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Disinfecting Capabilities of Oxychlorine Compounds

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The bacterial virus f2 was inactivated by chlorine dioxide at acidic, neutral, and alkaline pH values. The rate of inactivation increased with increasing pH. Chlorine dioxide disproportionation products, chlorite and chlorate, were not active disinfectants. As chlorine dioxide solutions were degraded under alkaline conditions, they displayed reduced viricidal effectiveness, thereby confirming the chlorine dioxide free radical as the active disinfecting species.

In recent years, chlorination practice has been implicated in the production of low concentrations of hazardous pollutants. Those pollutants, generally grouped as trihalomethanes, are products of halogen reactions with naturally occurring organic substrates. It is the contention of present-day regulators that these compounds, which exist in drinking water, produce a potential health hazard and place the public at risk.

As a result of these concerns, the use of chlorine dioxide as a water disinfectant has become widespread. Its use is desirable since chlorine dioxide does not produce trihalomethanes. Its mechanism of action as a disinfectant, however, is not understood. Alvarez and O'Brien (3) have reported that chlorine dioxide inactivated poliovirus more rapidly as the pH was increased. Since chlorine dioxide disproportionates at alkaline pH values, they concluded that the chlorine dioxide disproportionation products, chlorite and chlorate, were probably responsible for the increased inactivation rates. The purported inactivation of virus by disproportionation products of chlorine dioxide is unlikely; Emerich has indicated that the autolytic degradation of chlorine dioxide occurs too slowly at pH values less than or equal to 10 (D. E. Emerich, Ph.D. thesis, Miami University, 1981). Therefore, this work was completed to ascertain the relative viricidal effectiveness of chlorite, chlorate, solutions of disproportionated chlorine dioxide, and chlorine dioxide. These effects of oxychlorine compounds on f2 virus are representative of their oxidative capacity and do not necessarily reflect the reactions one may observe in in vivo toxicological studies. Chronic toxicity of oxychlorine compounds has been studied elsewhere (1, 5, 8).

MATERIALS AND METHODS

Preparation of chlorine dioxide. Chlorine dioxide was prepared by the method of Granstrom and Lee (6), with minor modifications. High-purity nitrogen gas was bubbled through three gas-washing bottles connected in series. The first bottle contained sodium chlorite at 16% (wt/vol) and potassium persulfate at 4% (wt/vol) in triple-distilled water. The second gas-washing bottle was maintained on ice. It contained triple-distilled water for the collection of chlorine dioxide. The third bottle contained double-strength nutrient broth and an antifoaming agent (HODAG FD-82). Its purpose was to trap the chlorine dioxide that spilled over from the second gas-washing bottle. Chlorine dioxide solutions prepared in this fashion were generally near 4,000 mg/liter. Stock solutions were stored at 4°C in brown glass bottles until working solutions were prepared. Since significant volatilization can occur in several minutes, working solutions were made from stock solutions just before use.

Sodium chlorite and sodium chlorate were purchased from Eastman Chemical Products, Inc. (Kingsport, Tenn.) and were used as received. Test solutions were prepared by dissolving the appropriate chemical in chlorine dioxide demand-free water.

Measurement of chlorine dioxide. Chlorine dioxide concentrations were determined spectrophotometrically and colorimetrically. Direct spectrophotometric measurements were made according to the method of Granstrom and Lee (6).

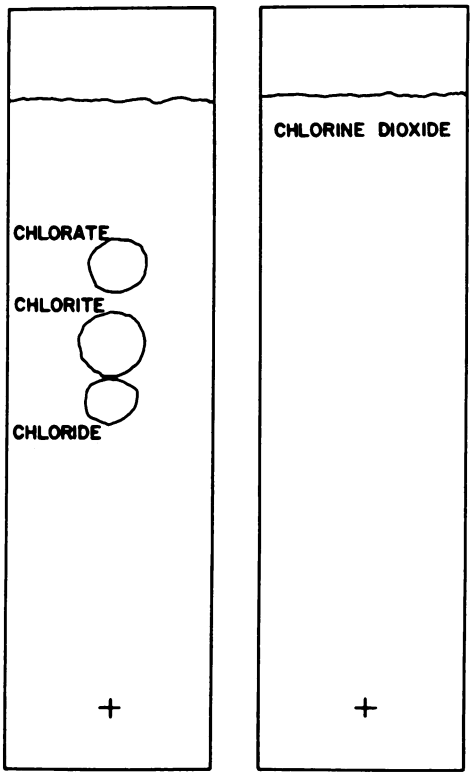

FIG. 1. Chlorate, chlorite, and chloride (2 μg each) and 6.3 μg of chlorine dioxide were chromatographed on silica gel. R_f values were 0.74, 0.61, and 0.51 for chlorate, chlorite, and chloride, respectively. +, Origin.

