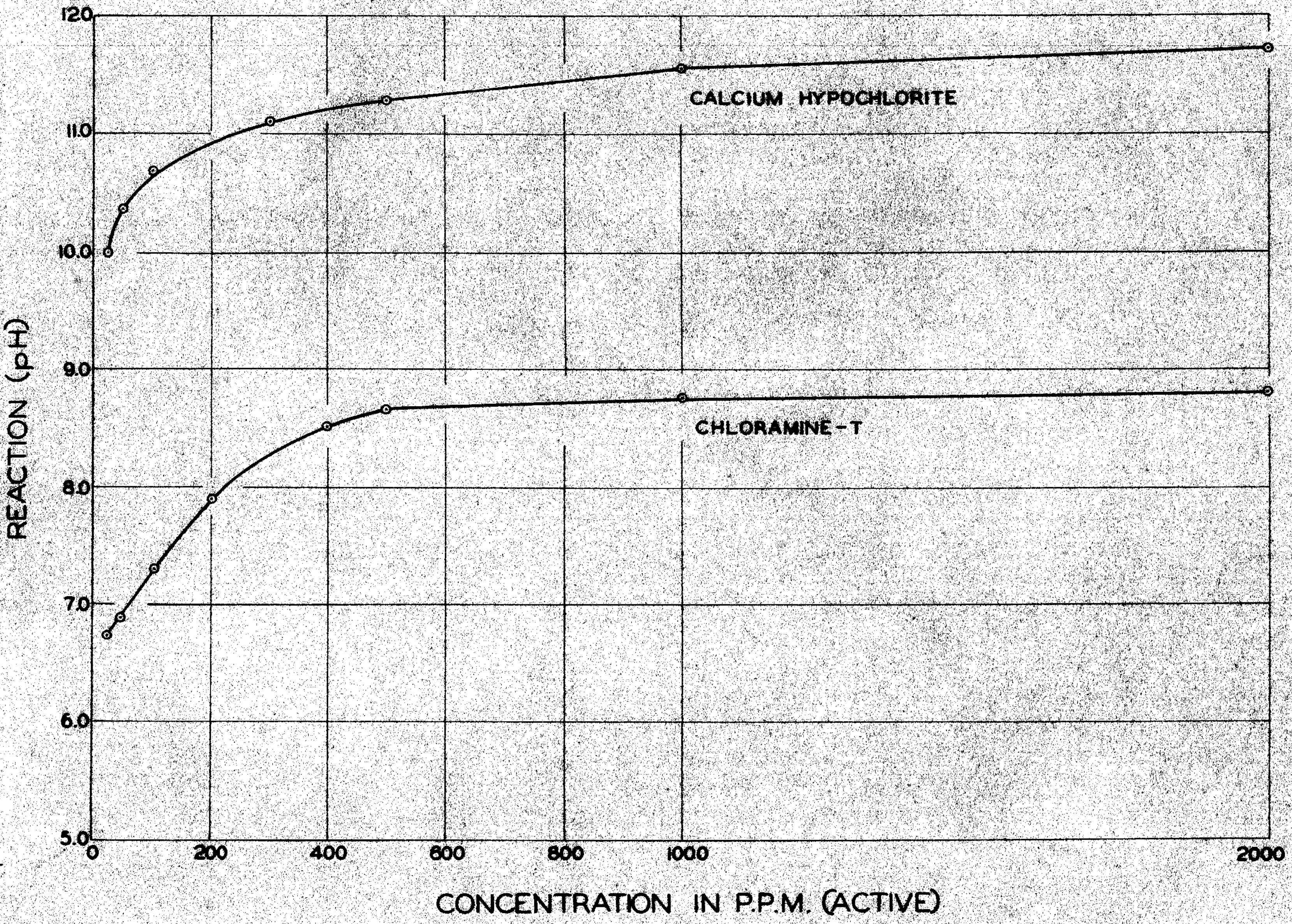


Table XVIII

Change of pH with Increasing Concentration of Calcium Hypochlorite

Concentration in p.p.m. (active)	pH	Oxidation Potential in volts
25	10.00	
50	10.38	0.6170
100	10.69	
300	11.12	
500	11.30	0.6063
1000	11.56	
2000	11.72	

The calcium hypochlorite displays the same properties as present by Chloramine-T. The pH increases sharply from 25 p.p.m. to 500 p.p.m. after which the slope is gentle.

The fact that the pH of both types of active chlorine compounds show varying pH values depending upon the concentration is of interest in the actual application of these compounds. In the lower concentrations the oxidation potentials are actually greater and these members should show a greater tendency to undergo reaction. The more concentrated solutions have a lower oxidation potential and should be thus less reactive. This fact may be substantiated by the addition of potassium iodide. The lower concentrations liberate more iodine than the concentrated solutions do prior to acidification.

H. INFLUENCE OF pH ON THE DISSOCIATION CONSTANT OF HYPOCHLOROUS ACID

The dissociation constant of hypochlorous acid has been measured by various investigators. Noyes and Wilson (24) have measured the conductivity of sodium hydroxide and sodium hypochlorite in aqueous solutions and found the value of 6.70×10^{-10} at 25° for the acidic ionization constant of hypochlorous acid. Sand (33) from measurements of the solubility of carbon dioxide in a solution of sodium hypochlorite found the value of 4.4×10^{-8}. Soper (39) also measured the ionization constant of the free hypochlorous acid in NaOCl solution by distillation methods and observed a value of 1.0×10^{-8} at 25° C. Recently Britton and Dodd (2) have measured the dissociation constant for this acid by means of the glass electrode and observed the value of 3.2×10^{-8} at 15° C.

