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Inactivation of Poliovirus by Chloramine-T

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Since concern has recently been expressed about the presence of genotoxic substances due to chlorination of water and wastewater, chloramine-T (CAT) is proposed as an alternative disinfectant to chlorine. The viricidal properties of chlorine and CAT were compared. Kinetics of inactivation of poliovirus type 2 by chlorine and CAT in chlorine demand-free water were investigated by using a kinetic apparatus. Inactivation of the virus by chlorine and CAT occurred in two steps. The initial linear part of the inactivation curve followed a pseudo-first-order reaction with the virus. An obvious dose-response relationship was demonstrated with CAT. The rate of inactivation of the virus by CAT was faster in acid medium than in alkaline medium. Inactivation kinetic studies were performed at different temperatures, and the kinetic, Arrhenius, and thermodynamic parameters were evaluated. The rate of inactivation of poliovirus type 2 by chlorine was faster than that by CAT under identical conditions. A mechanism for the viral inactivation in acid conditions was proposed which led to a rate equation consistent with the experimental results. The results indicate that CAT may be an effective viricide against poliovirus type 2 in an acid medium.

Ever since the disinfection process became a standard part of drinking water and wastewater treatment in the United States and other developed nations, chlorine has been the predominant disinfectant. Recently, concern has been expressed by several investigators (2, 14, 18, 27, 28, 30, 31, 35) about the presence of potentially genotoxic substances in drinking water. Studies have shown organic compounds recovered from chlorine-treated waters in the United States and Japan to be mutagenic (14, 27). Most evidence suggests that the formation of mutagenic substances takes place during the water and wastewater disinfection processes. Chlorination, which is known to produce halogenated substances, appears to be the cause of the genotoxic activity in drinking water (27). Hence, there is need for an alternate effective disinfectant which forms a minimum of genotoxic compounds in water and wastewater. A review of the literature suggests that chemicals such as chlorine dioxide, inorganic chloramines, and ozone have been proposed or used as alternatives to chlorine for disinfection of drinking water (19, 21). Inorganic chloramine- or ozone-treated and recycled water were shown to be mutagenic in bacteria (18, 25). Chloramine-T (CAT), p-$CH_3C_6H_4SO_2NClNa \cdot 3H_2O$, may be an alternative disinfectant to standard chlorine. It behaves chemically more as an oxidant than a chlorinating agent. Thus, it would be much less likely to form genotoxic compounds. These positive qualities have led to an interest in CAT as a disinfectant of water. One of the essential features of a disinfectant is its viricidal properties.

To our knowledge, no basic research has been done on the inactivation kinetics of viruses by CAT. Inactivation kinetic studies of poliovirus have been carried out with formaldehyde (33), chlorine, hypobromite, molecular bromine, inorganic haloamines (7-9), chlorine dioxide (19, 32), and ozone (21, 22).

In this study, the viricidal properties of CAT and chlorine are compared. Specifically, we investigated the kinetic and mechanistic aspects of inactivation of poliovirus type 2 by CAT by determining (i) 99% inactivation times and rate constants at varying CAT concentrations and pH's and (ii) kinetic and thermodymanic parameters. Mechanisms of poliovirus inactivation are proposed, and rate expressions are derived which are in agreement with the experimental data and those of other investigations (21, 22). A simple method of concentration and purification was used for the first time to achieve a chlorine demand-free poliovirus type 2 suspension of high titer.

MATERIALS AND METHODS

Poliovirus stock. The same stock of poliovirus type 2 was used throughout the study. The virus was grown on human amnion WISH cells (Flow Labora-

tories, Inc., Rockville, Md) and accumulated. Cells were grown to monolayers in Eagle minimal essential medium containing Hanks salts supplemented with 10% fetal bovine serum, 100 μg of streptomycin per ml, and 100 U of penicillin per ml and infected with five 50% infective doses ($TCID_{50}$) per cell. The virus was harvested when 75 to 100% of the cells in culture showed cytopathology. Then the virus-containing fluids were frozen and thawed once and centrifuged at 1,000 × *g* for 15 min to remove cell debris.

