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Role of Disinfectant Concentration and pH in the Inactivation Kinetics of *Cryptosporidium parvum* Oocysts with Ozone and Monochloramine

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The objective of this study was to investigate the effect of disinfectant concentration and pH on the inactivation kinetics of *Cryptosporidium parvum* oocysts with ozone, monochloramine, and ozone/monochloramine at 20 °C. Experimental results revealed that the *CT* (product of disinfectant concentration and contact time) required to achieve a certain level of *C. parvum* inactivation was unique, thus demonstrating the validity of the *CT* concept for these single disinfectant and sequential disinfection processes for the range of experimental conditions investigated. Inactivation curves were represented accurately by a delayed Chick−Watson expression consistent with the *CT* concept. No pH dependence was observed for primary inactivation with ozone in the pH range of 6−10 or primary and secondary inactivation with monochloramine at pH values of 8 and 10. Oocyst resistance to chemical disinfectant attack was found to vary among oocysts lots as well as with oocyst aging within a given lot. The synergy observed for sequential disinfection with ozone/monochloramine suggested that monochloramine might be reacting with some of the same chemical constituents, both vital and nonvital, of the oocyst wall and/or cavity that also react with ozone. If so, partial completion of these reactions by the primary disinfectant would have resulted in the disappearance of the lag phase and the faster rate of inactivation observed for the secondary disinfectant.

Introduction

Ozone is currently considered an effective chemical disinfectant for the control of *Cryptosporidium parvum* oocysts in drinking water. The kinetics of *C. parvum* oocyst inactivation with ozone has been under investigation for over a decade (*1*−*8*). However, despite these efforts, there are still several important issues that remain to be resolved. Two of these issues are the effects of disinfectant concentration and pH on the inactivation kinetics. The current regulatory approach is based on the *CT* (product of disinfectant concentration and contact time) concept. In essence, this approach assumes that the level of inactivation corresponding to a certain *CT* value is unique independently of the combination of disinfectant concentration and contact time used to achieve that *CT*. Furthermore, it has been previously assumed that the *CT* required to achieve a certain level of inactivation efficiency is independent of pH within the range typically encountered in drinking water (*5*, *7*, *8*). However, the validity of the *CT* concept is still being challenged, and the pH independence has not been fully demonstrated (*6*). A major reason behind the challenge to the *CT* concept is that the inactivation kinetics of *C. parvum* oocysts with ozone tends to deviate from the classic first-order Chick−Watson expression, $\ln(N/N_0) = -kCT$, in which N/N_0 is the oocyst survival ratio after contact with the disinfectant at an average concentration C for a period of time T, and k is the inactivation rate constant.

C. parvum inactivation curves can be characterized by an initial lag phase or shoulder, during which little or no measurable inactivation occurs, followed by a pseudo-first-order inactivation rate with or without tailing effects. The shape of the inactivation curves might be the result of a process involving the reactive transport of the disinfectant through the various layers of the protective wall and inner oocyst constituents, as previously argued for bacteria (*9*). A certain degree of variability in morphology and reactivity is consistent with the early "mechanistic" conception of the disinfection process (*10*, *11*). Alternative empirical expressions proposed (*6*) to represent these types of inactivation curves include the classic nth-order Chick−Watson expression, $\ln(N/N_0) = -kC^nT$, and the Hom equation, $\ln(N/N_0) = -kC^nT^m$, in which n and m are constant coefficients. The validity of these expressions would imply that, in general, the *CT* required to achieve a certain level of inactivation varies with disinfectant concentration, and thus it is not unique.

An important point to be made is that although the validity of the first-order ($n = 1$) Chick−Watson model implies that the *CT* concept is valid, the reverse is not generally true since there are many other models that are also consistent with the *CT* concept. For example, the *CT* concept is valid for the Hom model with $n = m$ (*6*, *7*) and the Series−Event model (*12*) because in both of them a certain level of inactivation corresponds to a unique *CT* value. Whatever the mathematical expression of the model, the requirement for applicability of the *CT* concept is that the product *CT* should be the only independent variable accounting for disinfectant concentration and time effects in the expression.