Holwerda (15) in an effort to study the influence of the pH on the chemical equilibrium made the following calculation:

> "This we can find quantitatively in the following way. The hydrolytic constant of the ClO-ion at 25° C. may be calculated from the dissociation-constant of the hypochlorous acid:
>
> $K_{HClO} = 4.4.10^{-8}$ at 25° C. (Sand.Zeitschr.phys.chem.48, 610 (1904)) and the dissociation-constant of water at 25° C: $C:K_{H_2O} = 1.04 \times 10^{-14}$.
>
> $$K_{hydr.} = \frac{(HClO)\ (OH-)}{(ClO-)} = \frac{K_{H_2O}}{K_{HClO}} = 2.4.10^{-7} \text{ (at 25° C.)}$$

It follows that: $\frac{(HC1O)}{(C1O^-)} = \frac{2.4 \times 10^{-7}}{(OH^-)} = 2.4 \times 10^{-7} \times (H^+) = 2.3 \times 10^{-7} \times H^+$

Accordingly we get for pH = 4; $\frac{(HC1O)}{(C1O^-)} = 2300$ or almost complete hydrolysis.

for pH = 5; (HC10) = 230 or 99.6 per cent hydrolytically diss.
for pH = 6; (HC10) = 25 or 95.8 " " " "
for pH = 7; (HC10) = 2.3 or 69.7 " " " "
for pH = 8; (HC10) = 0.23 " 18.7 " " " "
for pH = 9; (HC10) =0.023 " 2.2 " " " "
for pH = 10;(HC10) =0.0023" 0.22 " " " "

From this we see that at a pH = 7 of the hypochlorite added, 69.7 per cent is present in the form of HC10, at a pH = 8, however, only 18.7 per cent, while this decreases very quickly according to the pH getting higher; below a pH = 6 almost the whole amount of hypochlorite is present in the form of HC10."

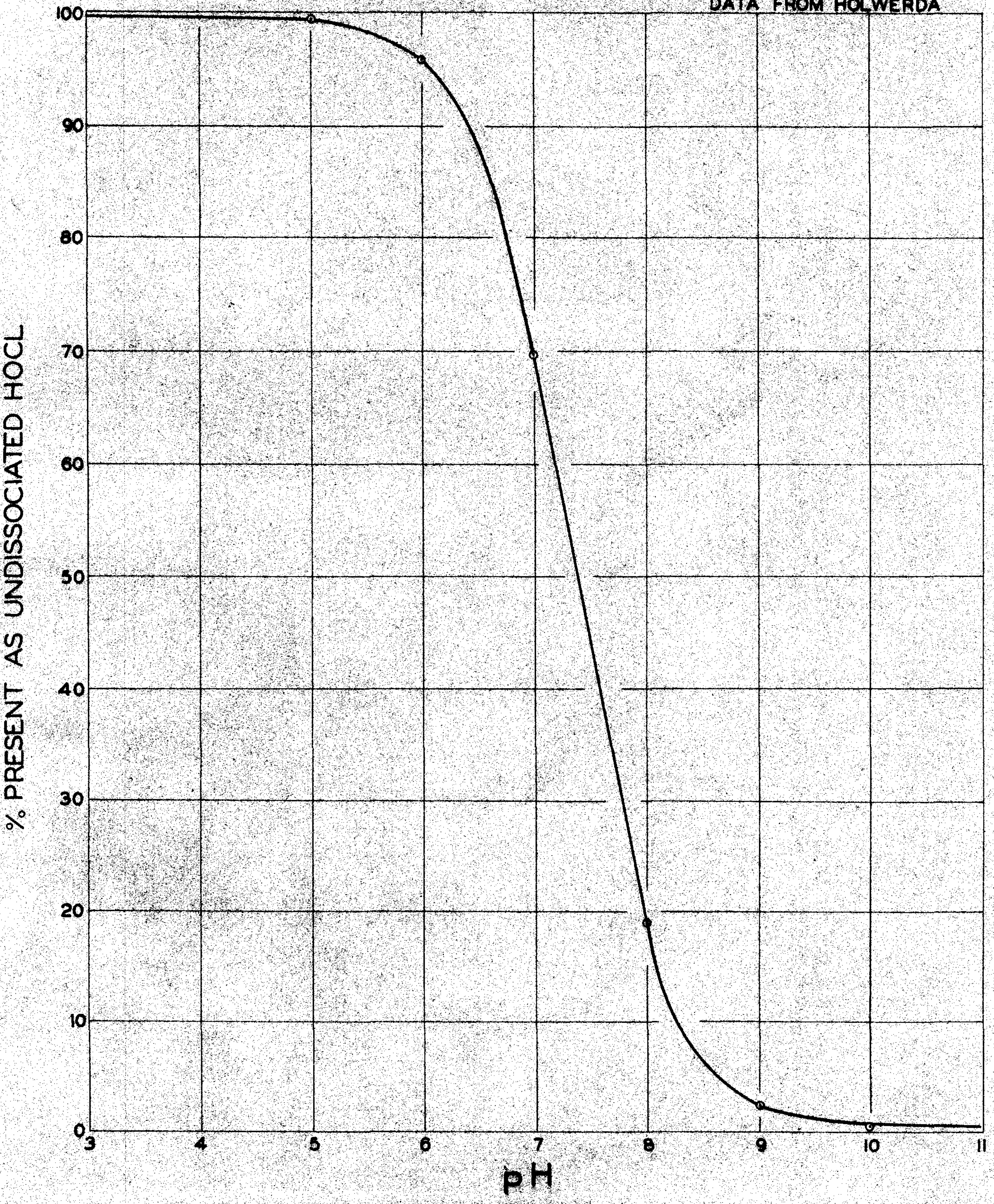
These calculations show that at any given pH value there is a certain percentage of the positive chlorine which may be present as the undissociated hypochlorous acid. It must be borne in mind, however, that the calculations as presented do not state what per cent of the theoretical positive chlorine is actually present. It only signifies the molecular state of the chlorine that is in the HOCl or OCl^- state.

The calculations as given by Holwerda are presented graphically by the author in Figure 13, which illustrates the large change in going from the acid pH to that of alkaline pH.

FIG. 13

INFLUENCE OF pH ON THE % DISSOCIATION OF HYPOCHLOROUS ACID

DATA FROM HOLWERDA



1. Comparison of the Percentage Dissociation and Germicidal Efficiency.

Rideal and Evans (30) have stated as follows.

> "The effect of acidity on the oxidizing power of hypochlorite solutions is particularly marked, probably because, as has been suggested by one of us, hypochlorous acid is the effective oxidizing agent, the oxidizing power of the salts and of the anion (OCl^-) being but feeble. Since the germicidal properties of the substance are in part due to its oxidizing power, it seems likely that these also will be affected by the hydrogen ion concentration of the water."