Preparation of chlorine demand-free poliovirus. The sodium dextran sulfate(SDS)-polyethylene glycol (PEG) phase separation method of Albertsson (1) with slight modification was used to concentrate the virus (preparation of reagents is shown below). The PEG solution (210 g) containing NaCl and 20% SDS solution (10.5 g) were thoroughly mixed. The mixture was added, in equal portions (~10 ml each) to a liter separatory funnel containing 700 ml of poliovirus suspension. After each addition, the funnel was thoroughly shaken. Then the system was allowed to stand in the cold (4°C) for 24 h. The bottom phase (4 ml) harvested was treated with 3 M KCl (3.5 ml) to precipitate SDS. Since the highly purified and concentrated virus suspension harvested from the lower phase had a high chlorine demand even after removal of most of the SDS by precipitation with KCl (Table 1), we suspected that residual SDS may have been responsible. The residual SDS was removed, therefore, by treating the virus suspension with the enzyme dextranase, which degrades SDS into low-molecular-weight fragments (20) that would pass through an Amicon filter. Specifically, the purified and concentrated virus suspension obtained was diluted 10 times with phosphate buffer (0.1 M KH_2PO_4 + 0.1 M NaOH), pH 6, and treated with 4 U of the enzyme (20) per ml of virus suspension and incubated for 2 h at 37°C. Then the resultant suspension was filtered through an Amicon filter (10×M 300, 43 mm; Diaflo ultrafiltration membranes, Amicon Corp., Lexington, Mass.) and washed several times with 10-ml portions of sterile distilled water. The pure virus concentrate, shown to be virtually chlorine demand-free (Table 1) was diluted with chlorine demand-free phosphate-buffered saline (0.15 M) containing 1 M $MgCl_2$ and stored in 5-ml vials at −70°C. The titer of the purified virus concentrate was approximately 10^{10} $TCID_{50}$ per ml.

TABLE 1. *Preparation of chlorine demand-free poliovirus*

Poliovirus suspensions	Vol (ml)	Titer ($TCID_{50}$/ml)	Chlorine demand[a] (mg/liter)
Crude	700	3×10^7	1.65
PEG-SDS two-phase separation. Bottom phase:			
(i) Without KCl precipitation	4	10^{10}	0.80
(ii) With KCl precipitation	6	10^{10}	0.56
Enzyme (dextranase) treatment and Amicon filtration of (ii)	5	10^{10}	0.10
Addition of 45 ml of 1 M $MgCl_2$	50	10^9	0.08

[a] Chlorine used, 2 mg/liter; contact time, 60 min.

Enzyme solution. One hundred units of dextranase (α-1,6-glucan 6-glucanohydrolase) obtained from a *Penicillium* species (Sigma Chemical Co., St. Louis, Mo) was dissolved in 0.5 ml of tris(hydroxymethyl)-aminomethane-hydrochloride buffer of pH 6, and this solution was used in purifying poliovirus type 2.

Virus assays. All virus was diluted in Eagle minimal essential medium containing Earle salts supplemented with 2% fetal bovine serum and antibiotics (100 μg of streptomycin and 100 U of penicillin per ml). The virus assays (34) were performed on WISH cell monolayers grown in 96-well microtiter plates. One-tenth milliliter of each serial $\log_{10}$ dilution of each sample was inoculated into four replicate wells. The microtiter plates were then incubated for 48 h at 37°C in a 5% CO_2 atmosphere. The cultures were stained with crystal violet solution (1:100, wt/vol) in 20% methyl alcohol in water, and the cytopathic effect was read. The mean $TCID_{50}$ per milliliter was calculated by the Kärber method (29).