A delayed first-order Chick−Watson expression also consistent with the *CT* concept, while at the same time allowing for the occurrence of a lag phase in inactivation curves, has been proposed to represent the inactivation of protozoan cysts (*13*). A simplified version of this model, in which no inactivation takes place during the lag phase, has been used to represent the inactivation kinetics of *C. parvum* oocysts with chemical disinfectants (*4*, *5*, *7*, *8*):

$$\frac{N}{N_0} = \begin{cases} \left(\frac{N}{N_0}\right)_c & \text{if } CT \le CT_{\text{lag}} = \frac{1}{k}\ln\left\{\left(\frac{N_1}{N_0}\right)\left(\frac{N_0}{N}\right)_c\right\} \\ \frac{N_1}{N_0}\exp(-kCT) & \text{if } CT > CT_{\text{lag}} = \frac{1}{k}\ln\left\{\left(\frac{N_1}{N_0}\right)\left(\frac{N_0}{N}\right)_c\right\} \end{cases} \quad (1)$$

where k is the post-shoulder inactivation rate constant, N_1/N_0 is the intercept with the ordinate axis resulting from extrapolation of the pseudo-first-order line, $(N/N_0)_c$ is the viability of the control, and CT_{lag} is the lag phase *CT*. Equation 1 was selected among expressions consistent with the *CT*

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TABLE 1. Summary of Single-Step and Sequential Disinfection Experiments (T = 20 °C)

		time elapsed from oocyst shedding (days)			control		ozone				monochloramine		
test	lot	shipment	cleaning	testing	S_c/EO_c	$(N/N_0)_c$	C (mg/L)	T (min)	pH	N/N_0	C (mg/L)	T (min)	pH
O-1	A	29	30	58	2.52	0.714	0.24	2.1−18.8	7.0				
O-2	A	29	30	57	2.82	0.713	1.01	0.5−4.5	7.0				
O-3	A	29	30	90	2.56	0.681	4.13	0.1−1.1	7.0				
O-4	B	26	27	53	1.84	0.560	0.41	0.61−1.83	6.0				
					2.24	0.472		2.44−4.39					
					1.93	0.550		5.37−7.32					
O-5	B	26	27	36	2.23	0.521	0.82	0.30−0.91	8.0				
					2.00	0.552		1.22−2.44					
					1.66	0.723		3.35−4.27					
O-6	B	26	27	51	2.29	0.505	0.42	0.60−1.79	10.0				
					2.38	0.530		2.38−4.29					
					2.54	0.489		5.23−7.14					
O-7	D	25	26	35	2.10	0.840	0.53	0.97−10.16	7.0				
M-1	A	29	30	37	2.98	0.636					4.21	480−2880	8.0
M-2	A	29	30	80	2.75	0.661					8.23	188−1125	8.0
M-3	C	11	14	65	2.77	0.529					7.90	180−710	8.0
				66	3.30	0.495					7.88	895−1440	
M-4	C	11	14	68	2.79	0.573					4.00	331−2880	8.0
M-5	C	11	14	73	2.70	0.492					1.03	1440−11 495	8.0
M-6	C	11	14	74	3.08	0.570					8.00	182−660	10.0
				75	3.62	0.476					8.00	900−1440	
M-7	D	25	26	35	2.34	0.718					8.19	196−1556	8.0
OM-1	A	29	30	108	3.51	0.656	1.17	1.63	7.0	0.119	1.04	50−400	8.0
OM-2	A	29	30	105	2.80	0.675	1.17	1.63	7.0	0.161	4.10	12.5−87.5	8.0
OM-3	A	29	30	102	2.86	0.639	1.28	1.48	7.0	0.147	8.23	8−48	8.0
OM-4	D	25	26	41	2.63	0.668	0.47	4.30	7.0	0.096	8.10	18−150	8.0
OM-5	D	25	26	63	2.84	0.728	0.47	4.30	7.0	0.088	8.17	17−135	10.0

concept mainly because it is more amenable for modeling the inactivation kinetics of *C. parvum* oocysts in flow-through reactors (*14*).

Ozone, in addition to reacting with pathogens, also reacts with many other constituents commonly present in natural waters (*15*). As a result, the dissolved ozone decays at a relatively fast rate, thus resulting in a lack of residual in the treated water. However, the recently promulgated Disinfectants/Disinfection By-Products Rule (*16*) requires the presence of a disinfectant residual in the distribution system. This requirement is commonly satisfied by the application of free or combined chlorine as a secondary disinfectant, following primary disinfection with ozone. Free chlorine and monochloramine have been found to be practically ineffective for inactivating *C. parvum* oocysts when used as single disinfectants (*2*, *5*, *7*, *8*, *17*). However, synergy has been shown to occur when these disinfectants are used in sequential disinfection schemes following ozone pretreatment (*5*, *7*, *8*). Utilities practicing such sequential disinfection schemes and providing sufficient contact time with secondary disinfectants might be achieving significantly greater disinfection efficiencies as compared to those attained by primary disinfection with ozone.