Holwerda (15) states that accordingly for the disinfection of drinking water by means of chlorine or hypochlorite, we have practically only to do with the HOCl molecule and the OCl^- ion and that the ratio $\frac{(HClO)}{(OCl^-)}$ is entirely determined by the pH which develops after the establishment of equilibrium. He continues to state that if we consider the results obtained in the disinfection experiments, we find that an increasing disinfection goes parallel with an increase of the (HOCl) concentration.

To support this observation of Holwerda the time to kill 99 per cent of the bacterial spores with Chloramine-T at 1000 p.p.m. (active) is compared with the percentage present as undissociated HOCl. Table XIX presents these values, and they are shown graphically in Figure 14.

FIG. 14

RELATIONSHIP OF % PRESENT AS UNDISSOCIATED HOCL (CHLORAMINE-T AT 1000 P.P.M. (ACTIVE)) AND THE TIME TO KILL 99% OF BACTERIAL SPORES

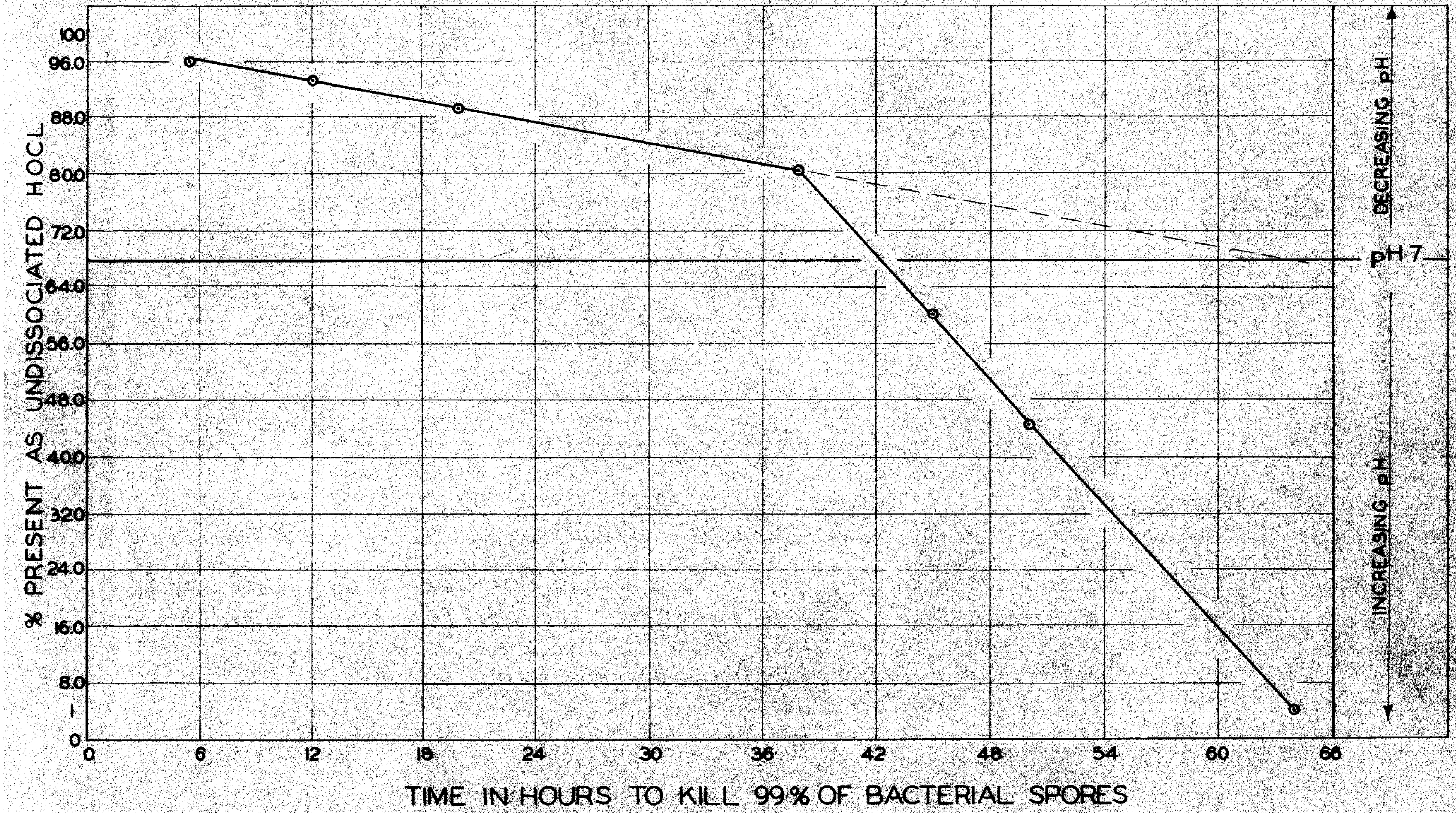


Table XIX

Time to Kill 99 per cent Bacterial Spores
with Chloramine-T at 1000 p.p.m. (active)
at Varying Percentage of Undissociated HOCl

pH	Percentage present as undissociated HOCl	*Time to kill 99 per cent of spores
6.0	95.8	5.4
6.2	93.5	12.0
6.38	90.5	19.8
6.74	80.0	37.8
7.17	60.0	45.0
7.51	44.5	50.0
8.78	4.0	64.0

Figure 14 shows that in the acid range the killing time is a linear function of the percentage present as undissociated hypochlorous acid. The derivation on the alkaline side is, no doubt, due to the previously discussed fact that the initial pH values are not constant but have a tendency to become more acid.

The observed fact that the killing time as determined is linear in respect to the percentage present as undissociated hypochlorous acid becomes of extreme interest when we consider our present means of determining the strength

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* The killing times denoted were obtained from Mr. D. B. Charlton (3).

of chlorine compounds or solutions.