Chlorine. Sodium hypochlorite solution (4 to 6%; Fisher Scientific Co., Pittsburgh, Pa.) was standardized iodometrically (2), diluted to a concentration of 100 mg of chlorine per liter, and stored as a stock solution at 4°C. The concentration of the stock solution was also checked spectrophotometrically by the standard orthotolidine-arsenite method (2).

CAT. CAT (Eastman Kodak Co., Rochester, N.Y.) was freed from possible *p*-toluene sulfonamide and dichloro contaminants by washing it several times with carbon tetrachloride and dried in a vacuum desiccator over $CaCl_2$. The purity of the sample determined iodometrically was >99%, as reported by Gowda et al. (15). The stock solutions of CAT were prepared by dissolving the solid (2 g/liter) in chlorine demand-free distilled water and stored at 4°C.

Determination of chlorine demand. The chlorine demand of sterile distilled water (Abbott Laboratories, North Chicago, Ill.) used as a diluent, and of the purified poliovirus suspension was determined spectrophotometrically by the standard orthotolidine-arsenite method (2). Spectrophotometry was carried out with a Gilford model 250 dual-source spectrophotometer fitted with digital readout.

Buffer solutions. Aqueous solutions of 0.5 M monobasic sodium phosphate (A) and 0.5 M dibasic sodium phosphate (B) were prepared in chlorine demand-free distilled water, autoclaved for 30 min, and stored at 4°C. Forty-milliliter buffers having the desired pH were prepared from A and B as follows (16): for pH 6.0, 35.08 ml of A and 4.92 ml of B; for pH 7.0, 15.6 ml of A and 24.4 ml of B; for pH 7.8, 3.4 ml of A and 36.6 ml of B; for pH 10.0, 40 ml of B. After mixing, the solutions were diluted to 400 ml with water. For the pH 10 buffer, 40 ml of B was diluted to 400 ml, and the pH was adjusted with a small volume (0.32 ml) of 2 M NaOH.

SDS. An aqueous solution of 20% SDS (Sigma) was prepared and autoclaved.

PEG. A solution of PEG 6000 (Fisher Scientific Co.) was prepared by dissolving 120 g of the polymer in 360 g of distilled water containing 24.93 g of NaCl and autoclaved.

Potassium chloride. An aqueous solution of 3 M

KCl (Fisher Scientific Co.) was prepared and autoclaved. This reagent was used to precipitate the SDS.

Chlorine demand-free glassware. Preliminary experiments showed that the use of chlorine demand-free glass- and metalware is essential for maintaining a constant concentration of chlorine during the experiments. Therefore, all glassware was first treated with chromic acid and washed with detergent. Then the glassware and metalware were soaked with 1% chlorine water, scrubbed with water, rinsed with chlorine demand-free distilled water, dried, and autoclaved.

Viral inactivation kinetics experiments. Experiments for the kinetics of inactivation of poliovirus type 2 were performed by using a kinetic apparatus (32). This apparatus consisted of six stainless-steel beakers of 600-ml capacity, a water bath, a thermoregulator, and six stainless-steel stirring rods connected to an overhead stirring device. Two beakers containing control solutions were used to determine the effects of the temperature and pH of the medium on virus inactivation. The remaining beakers contained test solutions. The solutions in all beakers were stirred throughout the experiment and maintained at the desired temperature in the carefully regulated water bath. A known volume of the stock solution of chlorine or CAT and 40 ml of a mixture of the two phosphate solutions (0.5 M each) were added to all beakers except the one used as a temperature control, which contained the same volume of water. Next, the solution was diluted to 399.5 ml with sterile, chlorine demand-free water. The actual timed experiment began at the inoculation of the test virus (0.5 ml) into the rapidly stirring (100 rpm) solutions. Five-milliliter portions of the solutions containing CAT or chlorine were withdrawn at definite contact time intervals and mixed immediately with 5 ml of thiosulfate. The concentration of thiosulfate solution used was adjusted to neutralize the toxic effects of CAT or chlorine without leaving excess residual thiosulfate which was toxic to cells (1.15 ppm of $Na_2S_2O_3$ [1.15 mg of $Na_2S_2O_3$ per liter]/ppm of CAT; 4.45 ppm of $Na_2S_2O_3$/ppm of chlorine) used for virus assay. In addition, since complete neutralization of the reactants was not observed at alkaline pH, the thiosulfate solutions used under these conditions were prepared in ~0.04 M HCl. In the case of control solutions, the toxic thiosulfate solution was replaced by the same volume of water, since control studies showed that this variation had no effect on virus titers obtained.

