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Inactivation of Human and Simian Rotaviruses by Ozone

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The inactivation of simian rotavirus SA-11 and human rotavirus type 2 (Wa) by ozone was compared at 4°C by using single-particle virus stocks. Although the human strain was clearly more sensitive, both virus types were rapidly inactivated by ozone concentrations of 0.25 mg/liter or greater at all pH levels tested. Comparison of the virucidal activity of ozone with that of chlorine in identical experiments indicated little significant difference in rotavirus-inactivating efficiencies when the disinfectants were used at concentrations of 0.25 mg/liter or greater.

The use of chlorine for the disinfection of water and wastewater has come under considerable scrutiny because of the potential for the formation of chlorinated hydrocarbons in waters which contain organic compounds (16). The products most commonly formed include the carcinogenic trihalomethanes which result from the reaction of chlorine with low-molecular-weight compounds (<3,000; 9). Concern over the formation of such compounds has prompted a search for alternate modes of disinfection. Prominent among available alternates is ozone, considered by many to be both less hazardous to use and a more effective disinfectant than chlorine (4, 6, 15).

A critical measure of the utility of any candidate disinfectant is its effectiveness as a virucide. Studies have shown ozone to be effective against several virus types. Initial work by Kessel et al. (4) demonstrated the rapid inactivation of poliovirus type 1. Other viruses shown to be sensitive to ozone include vesicular stomatitis and encephalomyocarditis viruses (1), poliovirus types 2 and 3, coxsackievirus, echovirus, and adenoviruses (5, 11). In the last study, strain susceptibility variations were noted, with echovirus type 29 the most sensitive and echovirus type 12 the most resistant to ozone treatment. Most recent studies have dealt with inactivation kinetics of poliovirus in pure culture (3) and in mixed cultures of organisms (2).

Studies have not focused on the effectiveness of ozone in inactivating those viruses most commonly identified in outbreaks of waterborne disease (i.e., hepatitis type A virus, Norwalk virus, and human rotavirus [HRV]). Rotaviruses were the subjects of the present study, in which the inactivation rates of ozone-treated, single-particle suspensions of simian rotavirus (SA-11) and HRV type 2 Wa were determined over a range of disinfectant concentrations and pH levels. The resulting data were then compared with those obtained from a similar study of rotavirus inactivation by chlorine (14) which had been conducted under identical experimental conditions.

MATERIALS AND METHODS

Simian rotavirus SA-11 was obtained from Charles Gerba, University of Arizona, Tucson. HRV type 2 Wa was purchased from Biotech Research Laboratories, Rockville, Md. Host cell cultures (MA-104) were purchased from Microbiological Associates, Walkersville, Md. Methods for virus propagation, purification, and assay, as well as those for the preparation of single-particle virus stocks, have recently been described (14).

Immediately before each experiment, stock ozone solutions (0.5 to 1.0 mg/liter) were prepared in ozone-demand-free phosphate-carbonate buffer (3) which had been readied at the desired pH and ionic strength by the formulations of Sharp and Leong (10). Ozone was generated from a Welsbach ozonator operating at 80 V. Dissolved ozone levels were determined by the spectrophotometric method of Schecter (8). Ozone concentrations in stock solutions maintained at 4°C were stable for 5 to 6 min.

All inactivation experiments were conducted at 4°C. The procedures used were identical for both virus types. Ozone-treated buffer (100-ml portions) at the desired pH and ozone residual was inoculated with 1 ml of dialyzed single-particle virus stock ($\sim 10^7$ PFU/ml) and gently mixed on a magnetic stirrer. Samples (10 ml) were collected at 10-, 20-, 30-, 45-, and 60-s intervals and placed in test tubes containing 0.1 ml of 0.5 M sodium thiosulfate to reduce the ozone. The ozone residuals at the end of each experiment were measured to determine dissipation levels. Zero time control experiments (i.e., initial virus concentrations) were carried out in 100-ml portions of ozone-free buffer inoculated with 1 ml of the same virus stock. All samples were then treated with 0.5 ml of chloroform for 10 min to eliminate microbial contamination, diluted in Tris-buffered saline, and assayed.

Positive and negative rotavirus controls were used in each assay to verify that host cells remained susceptible and virus-contaminant free. Experiments were repeated several times to assure consistency of the results. When appropriate, the data were statistically analyzed by the method described by Sokol and Rohlf (12) and Steel and Torrie (13). Statistical analyses and graphics were performed on a Hewlett-Packard HP8945B computer (Hewlett-Packard Co., Palo Alto, Calif.) with preprogrammed statistical software.