The two methods now used both depend upon the oxidizing power of the hypochlorous acid. In the Bunsen method the acidity is fixed at a low pH by means of an excess of acetic acid. In the *ortho*-tolidine method the reaction is fixed by a large amount of hydrochloric acid. This then signifies that we are not determining the oxidizing power under the conditions the chlorine solution is to be used, but under a condition where the maximum oxidizing power is noted. This obviously denotes that the germicidal efficiency of an active chlorine compound is not in accord with either the Bunsen or *ortho*-tolidine methods of determining the strength of chlorine compounds.

I. A STUDY OF THE RATE OF BLEACHING OF MERCUROCHROME.

It has been shown in the previous section that the Bunsen and *ortho*-tolidine methods determine the strength of chlorine solutions when the menstrum is highly acid and do not show the amount of active chlorine which may act as a germicide when used in slightly acid or in alkaline solutions.

Due to the above stated condition an attempt to develop a unit of measure for active chlorine compounds which might correlate with the activity which these compounds display under varying conditions has been made. As has been previously shown, the germicidal efficiency of Chloramine-T is a linear function of the per cent undissociated hypochlorous acid between pH 6 and 7. The hypochlorous acid represents the active oxidizing agent. Thus the proposed method should in some manner measure the oxidizing power (HOCl) of the solution as actually used and not after it has attained its maximum oxidizing power by acidification as in the Bunsen and orthotolidine methods.

Other investigators have attempted methods for determining active chlorine. Massink (23) has suggested the idea of quantitatively estimating the active chlorine by passing a methyl orange solution from a burette in the acidulated solution and measuring the quantity of standard methyl orange solution which is decolorized in this way. This method, however, does not give the amount of active oxidizing agent (HOCl) at the pH at which it is used.

Holwerda (15) has developed a method of determining active chlorine by the oxidation of $K_4Fe(CN)_6$. He found, however, that this reagent was also affected by other chlorine derivatives and thus did not measure exclusively the active chlorine.

Besemann (1) has also suggested the oxidation of methyl orange as a test for active chlorine in water. However, he states that the test fails in the presence of chloramines. Winker (43) has suggested the use of methyl red as a test for free chlorine.

Holwerda (15) has developed a method for the analysis of active chlorine with methyl orange. His method consists essentially in titrating the chlorine solution with a standard methyl orange solution until the color of methyl orange remains. He has observed that the amount of methyl orange solution used depends upon the speed with which the methyl orange has been added. To overcome this fact his method requires three titrations to obtain the value for the active chlorine. His method is designed to determine the amount of active chlorine at any given time. However, it does not indicate the rate at which the chlorine is available to act as an oxidizing agent.

The previously stated authors have been concerned with only small amounts of chlorine such as 0.1 p.p.m. to 5.0 p.p.m. as used in water sterilization. Nevertheless, when the previous methods were tried with such concentrations as were used in this work certain difficulties were encountered. The dyes such as had been used previously as well as many others were found to be unsuitable. The dyes are acid, base indicators and the solutions were different in shade from the standard and thus difficult to compare. The rate of disappearance of the color in this type of dye appeared to be two fold, namely, that the original color was quickly oxidized to a yellow color which was then oxidized to colorless. This yellow color was so very foreign to the standard solutions that comparisons were impossible. Methyl orange was found fairly satisfactory although due to its low solubility such a large volume of the water solution of the dye had to be added that there was a resulting change in reaction.

In an effort to find a suitable reagent the author tried mercurochrome. It had been previously observed that sodium hypochlorite would bleach mercurochrome stains on human tissue. With this in mind mercurochrome was tried and was found satisfactory. However, this reagent was found not to be ideal in that a precipitate is observed in solutions of high alkalinity. The mercurochrome used was the "220 soluble" ($NaOOC.C_6H_4C:C_6H_2Br:OC_6HBr:OC_6HBr(ONa)(HgOH.3H_2O)$, manufactured by Hynson, Westcott, and Dunning.

The following is the procedure as developed to determine the rate of bleaching of the mercurochrome solutions. One hundred cubic centimeters of the chlorine solution was placed in a glass stoppered (iodine) flask which had previously been swept out with nitrogen. To this solution was added 4 c.c. of mercurochrome solution (500 mg. to 250 c.c. of H_2O), and the time was noted. The flasks were rotated to insure an intimate mixture and then placed in the constant temperature box. The blank solution was prepared in like manner, the same quantity of the mercurochrome solution being added to 100 cubic centimeters of conductivity water.

After a definite time had elapsed, the test solutions were compared to the blank by means of a colorimeter

(Bausch and Lomb No. 3690) using as a constant light source a nephelometer attachment (Bausch and Lomb No. 3696).

The readings were made at definite time intervals and the readings at each time interval and for each solution were taken in triplicate. The ratio of the amount of color remaining to that of the standard was calculated by the formula given by Scott (38).

$$\frac{\text{Color of test solution}}{\text{Color of standard solution}} = \frac{\text{Height of standard solution}}{\text{Height of solution to be tested}}$$

The ratio values at different time intervals for Chloramine-T at 500 p.p.m. (active) at various pH values are given in Table XX.

Table XX

Rate of Bleaching of Mercurochrome Expressed as Ratio of $\frac{\text{Height of Standard Solution}}{\text{Height of Unknown Solution}}$ for Chloramine-T at at 500 p.p.m. (active) at Various pH Values

Run No. I				
pH	Ratio at 1 hour	Ratio at 4 hours	Ratio at 11 hours	Ratio at 14 hours
6.82	0.588	0.507	0.433	0.342
6.73	0.555	0.466	0.381	0.245
6.40	0.449	0.330	0.236	0.193
6.17	0.400	0.270	0.190	0.151
6.02	0.291	0.195	0.143	0.106

Another run (Run No. II) at differing pH values is stated in Table XXI.

Table XXI

Run No. II

pH	Ratio at 1 hour	Ratio at 4 hours	Ratio at 6 hours	Ratio at hours	Ratio at 12 hours
7.95	0.500	0.466	0.450	0.416	0.401
6.92	0.475	0.454	0.420	0.386	0.360
6.65	0.445	0.401	--	--	0.300
6.35	0.387	0.310	0.288	--	0.200
6.02	0.300	0.230	0.194	0.150	0.115

Figure 15 represents graphically the data presented in Tables XX and XXI. It may be observed that the logarithm of the ratio is a linear function of time after the end of the first hour. If we consider that at zero time the ratio is one, then the relationship does not hold during the first hour. However, various other factors may be involved during the introduction of the mercurochrome solution.