Driedger et al. (*7*) found that the *CT* concept is valid for secondary inactivation with free chlorine at pH 6 and 20 °C following pre-ozonation. Consistent with what it has been reported previously for other microorganisms (*18*), these authors also demonstrated the occurrence of a strong pH effect on the secondary inactivation kinetics, revealing that hypochlorous acid was the main inactivating species in the pH range of 6.0−8.5. A similar study assessing the roles of disinfectant concentration and pH in the kinetics of secondary inactivation with monochloramine, after ozone pretreatment, needs to be undertaken. The primary objectives of this study were to investigate the roles of disinfectant concentration and pH in the inactivation kinetics of *C. parvum* oocysts with ozone as primary disinfectant and monochloramine as both primary and secondary disinfectant.

Experimental Section

Oocysts. Experiments were performed with Iowa strain *C. parvum* oocysts propagated in bovine hosts at the Department of Veterinary Science, University of Arizona. Upon arrival to the University of Illinois, the oocyst suspensions were cleaned and stored following procedures described previously (*4*, *5*, *7*, *8*, *19*). Oocysts from four different shipments (lots A−D, Table 1) were used for the various experiments of the study. Times elapsed from oocyst shedding to shipment, cleaning, and testing are presented in Table 1.

Experimental Matrix. The effect of disinfectant concentration (0.24−4.13 mg/L) on the inactivation kinetics of *C. parvum* oocysts with ozone (tests O-1−3) was assessed at pH 7 with oocysts from lot A. The effect of pH (pH 6−10) on the ozone inactivation kinetics was investigated with oocysts from lot B (tests O-4−6). The dissolved ozone concentrations used for the pH tests were 0.41−0.82 mg/L. All ozone disinfection experiments were performed in a semi-batch reactor with the oocysts suspended in 0.01 M phosphate (pH 6−8) or borate (pH 10) buffer solution at 20 °C.

The roles of disinfectant concentration (1.03−7.90 mg/L as Cl_2) and pH (pH 8 and 10) in the inactivation kinetics of *C. parvum* oocysts with monochloramine used as a primary disinfectant were investigated with oocysts from lot C (tests M-3−5 and M-3,6, respectively). These tests served as the baseline for secondary inactivation experiments with monochloramine after ozone pretreatment. All experiments were performed in a batch reactor with 0.01 M phosphate (pH 8) or borate (pH 10) buffer solution with preformed monochloramine at 20 °C. pH 6 was not investigated to avoid potential interferences from other combined chlorine species (e.g., dichloramine) formed from monochloramine decomposition during the relatively long contact times investigated.

Sequential disinfection experiments were performed with oocysts from lots A and D. The oocysts were first treated with ozone under the conditions indicated in Table 1 and subsequently exposed to preformed monochloramine. Ex-

perimental variables were a secondary disinfectant concentration ranging from 1.04 to 8.23 mg/L as Cl_2 (tests OM-1−3 with lot A oocysts) and pH 8 and pH 10 (tests OM-4,5) with lot D oocysts. Ozone pretreatment was performed in a semi-batch reactor with 0.01 M phosphate buffer solution at pH 7 and 20 °C. Experimental conditions for secondary inactivation with monochloramine were the same as those previously described for primary treatment with this disinfectant. Tests O-7 and M-1,2,7 were performed with oocysts from lot A or lot D for further assessment of kinetic variability in the primary inactivation of oocysts from different lots with ozone or monochloramine, respectively.

Experimental Procedures. Ozone disinfection experiments were performed with a semi-batch reactor apparatus. The kinetics of primary and secondary inactivation with combined chlorine was investigated with a batch reactor system. Experimental components used and methods followed were described previously (*4*, *5*, *7*, *8*). Each test was designed to obtain an inactivation curve with generally 8−9 data points plus 1−3 control samples and a blank. A separate semi-batch reactor run was done to obtain each data point of an ozone inactivation curve. In contrast, serial sampling from a single batch reactor run was generally used to obtain all data points for a primary or secondary inactivation curve with monochloramine.