RESULTS

The average reductions in ozone residuals identified in the course of each experiment (i.e., 60 s) ranged from 0.02 to 0.08 mg/liter. All ozone concentrations reported below are those measured at the beginning of the indicated experiments. Datum points on each curve represent median values from several experimental runs.

SA-11 inactivation was most rapid at pH 6 (Fig. 1 to 3), with ozone residuals of 0.1 to 0.25 mg/liter causing complete inactivation (inactivation of $\geq 10^5$ PFU) within 30 s. With

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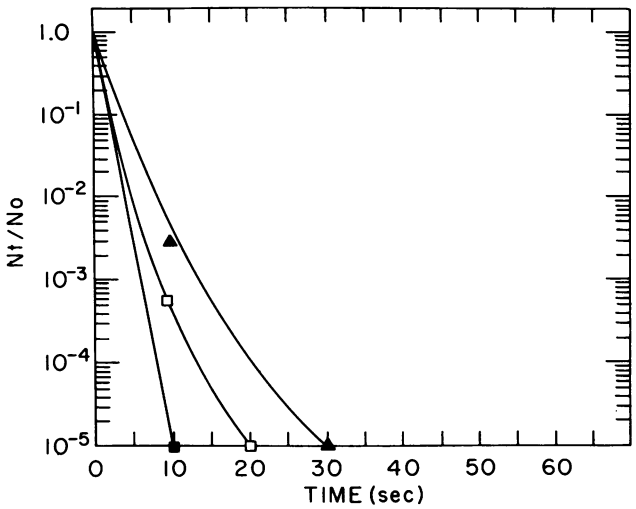

FIG. 1. Inactivation of SA-11 by ozone at pH 6.0. Ozone concentrations (milligrams per liter): ▲, 0.10; □, 0.17; ■, 0.25. N_t/N_0, Number of viruses at any time/number of viruses at zero time.

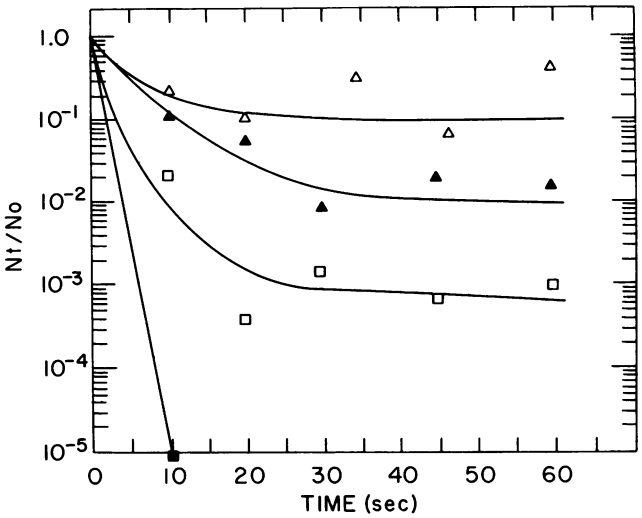

FIG. 3. Inactivation of SA-11 by ozone at pH 8.0. Ozone concentrations (milligrams per liter): △, 0.05; ▲, 0.11; □, 0.15; ■, 0.30. N_t/N_0, Number of viruses at any time/number of viruses at zero time.

increasing pH, a marked reduction in virucidal activity occurred, with initial ozone residuals at or below 0.15 mg/liter. pH effects were not observed at higher ozone input levels (0.18 mg/liter and above).

Inactivation of HRV by ozone was considerably more dramatic at all pH levels tested. Ozone residuals of as little as 0.05 mg/liter effected the complete inactivation (5 logs) of input virus at pHs 6 and 7 within 10 s (Fig. 4). Virus stability was only slightly enhanced at pH 8.0, at which 5-log inactivation was observed within 20 s at the same ozone residual (Fig. 5). Minimal enhancement of virus stability was noted when the system pH was further increased to 9.0 (Fig. 5), at which complete inactivation was achieved in 25 s with 0.1 mg of ozone per liter.