For each experiment, the time required for 99% inactivation of the virus and the first-order rate constant, k_l, were calculated from the initial linear part of the curve.

RESULTS

Inactivation of poliovirus type 2 by CAT. The kinetics of inactivation of poliovirus type 2 by CAT was investigated at several concentrations of the reactant. Assuming that inactivation of the virus is due to a chemical reaction between CAT and some susceptible site of the virus essential for infectivity, the kinetic laws of biomolecular reactions should be followed. Since CAT was used in great excess, pseudo-first-order kinetics yielding a straight line on semilogarithmic plot might be expected. However, in practice, departures from the expected linearity have been observed for chlorine. Gard (cited in reference 32) proposed that such deviations were due to reactions between chlorine and virus that did not result in inactivation of some of the virus particles. Similar to chlorine, the first part of the CAT inactivation plots obtained by plotting log n/n_0 (n = $TCID_{50}$) versus time were linear and thus obeyed a pseudo-first-order kinetics (Fig. 1). This indicates a first-order dependence of the inactivation rate on the virus (Table 2), since the CAT concentration was in large excess and remained constant throughout the reaction.

An interesting feature observed was the two-stage shape of the inactivation curve, the initial part showing a much faster rate than the second part. Our curves are in agreement with the kinetic curves reported in the literature for the inactivation of poliovirus (9, 21, 22). A possible interpretation for the two-stage reaction might be that 0.5 to 1% of the virus consists of clumps (11, 21).

Effect of CAT concentration on the inactivation rate of poliovirus type 2. The results in Table 2 show the dose-response relationship between the rate of inactivation of poliovirus type 2 and CAT concentration. A decrease in 99% inactivation time period was observed with increase in the concentration of CAT. A plot of log CAT concentration against log 99% inactivation time was linear with negative slope (Fig. 2), showing a direct correlation between the disinfectant concentration and the duration of 99% inactivation at pH 6.0, 7.0, and 7.8. The higher the CAT concentration, the shorter was the duration of the first part of the curve. In fact, at low CAT concentrations of 10 and 20 mg/liter, the two-stage inactivation did not even appear in Fig. 1. Furthermore, a plot of log k_l versus log [CAT] (molarity) gave a straight line

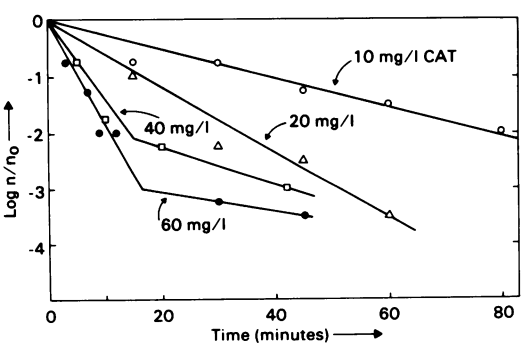

FIG. 1. *Kinetics of inactivation of poliovirus type 2 at the indicated concentrations of CAT at pH 6 and 5°C (where n/n_0 equals the surviving fraction).*

TABLE 2. *Effect of CAT concentration and pH on the rate of inactivation of poliovirus type 2 in water at 5°C*