In Figure 15 it may be observed that the values of the various solutions have been extrapolated to the point at which the mercurochrome would have been bleached to a water white solution. In this manner the time in hours and minutes required to completely bleach mercurochrome should be determined.

In summary Table XXII is given the time to bleach the mercurochrome to a water white solution for the various solutions whose physical constants are as tabulated.

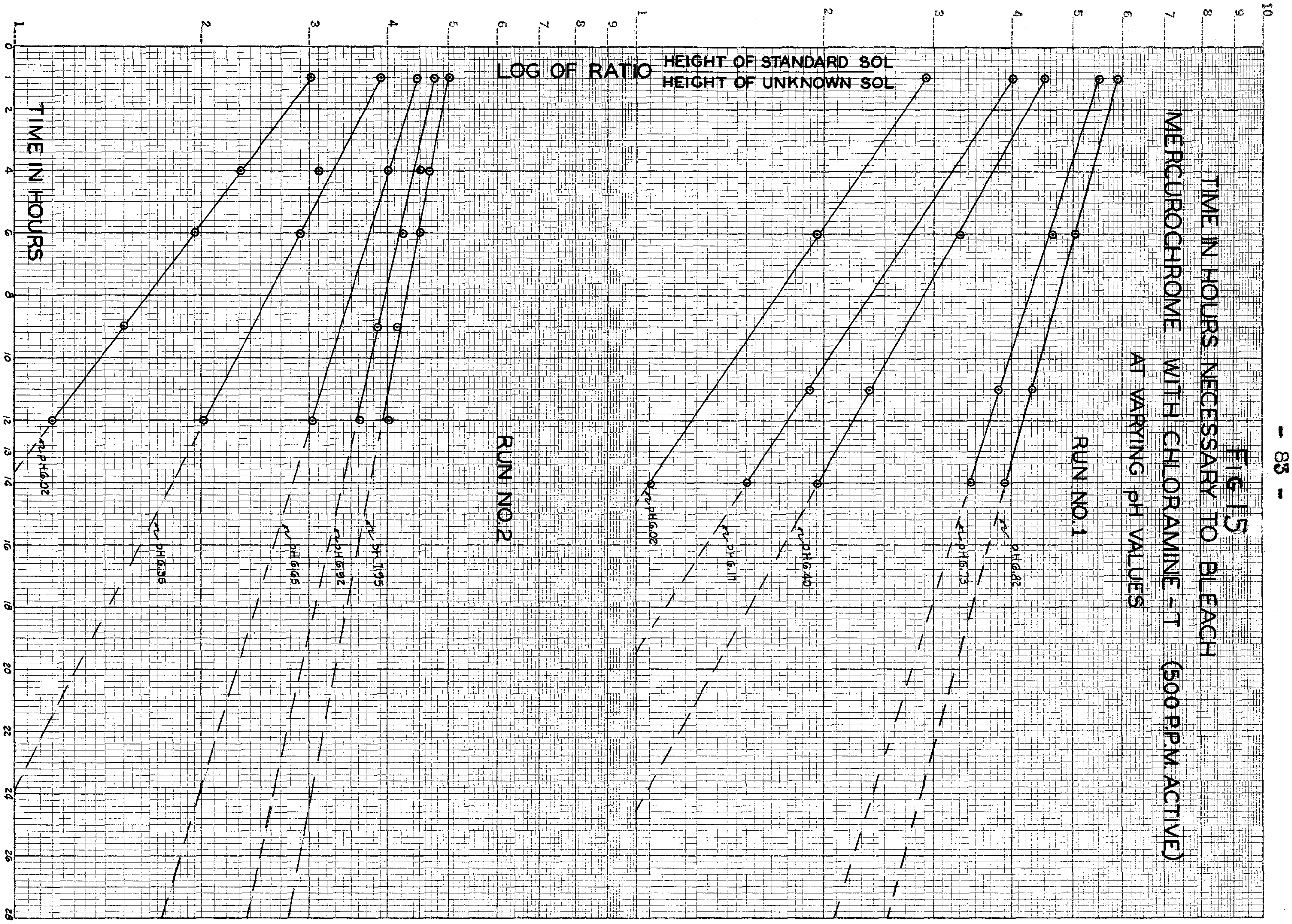

FIG 15
TIME IN HOURS NECESSARY TO BLEACH
MERCUROCHROME WITH CHLORAMINE - T (500 P.P.M. ACTIVE)
AT VARYING pH VALUES

Table XXII

Summary of Data for Chloramine-T at 500 p.p.m. (active)

	per cent present as undissociated HOCl	Oxidation Potential in volts	Time in Hrs & min. to bleach 100 per cent mercurochrome	Time in hrs & min. to kill 99 per cent bacterial spores
7.95	20.7	0.6350	61'	---
6.92	71.8	0.7558	51',48"	74'
6.82	74.2	0.7678	55',36"	---
6.73	77.0	0.7798	42'	---
6.65	79.0	0.7895	40'	---
6.40	85.2	0.8035	23'	43',24"
6.35	86.8	0.8258	23',48"	---
6.17	91.2	0.8490	18'	---
6.02	95.2	0.8525	13',36"	---
6.02	95.2	0.8525	14',24"	14',48"

1. As Influenced by Oxidation Potential.

In Figure 16 is shown the relationship between oxidation potential and time to bleach. It is very interesting that this relationship is a linear function. That is, the time to bleach 100 per cent is a straight line function of the oxidation potential. It may be noted that the oxidation potential values of 0.6350 and 0.7558 do not fall on the line. A possible explanation of this is that these two values represent solutions with alkaline pH values. Thus, the rate of oxidation is so very slow and the extrapolated