Viability Assessment. Oocyst viability of control and disinfected samples was assessed by the modified in vitro excystation method developed by Rennecker et al. (*4*) for all experimental sets presented in Table 1. The various steps comprising this method have been described in previous publications (*4*, *19*). Oocyst viability (N/N_0) was calculated with the expression (*4*)

$$\frac{N}{N_0} = \frac{S/X}{IO + EO} \tag{2}$$

where IO, EO, and S are the number of intact oocysts, excysted oocysts, and sporozoites, respectively, enumerated by phase contrast microscopy at a magnification of 1000×. X is the ratio of sporozoites to excysted oocysts in the control, S_c/EO_c, given in Table 1 for each test. The total number of entities, i.e., IO + EO + S, enumerated for each sample was at least 800 so that inactivation efficiencies as high as 99.5% could be determined with coefficients of variation within 50%. The number of sporozoites and excysted oocysts observed in control and treated samples were corrected to account for their presence in blank (i.e., oocyst stock) samples as described by Rennecker et al. (*4*). Microscopic observation of blank samples also confirmed the absence of clumps in all oocyst lots. Experimental results obtained with the modified in vitro excystation method have been shown to be consistent with infectivity data for primary disinfection with ozone (*4*, *5*) and chlorine dioxide (*19*, *20*) and secondary disinfection with monochloramine after ozone pretreatment (*5*).

Results and Discussion

Ozone Disinfection. Results obtained for the experiments (tests O-1−3) performed to assess the effect of disinfectant concentration on the inactivation kinetics of *C. parvum* oocysts (lot A) with ozone are presented in Figure 1. As depicted in the figure, there is good agreement among the three data sets when plotted in terms of oocyst survival ratio versus *CT*. These results confirm the applicability of the *CT* concept for the inactivation kinetics of *C. parvum* oocysts with ozone over the concentration range investigated.

The ozone inactivation curves were characterized by a lag phase followed by inactivation at an initially fast rate that subsequently slowed after approximately 1.5 log of inactivation. It is interesting to observe that the faster initial rate

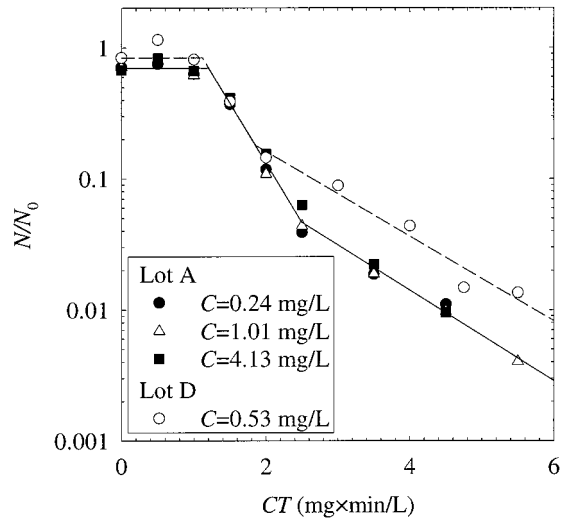

FIGURE 1. Effect of disinfectant concentration on the primary inactivation kinetics of *C. parvum* oocysts with ozone at pH 7 and 20 °C.

corresponded to a rate constant $k_1 = 2.10 \pm 0.17$ L/(mg·min), a value within 28% of the value 1.65 ± 0.21 L/(mg·min) reported by Driedger et al. (*8*) at 20 °C for a different lot of Iowa strain *C. parvum* oocysts with weaker resistance to ozone as compared to that previously observed with other lots (*4*, *5*). Furthermore, the constant for the subsequent slower rate of inactivation, $k_2 = 0.797 \pm 0.049$ L/(mg·min), was within 1−24% of the range of 0.804 ± 0.053 to 1.04 ± 0.08 L/(mg·min) observed by Rennecker et al. (*4*, *5*) at 20 °C with lots of the stronger Iowa strain *C. parvum* oocysts.

Consistent with previous observations (*4*), the excystation efficiency shown in Figure 1 for samples with relatively low exposure to ozone ($CT = 0.5$ mg·min/L) was greater (5−37%) than that of the corresponding control, i.e., data at $CT = 0$ mg·min/L. Such enhancement of excystation efficiency, though not seen for all oocyst lots, has also been observed with chlorine dioxide (*19*) and sodium hypochlorite (*4*, *21*). In agreement with these observations, Neumann et al. (*22*) found that unexcysted oocysts following in vitro excystation of control samples can be infective.