The overall pattern (i.e., rapid inactivation and pH effects) of rotavirus inactivation by ozone was not unlike that recently described for chlorine (14). Both studies used identical virus types and experimental conditions (average chlorine reductions, 0.03 to 0.07 mg/liter over 60 s, were similar to those identified for ozone), a situation which made feasible a fairly precise comparison of the relative virucidal efficiencies of chlorine and ozone. Three-log (99.9%) virus inactivation times from the present study (Fig. 1 to 5) and those reported by Vaughn et al. (14) were derived by extending a line through the y axis (N_t/N_0 [number of viruses at any time/number of viruses at zero time]) to its point of intersection on each inactivation curve and then vertically to its corresponding point on the x axis (time). Data were compared on the basis of disinfectant concentration and pH level (Table 1). In some instances inactivation occurred almost instantaneously, precluding any accurate measurement of 3-log reduction. These were assigned a maximum value of 6.0 s in Table 1 (although the actual value is probably much lower). As previously noted (14), SA-11 and HRV inactivation times under chlorine stress were similar. Values for SA-11 inactivation by ozone were not consistent with the chlorine data at each pH level. Chlorine appeared to be the more effective virucide at pH 7, whereas ozone induced more rapid inactivation at pHs 6 and 8. HRV inactivation times were considerably reduced at all ozone concentrations and pH levels tested, demonstrating the increased sensitivity of this strain to ozone.

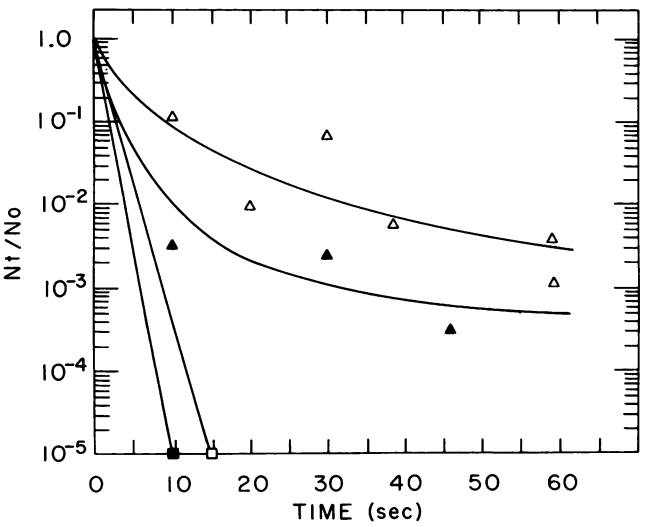

FIG. 2. Inactivation of SA-11 by ozone at pH 7.0. Ozone concentrations (milligrams per liter): △, 0.07; ▲, 0.12; □, 0.18; ■, 0.25. N_t/N_0, Number of viruses at any time/number of viruses at zero time.

DISCUSSION

The inactivation of human viruses, principally enteroviruses, by ozone has been the subject of a number of laboratory investigations. Experiments carried out in solutions ranging from distilled water to primary and secondary wastewater effluents and river water have demonstrated rapid virus inactivation with ozone residuals of 0.06 to 1 mg/liter (1, 3, 5, 7). Few such studies made use of single-particle virus preparations, and none addressed the inactivation kinetics of HRVs.

In the present study, simian rotavirus and HRV types were exposed as single particles to various ozone concentrations and pH levels at 4°C. HRV was considerably more

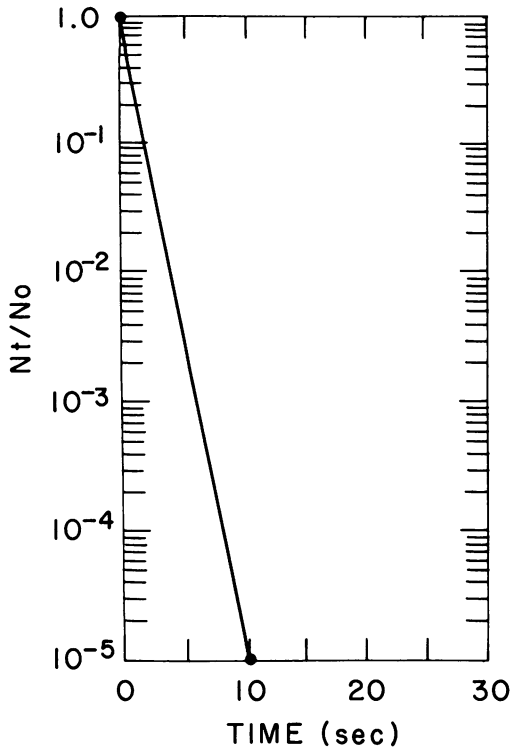

FIG. 4. Inactivation of HRV by ozone at pHs 6.0 and 7.0. The ozone concentration was 0.05 mg/liter. The results were identical at both pH levels. N_t/N_0, Number of viruses at any time/number of viruses at zero time.