CAT concn		pH 6		pH 7		pH 7.8		pH 10[a]	
mg/liter	M ($\times 10^4$)	Time[b] (min)	k_1 ($\times 10^3$) (s^{-1})	Time (h)	k_1 ($\times 10^4$) (s^{-1})	Time (h)	k_1 ($\times 10^4$) (s^{-1})	Time (h)	k_1 ($\times 10^5$) (s^{-1})
10	0.355	78	0.98	4.35	2.94	8.1	1.58	33	3.88
20	0.71	34	2.26			5.5	2.33	>8	
40	1.42	14	5.29	1.35	9.48	4.8	2.67	>6	
60	2.13	11	6.98	1	12.79	2.62	4.88	27.1	4.74

[a] The curves at pH 10 showed more scattering than at other pH's, so calculated parameters are less reliable here.

[b] 99% inactivation time.

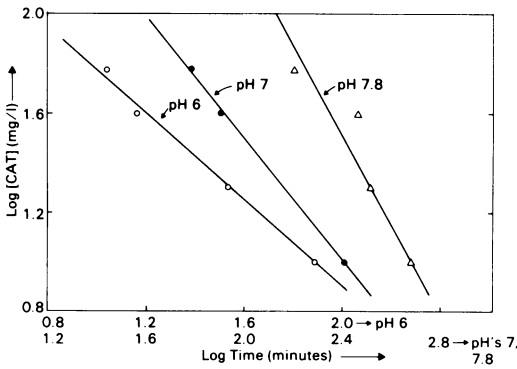

FIG. 2. *Time-concentration relationships for 99% inactivation of poliovirus type 2 at different pH's and 5°C (log-log scale).*

with a slope equal to 1.1 (Fig. 3), establishing a first-order dependence on the disinfectant concentration at pH 6 and 5°C. The same trend was observed at pH 7.0 and 7.8 (Table 3; Fig. 3). The deviation from unity may have been due to experimental error or to a change in mechanism at higher pH. Deviations of a similar magnitude have been observed previously for aqueous disinfectants (22).

Effect of pH on the inactivation rate of poliovirus type 2. The kinetics of inactivation of poliovirus type 2 by CAT at pH 6.0, 7.0, 7.8, and 10 were also two stage (Table 2). The inactivation rate decreased linearly with increase in pH, whereas the 99% viral inactivation time increased proportionately with increase in pH. Furthermore, at a CAT concentration of 10 mg/liter, a plot of log k_1 versus pH gave a straight line with a slope of −0.5, showing that the order with respect to $[OH^-]$ was a negative fraction, whereas the order with respect to $[H^+]$ was a positive fraction (Fig. 4). The same trend was noticed at higher CAT concentrations of 40 and 60 mg/liter (Table 3; Fig. 4).

Effect of temperature on the rate of inactivation of poliovirus type 2. The kinetics

TABLE 3. *Order of the inactivation reaction of poliovirus type 2 with CAT at 5°C*

CAT (mg/liter)	Order with respect to $[H^+]$	pH	Order with respect to [CAT]
10	0.47	6.0	1.10
40	0.72	7.0	0.86
60	0.64	7.8	0.60

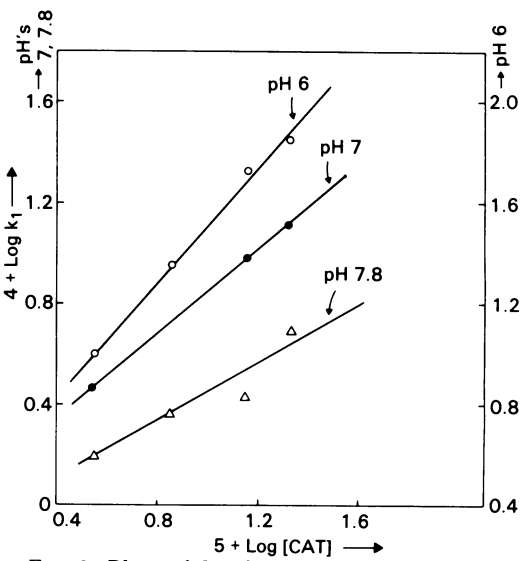

FIG. 3. *Plots of log k_1 (per second) versus log [CAT] (molar) at the indicated pH and 5°C.*