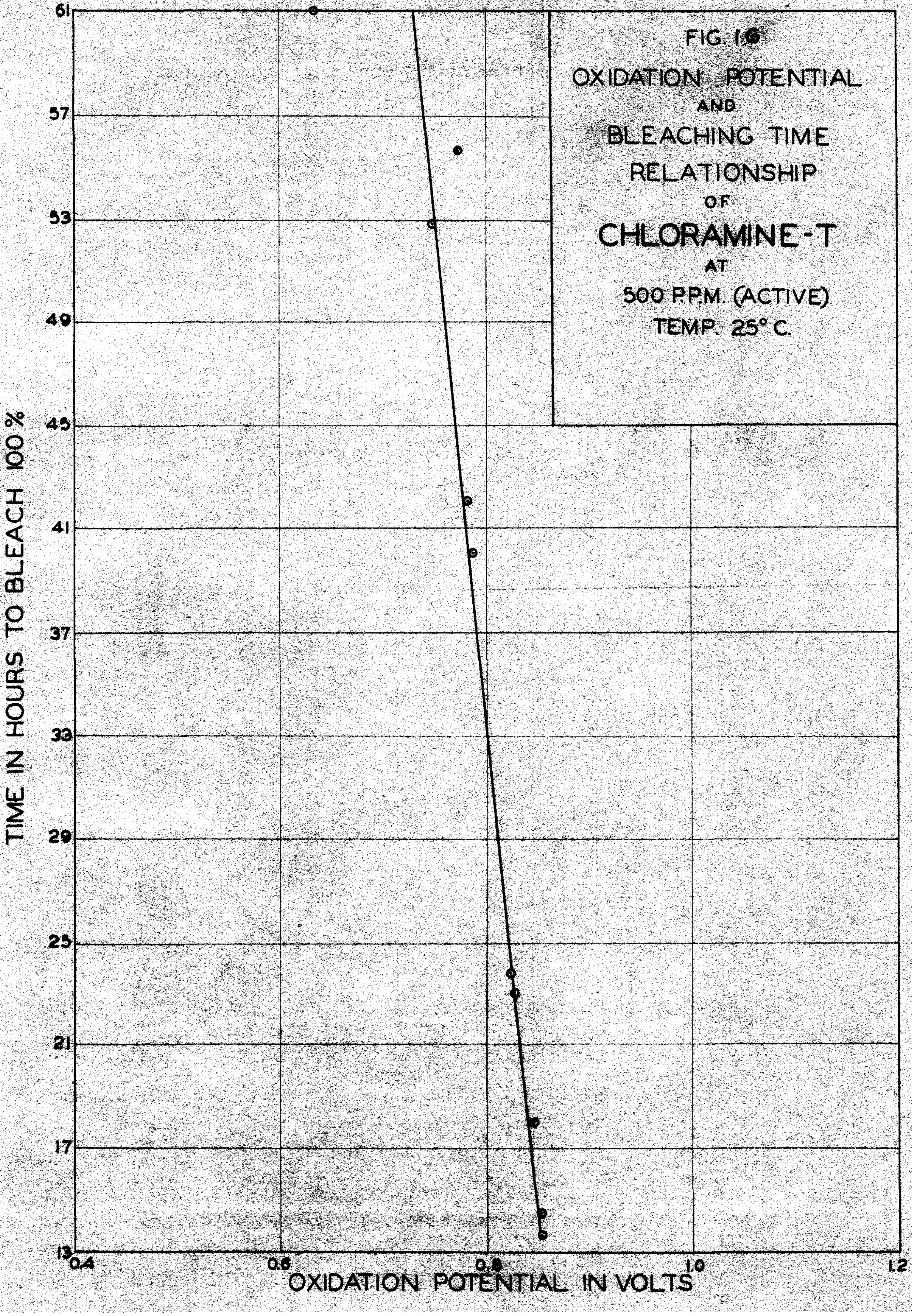

FIG. 16
OXIDATION POTENTIAL
AND
BLEACHING TIME
RELATIONSHIP
OF
CHLORAMINE-T
AT
500 P.P.M. (ACTIVE)
TEMP. 25° C.

value so large that the values areless reliable.

The foregoing observation is of interest and confirms the work of Ruis y Miro (31) who has demonstrated a perfect relationship between the potential of a platinum electrode submerged in a solution of a hypochlorite and the constant for the decolorization of methyl orange. He states that it is possible to deduce that potential which can be regulated by addition of acid or alkali the time necessary for decolorization. His conclusions may well be extended to that of bleaching of mercurochrome.

2. As Influenced by per cent Present as Undissociated Hypochlorous Acid.

In Figure 17 is shown graphically the per cent present as undissociated hypochlorous acid as plotted against time to decolorize mercurochrome 100 per cent.

It may be observed that the rate of bleaching is a function of the per cent of HOCl present as the undissociated acid (HOCl). This observation tends to illustrate the fact that in the case of mercurochrome the active bleaching agent is the undissociated molecule.

3. As Compared with Germicidal Efficiency.

The author does not possess sufficient data so that a direct comparison of bleaching time and time to kill