The curves in Figure 1 would be consistent with the oocysts from lot A being a mixture of two distinct populations of strong and weak oocysts. Interestingly, oocysts from lot D (test O-7) showed a similar trend in resistance to ozone with a similar lag phase and subsequent inactivation rates as also depicted in Figure 1. However, the fraction of stronger oocysts would be somewhat greater than that for oocysts from lot A. The slope of the portion of the inactivation curve with the slower rate was $k_2 = 0.744 \pm 0.093$ L/(mg·min), not a statistically different value from that for lot A oocysts.

Equation 1 can be extended to represent inactivation curves, such as those shown in Figure 1, with a lag phase and two segments of pseudo-first-order kinetics:

$$\frac{N}{N_0} = \begin{cases} \left(\frac{N}{N_0}\right)_c & \text{if } CT \le CT_{lag} = \frac{1}{k_1}\ln\left\{\left(\frac{N_1}{N_0}\right)\left(\frac{N_0}{N}\right)_c\right\} \\ \frac{N_1}{N_0}\exp(-k_1 CT) & \text{if } CT_{lag} \le CT \le CT_{12} \\ \frac{N_2}{N_0}\exp(-k_2 CT) & \text{if } CT \ge CT_{12} = \frac{1}{k_2 - k_1}\ln\left\{\left(\frac{N_2}{N_1}\right)\right\} \end{cases} \tag{3}$$

where k_1 and k_2 are the rate constants or slopes of the faster

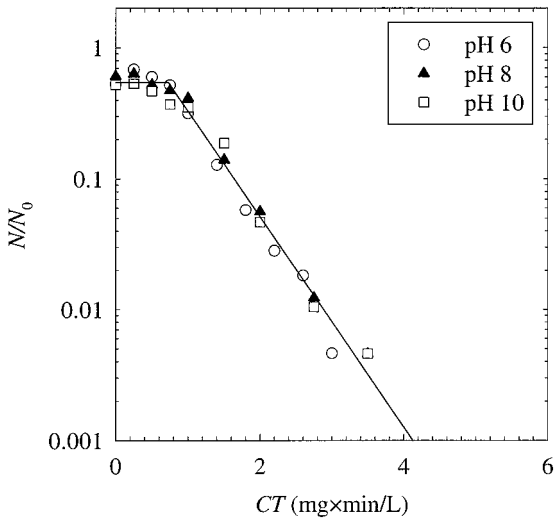

FIGURE 2. Effect of solution pH on the primary inactivation kinetics of *C. parvum* oocysts with ozone at 20 °C.

and slower pseudo-first-order lines [in L/(mg·min)], N_1/N_0 and N_2/N_0 are the respective intercepts with the ordinate axis resulting from extrapolation of the faster and slower pseudo-first-order lines, CT_{lag} is the lag phase *CT* (in mg·min/L), CT_{12} is the *CT* value corresponding to the intercept of the two pseudo-first-order lines (in mg·min/L), and $(N/N_0)_c$ is the viability of the control given in Table 1. Least-squares fitting of the data shown in Figure 1 with eq 3 resulted in the values reported in the preceding paragraphs for k_1 and k_2, $N_1/N_0 = 8.74 \pm 3.14$, and $N_2/N_0 = 0.342 \pm 0.064$ (lot A) or 0.713 ± 0.268 (lot D). It is interesting to notice that the value $N_1/N_0 = 8.74 \pm 3.14$ obtained for the weaker oocysts in this study is significantly greater than that observed by Driedger et al. (*8*), $N_1/N_0 = 1.69 \pm 0.92$, for oocysts having a comparable post-lag-phase rate of inactivation. This observation is consistent with that reported by Rennecker et al. (*5*) for the inactivation of three different lots of the stronger oocysts with ozone. Because no correlation is observed between the extent of the lag phase, CT_{lag}, and the corresponding post-lag-phase rate of inactivation with ozone, the term weaker or stronger oocyst used in this study will refer to oocysts with a faster or slower post-shoulder rate of inactivation, respectively, independent of the magnitude of the corresponding lag phase.

It is important to point out that the fitted lines shown in Figure 1, and in subsequent figures of this study, should not be considered to apply beyond the range for which experimental data is provided. Tailing effects in inactivation curves have been reported (*6*, *17*) at inactivation efficiencies greater than 99.5%, the limit of the viability assessment method used in this study.