of inactivation of poliovirus type 2 by CAT (10 mg/liter) at pH 6 (0.05 M phosphate) was determined at different temperatures. The 99% viral inactivation time and k_1 were calculated, and an Arrhenius plot (Fig. 5) was obtained by plotting log k_1 against $1/T$ (reciprocal of absolute temperature). Table 4 shows the kinetic, Arrhenius, and thermodynamic parameters evaluated in the standard manner (10).

The data in Table 4 include the values of kinetic parameters (99% inactivation time and first-order rate constant), Arrhenius parameter

(Arrhenius factor A), and thermodynamic parameters (enthalpy of activation, entropy of activation, and free energy of activation) obtained at four temperatures. However, Arrhenius and thermodynamic parameters were calculated based on the value of energy of activation, E_a (where E_a is 2.303 × the ideal gas constant, R × slope of Arrhenius plot) determined at 5 to 35°C. The low values of E_a and thermodynamic parameters, including a negative value for entropy of activation, suggest that poliovirus inactivation by CAT does not involve the rupture of many hydrogen bonds (5, 6, 12, 13).

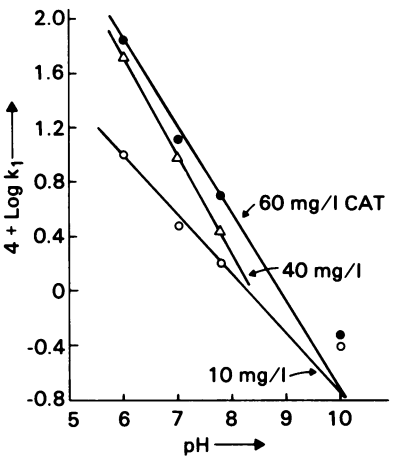

FIG. 4. *Plots of log k_1 (per second) versus pH of the medium for the indicated concentrations of CAT at 5°C.*

Inactivation of poliovirus type 2 by chlorine. For comparison with CAT, the inactivation of poliovirus type 2 by HOCl (0.5 mg of chlorine per liter) at pH 6 was observed at two different temperatures, 5 and 10°C, and two alkaline pH's, 7.8 and 10. Kinetic curves were obtained by plotting log n/n_0 against time (Fig. 6). It can be hypothesized that the kinetics of inactivation obey a pseudo-first-order reaction. Table 5 gives the kinetic, Arrhenius, and thermodynamic parameters determined from Fig. 6 and similar inactivation studies at 10°C (data not shown). It is interesting to note that the general shape of the kinetic curve of chlorine is similar to that of CAT.

DISCUSSION

The overall significance of these studies is that CAT may be a useful disinfectant for inactivat-

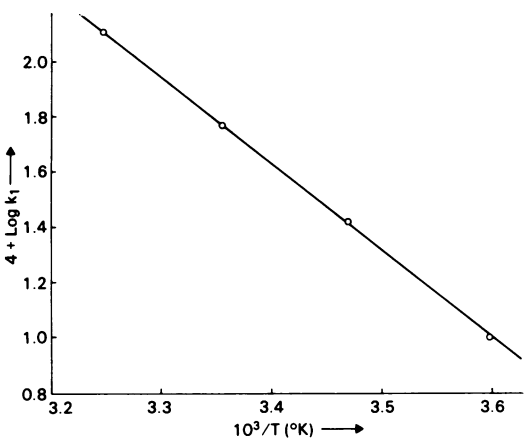

FIG. 5. *Arrhenius plot of log k_1 (per second) versus reciprocal of absolute temperature for 10 mg of CAT per liter at pH 6.*