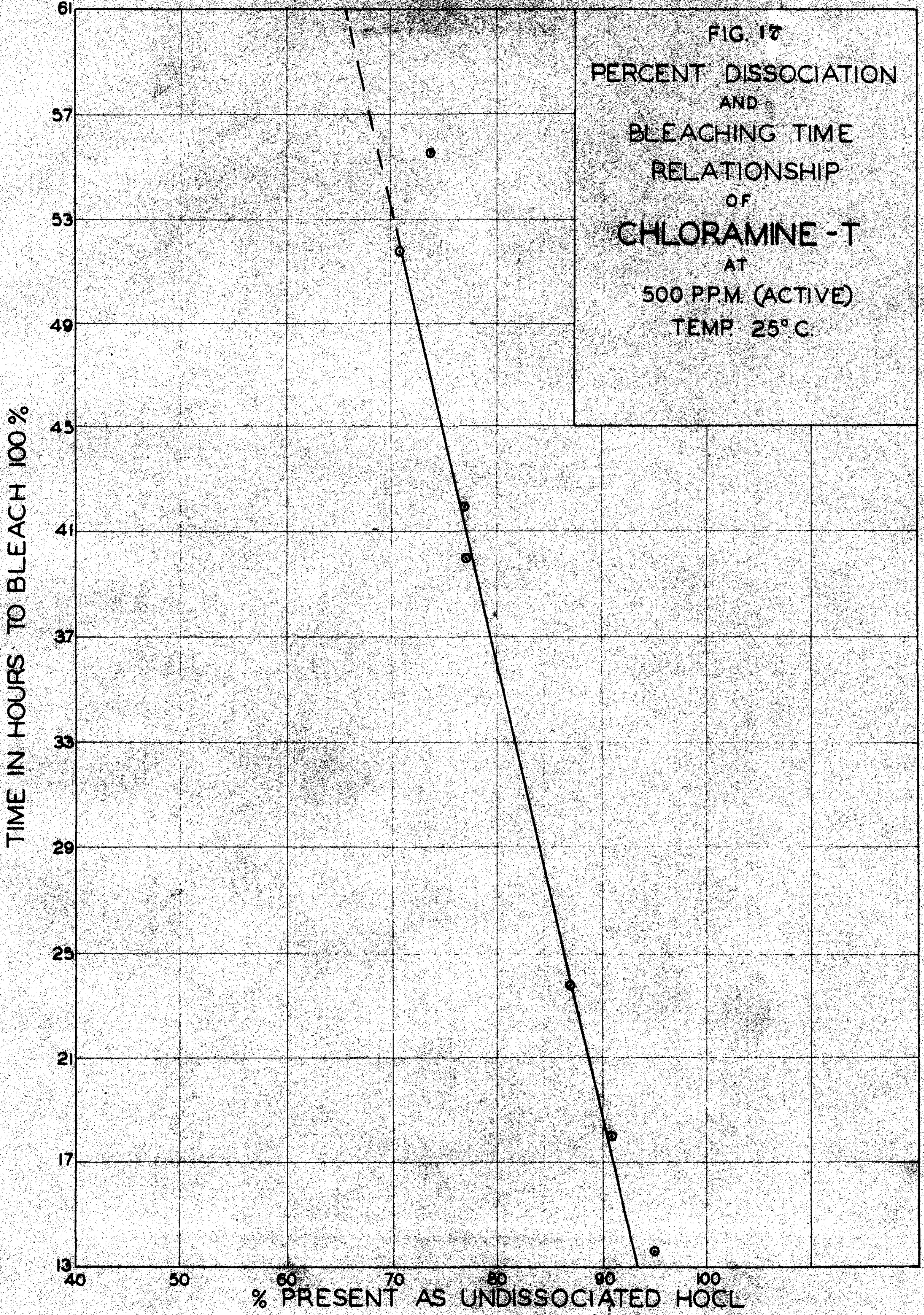

FIG. 17
PERCENT DISSOCIATION
AND
BLEACHING TIME
RELATIONSHIP
OF
CHLORAMINE-T
AT
500 P.P.M. (ACTIVE)
TEMP. 25° C.

bacterial spores can be noted. However, certain ideas may be presented as they are drawn from previous data.

In Figure 11 it has been shown that in the pH range from 6 to 7 the time to kill 99 per cent of the bacterial spores was a linear function of the oxidation potential. An explanation was also stated for the derivation of the killing time values as found on the alkaline side of the pH range. In Figure 16 it has also been shown that in the same pH range the time to decolorize mercurochrome is also a linear function of the oxidation potential. Thus time to decolorize mercurochrome and the time to kill 99 per cent of the bacterial spores are both linear functions of the same unit, namely, oxidation potential expressed in volts. From a purely mathematical reasoning the two time factors being linear in relationship to a common unit (oxidation potential) should be linear in respect to one another. If sufficient bactericidal values were available and the above relationship was found to hold experimentally the time to decolorize mercurochrome should form a fundamental basis for a test for active chlorine which would be in harmony with the observed germicidal efficiency.

The problem may be approached from another angle considering the per cent of hypochlorous acid present as the undissociated molecule. The time to decolorize mercurochrome and the time to kill 99 per cent of the bacterial spores have been previously shown to be linear functions of the per cent as undissociated hypochlorous acid. Thus both time factors must be dependent upon the same quantity, namely, the undissociated molecule. This fact would substantiate the conclusion that the rate of decolorization of mercurochrome might be developed into a suitable test for the germicidal efficiencies of active chlorine compounds.

It has been observed in this work that the germicidal power of the inorganic hypochlorites at the same concentration and pH was much greater than that of Chloramine-T. The same wide difference was observed in the decolorization of mercurochrome. At a pH of 11.3 a 500 p.p.m. (active) solution of calcium hypochlorite (using the same technique as with Chloramine-T) decolorized in the course of three hours. At a pH of approximately 10 the time was 30 minutes and in the pH range used in the Chloramine-T experiment (6 to 7) the decolorization was almost instantaneous with the inorganic hypochlorites.

We have previously discussed the greater oxidation potential of calcium hypochlorite as compared with Chloramine-T at the same concentration and at the same pH. We have also discussed the very different nature of the linkage of the positive chlorine to the nucleus in the chloramine type (:NCl) and the inorganic type (OCl)M. The decolorization of mercurochrome may augment our knowledge of these various linkages.

It has been shown previously that a solution of calcium hypochlorite at a pH of 7.0 will decolorize mercurochrome rapidly, whereas a like solution of Chloramine-T at a pH of 7 will decolorize it slowly.

If we consider the decolorization of mercurochrome to be instantaneous once the chlorine is present as hypochlorous acid, the relatively long time needed for Chloramine-T may be due to the slow release of the active chlorine from the residue. This gives an approximate measurement of the strength of the (:NCl) linkage. This reaction governs the rate at which the chlorine is liberated from its parent nucleus and is thus made available to decolorize a definite quantity of dye or to kill bacterial spores.

IV SUMMARY.

The linear relationship between pH and oxidation potential has been shown to hold for Chloramine-T in all the concentrations expressed in this work. The average slope was calculated to be -7.425. That is, for each change of one pH unit, there is an approximate change of 0.1400 volts in the oxidation potential value. This linear relationship does not hold over the entire pH range. The limits through which the relationship is constant are determined by the concentrations.

In all reactions involving oxidation or reduction, two forces come into play, namely, an intensity factor and a capacity factor. The oxidation potentials, as displayed by active chlorine compounds, may be considered only as a measure of the intensity factor, since the concentration of active chlorine appears to have very little effect upon the value of the potential developed.