Results obtained for the experiments performed with oocysts from lot B to assess the role of pH in the inactivation kinetics of *C. parvum* oocysts with ozone (tests O-4−6) are presented in Figure 2. As depicted in the figure, no significant effect of pH was observed within the range of pH 6−10. The three inactivation curves were characterized by a lag phase ($N_1/N_0 = 2.08 \pm 0.38$) followed by inactivation at a constant rate with $k = 1.85 \pm 0.08$ L/(mg·min). This rate constant value is within 14% of both that observed for the faster initial inactivation rate with oocysts from lots A and D in this study and the value reported by Driedger et al. (*8*) at the same temperature of 20 °C.

An interesting observation for the inactivation of *C. parvum* oocysts with ozone in this study as well as in previous investigations with both ozone (*4*, *5*) and chlorine dioxide (*19*) is that rather than a uniform distribution in resistance variability for oocysts from different lots, two distinct types or populations of oocysts, each with more limited variability, have been observed with the weaker oocysts having post-shoulder inactivation rates 1.6−2.6× faster than those observed for the stronger oocysts. Furthermore, the stronger oocysts have been observed to transform into weaker oocysts with aging, but rather than observing a gradual increase in the post-lag-phase inactivation rate, the change tends to happen suddenly after several months of storage (*5*). Sometimes, a new oocyst lot has the inactivation kinetics of the weaker oocysts from the beginning. Such was the case for the lot of oocysts used for the pH experiments with ozone in this study (Figure 2). Other times, lots of oocysts appear to be a mixture of stronger and weaker oocysts such as those used for the ozone concentration test of this study (Figure 1). It is important to emphasize once again that the terms stronger and weaker are used to differentiate between oocyst lots based on the post-shoulder rate of inactivation. Lot aging can also result in a decreasing number of viable oocysts in control samples as well as shortening of the shoulder, but such effects do not generally correlate with corresponding changes in the post-lag-phase rates of inactivation.

The lack of pH dependence for the ozone inactivation curves observed in this study can be interpreted by assessing the chemistry of this disinfectant in aqueous solution. The ozone disinfection experiments were performed in a semi-batch reactor in order to maintain constant the dissolved ozone concentration for the duration of the experiment. Under these conditions, the distribution of ozone decomposition intermediates, including the strong and unselective oxidizing agent hydroxyl radical, in solution should have been different at the two extreme pH values investigated (pH 6 and pH 10). The concentrations of these secondary species, including hydroxyl radical, were estimated based on the ozone decomposition mechanism proposed by Staehelin and Hoigné (*23*), Bühler et al. (*24*), and Staehelin et al. (*25*). The resulting ozone decomposition model was mathematically described by a system of ordinary differential equations and solved numerically as described elsewhere (*14*). The calculation for a synthetic phosphate-buffered solution revealed that the concentration of hydroxyl radical at pH 10 in the presence of dissolved ozone at a concentration of 0.5 mg/L was 3.4×10^{-9} M, a value at least 100 times higher than that estimated at pH 6 in the presence of the same dissolved ozone concentration. Consequently, it appeared that the rate of *C. parvum* oocysts inactivation was not affected by the presence of hydroxyl radicals at the low concentration levels estimated. It is anticipated that the same conclusion could be reached for natural waters for which carbonate and organic impurities, serving as hydroxyl radical scavengers, would result in the formation of comparable or even lower hydroxyl radical concentrations.

Primary Disinfection with Monochloramine. Results obtained for the experiments (tests M-3−5) performed to assess the effect of disinfectant concentration on the inactivation kinetics of *C. parvum* oocysts (lot C) with monochloramine are presented in Figure 3. The good agreement among the three data sets reveals that the *CT* concept is also valid for the inactivation kinetics of *C. parvum* oocysts with monochloramine within the concentration range of 1−8 mg/L as Cl_2 investigated. The monochloramine inactivation curve is characterized by a lag phase followed by a pseudo-first-order rate of inactivation. Least-squares fitting with eq 1 (continuous line in Figure 3) resulted in $k = 0.000854 \pm 0.000075$ L/(mg·min) and $N_1/N_0 = 48.5 \pm 35.1$. Also shown in Figure 3 is the inactivation curve for lot D oocysts (test M-7). As depicted in the figure, these oocysts had a control with higher viability (see Table 1) and were somewhat more resistant to monochloramine attack than lot C oocysts. However, the greater resistance for lot D oocysts