sensitive than SA-11, with near instantaneous inactivation of available HRV at ozone concentrations as low as 0.5 mg/liter in pH 6.0 and 7.0 buffer and residuals of 0.1 mg/liter effecting 99.999% inactivation within 25 s at pHs 8.0 and 9.0. Ozone residuals of 0.1 to 0.2 mg/liter were usually sufficient to assure similar (<25 s) inactivation of SA-11 at all pH levels

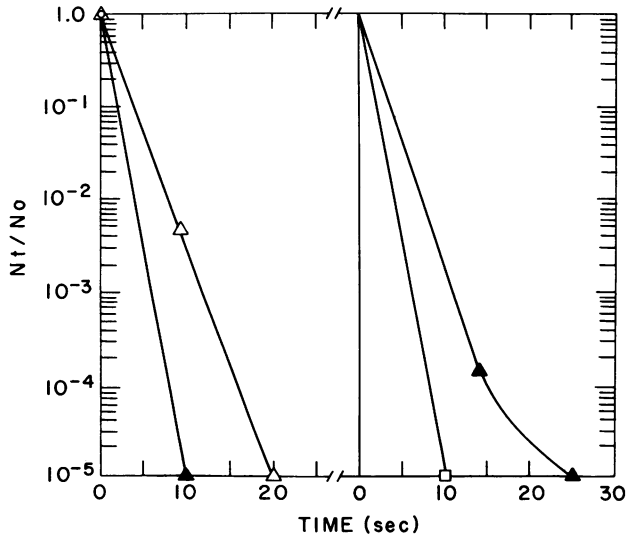

FIG. 5. Inactivation of HRV by ozone at pHs 8.0 and 9.0. The ozone concentrations (milligrams per liter) were as follows. pH 8.0: △, 0.05; ▲, 0.10. pH 9.0: ▲, 0.10; □, 0.20. N_t/N_0, Number of viruses at any time/number of viruses at zero time.

TABLE 1. Approximate times for 99.9% inactivation of SA-11 and HRV by comparable concentrations of chlorine and ozone (4°C)

Virus	pH	Disinfectant concn (mg/liter)	99.9% inactivation time (s)	
			Chlorine[a]	Ozone
SA-11	6.0	0.17	20.0	8.0
		0.25	6.0[b]	6.0
	7.0	0.1	21.0	32.0
		0.2	6.0	9.0
	8.0	0.1	28.5	6.0
HRV	6.0	0.1	46.0	6.0
		0.2	10.0	6.0
		0.3	7.0	6.0
	7.0	0.1	60.0	6.0
		0.2	8.0	6.0
		0.3	6.0	6.0
	8.0	0.1	39.0	6.0
		0.2	22.0	6.0
		0.3	10.0	6.0

[a] From data of Vaughn et al. (14).

[b] A value of 6.0 represents the shortest time which can be estimated from inactivation curves. Since 5-log inactivation was less than 10 s in these experiments, the actual 99.9% reduction was very likely <6.0 s.

tested. The disinfection efficiency of ozone for rotaviruses was such that pH effects (i.e., a reduction in inactivation rate with increasing pH) were negligible at ozone concentrations above 0.15 mg/liter.

Comparison of 99.9% virus inactivation times in the present study with those from a recent study with chlorine revealed similarities between inactivation of SA-11 and HRV by chlorine and SA-11 inactivation by ozone. Considerably lower values derived from HRV-ozone data demonstrated the superiority of ozone in the inactivation of the human strain. On the basis of these data alone, however, little advantage could be ascribed to the use of either disinfectant, especially when used at concentrations exceeding 0.25 mg/liter.

The present study provides preliminary information on the rates of HRV and simian rotavirus inactivation by ozone in a laboratory-controlled ozone-demand-free system. While the general principles elucidated during the study should remain constant, it is likely that rates will differ significantly in water samples representing more ecologically significant aquatic systems (i.e., treated drinking water, wastewater effluents, groundwater, etc.).

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