Evidence has been presented to illustrate the fact that the oxidation potential is influenced by the presence of other salts in the menstrum. At the same pH and temperature, the oxidation potential is actually smaller in a solution of 0.5 molar sodium chloride. However, if two solutions of Chloramine-T were used having the same pH and to the one sodium chloride was added, the pH of this would be decreased and, as a result, a higher oxidation potential would be obtained.

The foregoing point illustrates the fact that the oxidation potential values should be determined in a water menstrum, rather than in a solution of buffers.

What has previously been stated for the pH and oxidation potential relationship of Chloramine-T may be likewise extended to include the inorganic calcium hypochlorite. The potential values for calcium hypochlorite are much larger than for Chloramine-T. The slope for calcium hypochlorite signifies a change of approximately 0.700 volts for each change in pH unit. It has been shown in the curves expressing Chloramine-T values that the oxidation potential varied but little with the concentration. However, in the curve for calcium hypochlorite, a gradual change is noted in proceeding from 10 p.p.m. (active) to 500 p.p.m. (active). This change may be due to the large amount of inert material in the solutions of higher concentration.

Sodium hypochlorite which has the same general formula as that of the calcium salt has displayed like properties. The oxidation potential values and the slope value for the sodium compounds are approximately the same as for the calcium compound. This fact is of interest since it tends to illustrate that the two compounds having the general formula (OCl)M are similar in their properties.

By again referring to Figure 10, it becomes evident that the oxidation potential values of the two types of active chlorine compounds are very different. The variation in respective slopes is especially outstanding. To explain this fact, it has been stated that the ability of the positive chlorine (Cl^+) to hydrolyze and thus to form hypochlorous acid (HOCl), is dependent upon the strength of the bond with which the chlorine is attached to the (R) group. The strength of this bond may be considered as depending upon the negativity of the (R) groups. In this manner, an explanation has been presented to account for the wide differences in the nature of the organic and inorganic hypochlorites.

Due to the fact that the pH and oxidation potential are a linear function for the compounds used, equations have been developed by which the pH or the oxidation potential may be calculated the value of either one and the value for the slope of the line given. For determining the pH the equation may be stated:

$$pH = ME + b$$

For E (oxidation potential) the equation is stated:

$$E = \frac{pH - b}{M}$$

The results have shown that the oxidation potential and the time to kill 99 per cent of the bacterial spores are a linear function below a pH of 7. The cause for this variation on the alkaline side may be explained by the fact that the carbon dioxide of the air causes the weakly buffered solutions to become more acid, and thus the killing time would be likewise decreased.

A consideration of oxidation potential as a guide to germicidal efficiency brings out certain limitations. The comparisons can only be made with one type of compound of a given concentration with the pH and oxidation potential the only variables.

In the work on the significance of oxidation potential as a guide to germicidal efficiency an explanation has been offered to explain the manner in which the cells are destroyed with active chlorine. It has been shown that if the assumption is made that the active chlorine kills the cell by replacing the hydrogen atom or atoms of the amine group, then the process is one of replacement and does not involve oxidation.

The observation that the time to kill 99 per cent of the bacterial spores is a linear function of the per cent of active chlorine present as the undissociated hypochlorous acid is of extreme interest. It would tend to show that the active germicide is the undissociated molecule. The deviation experienced on the alkaline side may be due to the inability of maintaining a constant pH value. These results all indicate that our present methods of determining the strength of active chlorine compounds, namely, the Bunsen and the <u>ortho</u>-tolidine methods, do not denote the ability of the compound in regard to germicidal efficiency.

It was desired to find some chemical reaction that might be used as a guide to germicidal efficiency. The data point to the fact that the rate of decolorization of mercurochrome is a linear function of the oxidation potential and also a linear function of the per cent of $HOCl$ present as the undissociated molecule.

Reasoning mathematically the bleaching of mercurochrome and the killing times are both linear functions of the same unit, namely, the oxidation potential, and therefore must be linear in respect to each other. Thus the bleaching of mercurochrome affords a means of developing a suitable test for the germicidal efficiency of active chlorine compounds.

V CONCLUSIONS

1. Data have been presented to show that for Chloramine-T at 50, 500, 1000, and 2000 p.p.m. (active) the oxidation potential is a linear function of the pH between certain limiting values. The average slope is expressed by the value -7.425.

2. The oxidation potential of Chloramine-T is apparently independent of the concentration.

3. The oxidation potential of Chloramine-T (1000 p.p.m. active) in 0.5 molar sodium chloride is a linear function of the pH.

4. The solution of Chloramine-T (1000 p.p.m. active) in 0.5 molar sodium chloride has a lower oxidation potential at the same pH and temperature.

5. The oxidation potential for calcium hypochlorite has been shown to be a linear function of the pH. The average slope of the line is expressed by the value -14.37.

6. The same linear relationship between oxidation potential and pH has been observed in the case of sodium hypochlorite. The fact becomes evident that the two compounds having the general formula $(\mathrm{OCl})M$ are similar in their action regarding oxidation potential values.

7. The oxidation potential values for *para*-toluene sulfonamide are a linear function of the pH between the values 9.58 and 6.01.

8. A plausible explanation based on the negativity of the (R) group is given to account for the different oxidation potential values of the two types of active chlorine compounds.

9. The time to kill 99 per cent of the bacterial spores is a linear function of the oxidation potential below pH 7.

10. The oxidation potential can only be considered as an index of germicidal efficiency in a series of solutions consisting of one type of active chlorine compound at a given concentration. The only variables may be pH and oxidation potential.

11. The pH and oxidation potential values of both types of active chlorine compounds vary with the concentration.

12. The time to kill 99 per cent of the bacterial spores has been shown to be a linear function of the percentage of active chlorine present as the undissociated hypochlorous acid below pH 7.

13. The time necessary to decolorize a solution of mercurochrome with Chloramine-T has been shown to be a linear relationship of the oxidation potential.

14. The rate of bleaching is a function of the per cent of HOCl present as the undissociated acid (HOCl).

15. It has been shown that the time to kill 99 per cent of the bacterial spores and the time to bleach mercurochrome are parallel functions of the oxidation potential and thus should be a function of each other.

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