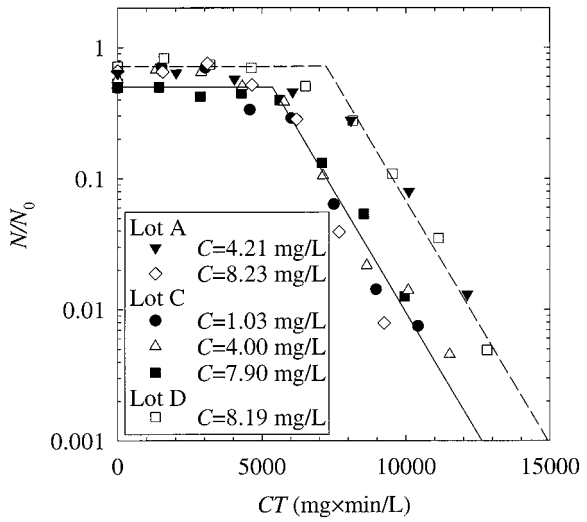

FIGURE 3. Effect of disinfectant concentration on the primary inactivation kinetics of *C. parvum* oocysts with monochloramine at pH 8 and 20 °C.

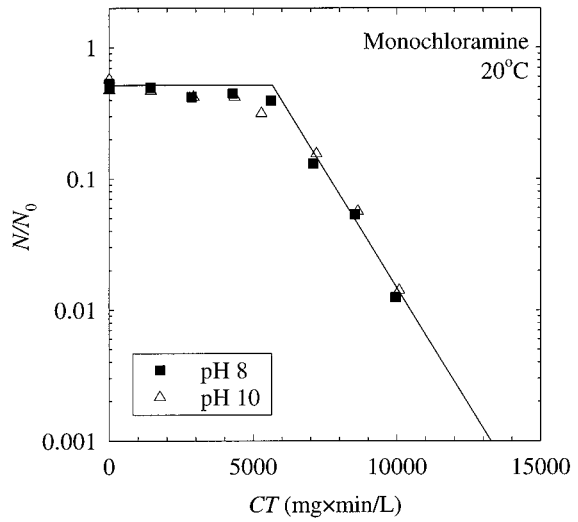

FIGURE 4. Effect of solution pH on the primary inactivation kinetics of *C. parvum* oocysts with monochloramine at 20 °C.

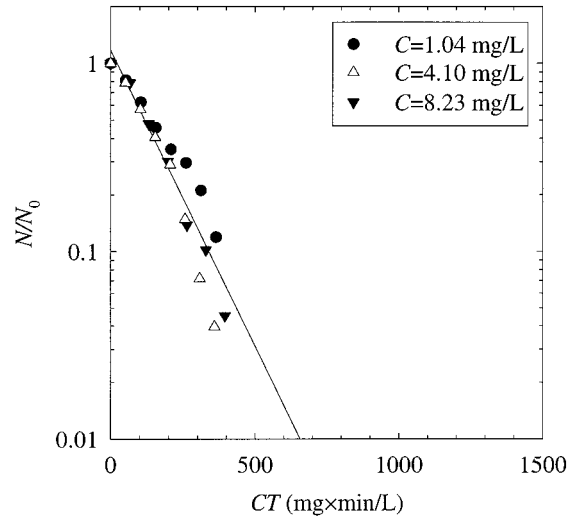

FIGURE 5. Effect of disinfectant concentration on the secondary inactivation kinetics of *C. parvum* oocysts with monochloramine at pH 8 and 20 °C, following ozone pretreament at pH 7 and 20 °C.

was primarily due to a longer lag phase with the subsequent inactivation rate being essentially the same for lots C and D oocysts. Fitting of the inactivation curve for lot D oocysts with eq 1 resulted in $k = 0.000854 \pm 0.000084$ L/(mg·min) and $N_1/N_0 = 343 \pm 307$.

Monochloramine inactivation curves were also obtained with oocysts from lot A (tests M-1,2) at two different times. As depicted in Figure 3, the results obtained when the oocysts were only 37 days old were consistent with those obtained for the stronger oocysts from lot D. The older oocysts from lot A (80 days old) had lower resistance to monochloramine attack matching closely the inactivation kinetics of the weaker oocysts from lot C.

The primary monochloramine inactivation curves obtained in this study for oocysts from lots A, C, and D are consistent with curves reported previously. The inactivation rate constant observed in this study is within 7% of the previously reported values $k = 0.000801 \pm 0.000109$ L/(mg·min) (*5*) and $k = 0.000836 \pm 0.000047$ L/(mg·min) (*8*) obtained at the same temperature of 20 °C.