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A_{357} was measured in a 1-cm quartz cuvette with a Heath-Schlumberger model 701 spectrophotometer. When low concentrations of chlorine dioxide were required, 10-cm cuvettes were used for absorbance measurements, or chlorine dioxide was measured with leuco crystal violet (4).

Determination of chlorine dioxide purity. Concentrated stock solutions of chlorine dioxide were chromatographed to check for the presence of chlorite and chlorate. Harrison and Rosenblatt (7) reported a paper chromatographic procedure for separation of oxychlorine species. This procedure was modified to increase the clarity of the chlorite and chlorate spots.

Prepackaged thin-layer silica chromatograms were spotted with 1 to 10 μg of the chlorine dioxide solution. Chromatograms were run for 8 h in a solvent consisting of 2-propanol, water, pyridine, and concentrated ammonium hydroxide at respective volumes of 15, 2, 2, and 2. Chlorite was detected by spraying the air-dried chromatograms with a reagent consisting of 50 ml of 3 M HCl, 50 ml of acetone, and 100 ml of 5% diphenylamine in ethanol. The immediate appearance of a blue spot was indicative of chlorite, and as the chromatogram dried, the presence of chlorate was detected by the appearance of a green spot. Chloride could also be detected separately by spraying a duplicate chromatogram with 0.2 N silver nitrate, allowing the chromatogram to dry, and then exposing the chromatogram to UV light for 2 min to develop a purple spot.

Oxychlorine species reaction system. All glassware was presoaked in chlorine dioxide solution to avoid extraneous oxidant demand. All experiments used triple-distilled water, which demonstrated negligible chlorine dioxide demand.

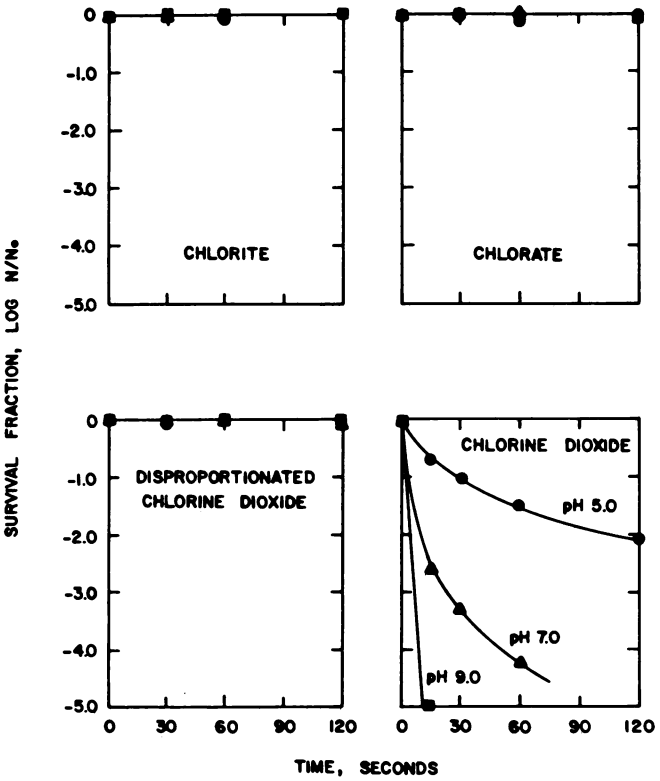

FIG. 2. Inactivation of f2 virus with 3 mg of chlorite, chlorate, disproportionation products of chlorine dioxide, and chlorine dioxide per liter at pH 5.0 (●), 7.0 (▲), and 9.0 (■) at 3°C.