Results obtained for the experiments performed with oocysts from lot C (test M-3,6) to assess the role of pH in the inactivation kinetics of *C. parvum* oocysts with monochloramine are presented in Figure 4. As depicted in the figure, the two curves obtained at pH 8 and pH 10 were essentially identical. Fitting of the two inactivation curves with eq 1 resulted in $N_1/N_0 = 54 \pm 31$ and $k = 0.000821 \pm 0.000067$ L/(mg·min). The value for the post-lag phase inactivation rate constant is within 4% of that observed for the concentration dependence tests with the same oocyst lot.

Secondary Disinfection with Monochloramine. Results obtained for experiments (tests OM-1−3) performed to assess the role of disinfectant concentration in the secondary inactivation kinetics of *C. parvum* oocysts (lot A) with monochloramine after ozone pretreatment are presented in Figure 5. Because the ozone pretreatment resulted in somewhat different primary inactivation efficiencies (Table 1), the secondary inactivation curves shown in Figure 5 were normalized by dividing all N/N_0 values by the corresponding survival ratio after ozone pretreatment. As depicted in the figure, the *CT* concept was also valid despite some variability in the results. As reported previously (*5*, *8*), the ozone pretreatment resulted in a disappearance of the lag phase. Furthermore, the subsequent rate of secondary inactivation with monochloramine, $k = 0.00726 \pm 0.00051$ L/(mg·min), is approximately 8.5× faster than that observed for primary monochloramine inactivation with oocysts from the same lot. This rate of secondary inactivation is 75−89% faster than the secondary inactivation rate constant values $k = 0.00385 \pm 0.00035$ (*5*) and 0.00415 ± 0.00019 L/(mg·min) (*8*) previously reported for stronger oocysts. Also, these differences are similar to that of 69% observed by Rennecker et al. (*5*) for the secondary monochloramine inactivation of oocysts from weaker and stronger lots at the experimental temperature of 4 °C. It is interesting to observe the occurrence of discrepancies between secondary inactivation rates for oocysts from weaker and stronger lots, even though the post-lag-phase rate for primary inactivation with the monochloramine is approximately the same for both types of oocysts. No explanation can be provided at this time for the different effects.

The synergy observed for sequential disinfection with ozone/monochloramine suggests that monochloramine might be reacting with some of the same chemical constituents, both vital and nonvital, of the oocyst wall and/or cavity that also react with ozone. Partial completion of these reactions by the primary disinfectant might have resulted in the disappearance of the lag phase and the faster rate of inactivation observed for the secondary disinfectant. Ad-

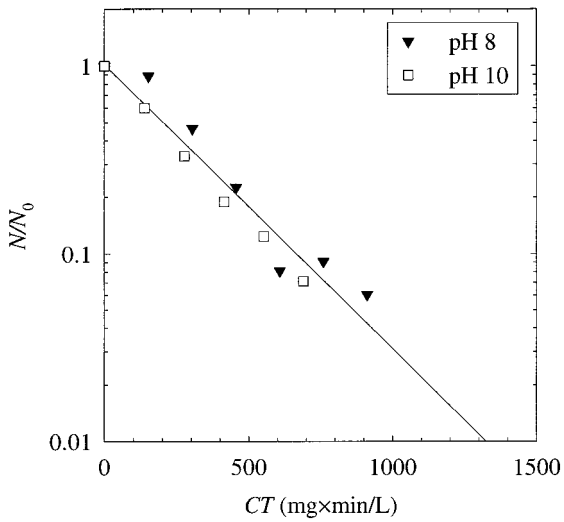

FIGURE 6. Effect of solution pH on the secondary inactivation kinetics of *C. parvum* oocysts with monochloramine at 20 °C, following ozone pretreament at pH 7 and 20 °C.

ditional research is needed to unveil the actual mechanisms responsible for these effects.

Results obtained for the experiments performed with oocysts from lot D (test OM-4,5) to assess the role of pH in the inactivation kinetics of *C. parvum* oocysts with monochloramine after ozone pretreatment are presented in Figure 6. Consistent with the results obtained for primary inactivation, the two curves obtained at pH 8 and pH 10 were essentially identical. The corresponding rate constant $k = 0.00349 \pm 0.00026$ L/(mg·min) was within 16% of those reported previously (*5*, *8*) for oocysts from stronger lots.

The lack of pH dependence for the primary and secondary inactivation of *C. parvum* oocysts with monochloramine is consistent with monochloramine being essentially the only chloramine species present in solution at the pH and relatively low chlorine-to-ammonia weight ratio (approximately 1:1) investigated (*26*).

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