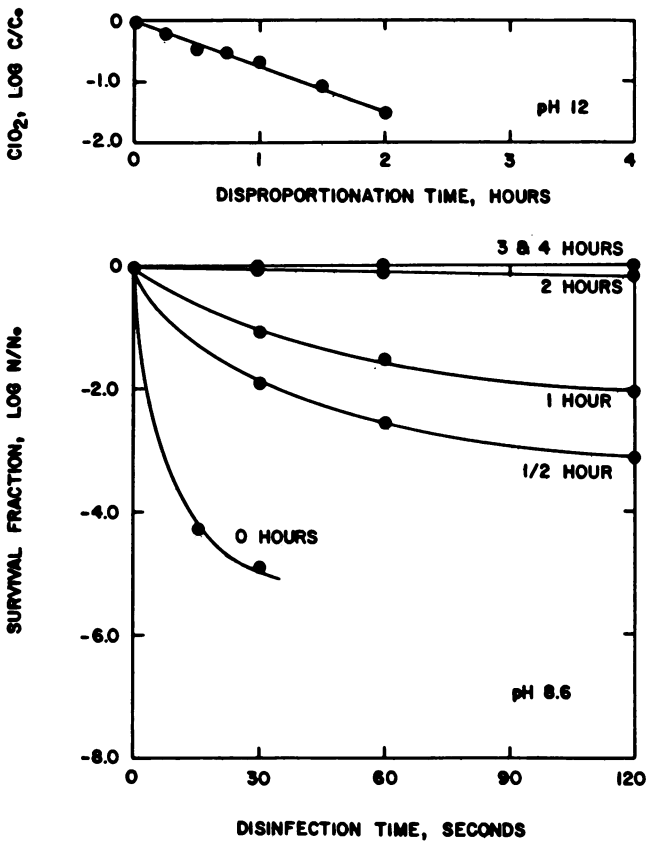

FIG. 3. Inactivation of f2 virus with chlorine dioxide disproportionation products at 3°C and pH 7.9 to 8.1. Chlorine dioxide at room temperature was adjusted to pH 12 for the time indicated and was mixed with equal volumes of 0.2 M phosphate buffer at pH 6.5 to lower the pH for inactivation studies.

Inactivation experiments were performed in a trypsinizing flask to increase turbulent mixing. Solutions containing oxychlorine compounds were brought to the desired temperature before f2 virus was added. The solution pH was controlled with 0.01 M acetate, phosphate, or borate buffer for an acidic, neutral, or alkaline pH value, respectively.

Preparation and assay of f2 virus. Large quantities of f2 bacterial virus (ATCC 15776-B) were prepared by the method of Loeb and Zinder (9). The virus was concentrated and purified according to the polyethylene glycol precipitation technique described by Yamamoto et al. (10). The virus was assayed by the agar overlay procedure of Adams (2), using *Escherichia coli* K13 (ATCC 15776) as the male host.

RESULTS AND DISCUSSION

Purity and chlorine dioxide solutions. The purity of chlorine dioxide stock solutions was checked chromatographically. Figure 1 shows a chromatogram spotted with 2 μg of chlorite and chlorate next to a chromatogram spotted with 6.3 μg of chlorine dioxide. No chlorite or chlorate was detected in the chlorine dioxide stock solution (0.1 μg was the lower limit of detection for chlorite and chlorate by this chromatographic procedure). After dilution of the stock solution, the concentration of chlorite or chlorate in the reaction system was calculated to be not greater than 13 μg/liter.

Effect of chlorite and chlorate on f2. Figure 2 shows that

chlorine dioxide was responsible for inactivation of f2 virus. Chlorite and chlorate, used at concentrations of 3 mg/liter, had no effect on f2 virus at pH 5.0, 7.0, and 9.0 at 3°C. Disproportionation products of chlorine dioxide also had no disinfecting capabilities. Only chlorine dioxide demonstrated viricidal activity. The inactivation of f2 virus by chlorine dioxide increased as the pH increased. This phenomenon is considered to be a function of a change in reactivity between chlorine dioxide and viral components and is not due to a change in the species of oxychlorine compound present.

Disproportionated chlorine dioxide had no effect on f2 virus at pH 8 (Fig. 3), where loss of viral inactivation capability decreased as chlorine dioxide degraded. When f2 virus was treated with 3 mg of chlorine dioxide per liter disproportionated at pH 12, it lost its disinfecting potential.

To determine the chlorine dioxide reactions that caused f2 bacterial virus inactivation, it was first necessary to demonstrate which oxychlorine compound was present. The method of chlorine dioxide generation described by Granstrom and Lee (6) indicated that pure chlorine dioxide was obtained by combining potassium persulfate with sodium chlorite. The chromatographic technique of Harrison and Rosenblatt (7) verified the purity of the chlorine dioxide solutions. When chlorine dioxide reacts with available substrate, however, it may be reduced to chlorite. For this reason, f2 virus was treated with chlorite, which was shown to have no effect on viability.

The data presented herein demonstrate that inactivation of f2 bacterial virus was caused by chlorine dioxide and was not the result of disproportionation products such as chlorite or chlorate between pH values of 5 and 9. Therefore, changes in the chemical moieties that constitute an f2 virion may account for increased chlorine dioxide reactivity, causing increased viral inactivation. This need not be the only explanation for increased inactivation associated with the elevation of pH. Also likely is the possibility that hydroxyl ions are necessary for a reaction to occur, thus creating a rate-limiting step that lowers the rate of disinfection as the pH decreases.

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