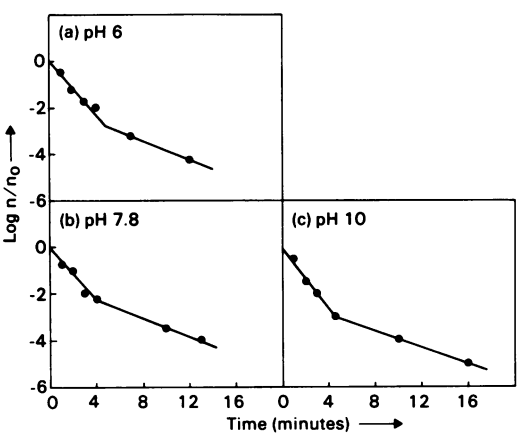

FIG. 6. *Kinetics of inactivation of poliovirus type 2 by 0.5 mg of chlorine per liter at the indicated pH and 5°C (where n/n_0 equals the surviving fraction).*

TABLE 4. *Kinetic, Arrhenius, and thermodynamic parameters for the inactivation of poliovirus type 2 by CAT in water*[a]

Temp (°C)	99% inactivation time (min)	k_1 (× 10^3) (s^{-1})	log A	$\Delta H‡$ (kcal/mol)	$\Delta S‡$ (e.u.)	$\Delta G‡$ (kcal/mol)
5	78	0.984	8.23	13.76	−20.73	19.52
15	30	2.559	8.27	13.74	−20.64	19.69
25	13	5.905	8.26	13.72	−20.75	19.91
35	6	12.791	8.26	13.70	−20.81	20.11

[a] CAT concentration, 10 mg/liter, pH 6. E_a, 14.31 kcal/mol; A, Arrhenius factor; $\Delta H‡$, enthalpy of activation; $\Delta S‡$, entropy of activation; $\Delta G‡$, free energy of activation; e.u., entropy units. 1 cal = 4.185 J.

TABLE 5. *Kinetic, Arrhenius, and thermodynamic parameters for the inactivation of poliovirus type 2 by chlorine in water*[a]

Temp (°C)	99% inactivation time (min)	k_1 ($\times 10^2$) (s^{-1})	log A	$\Delta H\ddagger$ (kcal/mol)	$\Delta S\ddagger$ (e.u.)	$\Delta G\ddagger$ (kcal/mol)
5	3.5	2.193	9.58	14.73	−13.07	18.36
10	2.15	3.571	9.60	14.72	−13.10	18.43

[a] Chlorine concentration, 0.5 mg/liter, pH 6. *Ea*, 15.28 kcal/mol. Abbreviations as in Table 4.

ing poliovirus in slightly acidic (around pH 6) water. The experimental results suggest that the overall reaction for the inactivation of poliovirus type 2 by CAT is complex, being first order with respect to the virus (P) and CAT, each having a fractional order with respect to [H^+] or [OH^-]. The rate laws are given by equations 1 and 2, where rates are designated by v_1 and v_2 and the rate constants are designated by k_1 and k'_1 for acid and alkaline conditions, respectively.

$$v_1 = k_1 [P] [CAT] [H^+]^{0.5} \text{ (acid conditions)} \quad (1)$$

$$v_2 = k'_1 [P] [CAT]/[OH^-]^{0.5} \text{ (alkaline conditions)} \quad (2)$$

The reaction mechanism responsible for the inactivation of poliovirus type 2 by CAT in acid medium was arrived at as follows. Bishop and Jennings (4) have shown that CAT (CH_3-C_6H_4-SO_2NClNa; RNClNa) behaves like a strong electrolyte in aqueous medium, dissociating as:

$$RNClNa \rightleftharpoons RNCl^- + Na^+ \quad (3)$$

The protonation of the anion in acid solutions gives the free acid, RNHCl, as follows:

$$RNCl^- + H^+ \rightleftharpoons RNHCl;\ K = 3.8 \times 10^4;\ 25°C \quad (4)$$

Although the free acid has not been isolated, there is ample experimental evidence for its existence in acidic solutions (17). Bishop and Jennings have shown that in a 0.05 M solution of CAT, [RNHCl] $\approx 10^{-2}$ in the range of pH 0 to 1, whereas [HOCl] $\approx 10^{-7}$. In aqueous alkaline medium, CAT undergoes hydrolysis to form RNHCl as follows (4, 26):

$$RNCl^- + H_2O \rightleftharpoons RNHCl + OH^- \quad (5)$$

It has been proposed by Mahadevappa et al. (26) that RNHCl, in fact, is the main reactive species for the alkali-retarded chloraminometric reactions. Therefore, RNHCl, the acid form of CAT, is most likely to be the main reactive species in both acid and alkaline media. The possibility of other species of CAT, such as $RNCl_2$ (dichloramine-T) and HOCl in acidic solutions and OCl^- and $RNCl^-$ (CAT) itself in alkaline solutions, being the reactive species can be discounted based on the experimental results. We propose the following mechanism to account for the observed kinetics of inactivation of poliovirus type 2 by CAT in acid conditions:

$$RNCl^- + H^+ \underset{}{\overset{K_1}{\rightleftharpoons}} RNHCl;\ \text{fast} \quad \text{(i)}$$

$$RNHCl + P \underset{}{\overset{K_2}{\rightleftharpoons}} X;\ \text{slow} \quad \text{(ii)}$$

$$X \xrightarrow{k_3} X';\ \text{slow and rate determining} \quad \text{(iii)}$$

$$RNHCl + X' \xrightarrow{k_4} P_i + \text{products};\ \text{fast} \quad \text{(iv)}$$

where P_i is the inactivated poliovirus and X and X' are activated complex and reaction intermediates, respectively. Considering the first two equilibrium reactions (given above) and the expression for total concentration of CAT (equation 6), the rate expression is obtained as in equation 7:

$$[CAT]_T = [RNCl^-] + [RNHCl] + [X] \quad (6)$$

$$v = \frac{K_1 K_2 k_3 [P] [CAT]_T [H^+]}{1 + K_1 [H^+] + K_1 K_2 [H^+] [P]} \quad (7)$$

Since K_2 is small, the following equation (8) could be obtained by making the reasonable assumption $K_1 [H^+] \gg K_1 K_2 [H^+] [P]$:

$$v = \frac{K_1 K_2 k_3 [P] [CAT]_T [H^+]}{1 + K_1 [H^+]} \quad (8)$$

$$\text{or } v = \frac{k [P] [CAT]_T [H^+]}{1 + K_1 [H^+]} \quad (9)$$

where $k = K_1 K_2 k_3$ and v is the rate of inactivation. Based on similar arguments and considering a similar mechanism, a similar rate expression for alkaline conditions can be derived (details will be published elsewhere).

The observed fractional order dependence on [H^+] can be explained by equation 9. The omission of the first term makes the order with respect to [H^+] zero whereas omission of the second term produces an equation with order 1 for [H^+], showing that the reaction actually operates between the two values. The proposed machanism and the derived rate law (equation 9) are in agreement with the experimental results (equation 1). The proposed mechanism and assertion that RNHCl is the active form of CAT in both acid and alkaline media are consistent

with the fact that the rate constant in acid inactivation by CAT is much higher than for alkaline inactivation.

The most likely interaction of CAT with infectious virus that leads to the virus inactivation can be determined from the Arrhenius and thermodynamic parameters. The values of these parameters (Table 4) determined for the inactivation of poliovirus type 2 by CAT are compatible with those of denaturation of the ribonucleic acid (RNA) of viruses (12, 13). The low values of energy of activation (14.31 kcal/mol [59.89 kJ/mol]), enthalpy of activation (13.73 kcal/mol [57.46 kJ/mol]), and free energy of activation (19.81 kcal/mol [82.91 kJ/mol]) and the negative value of entropy of activation (−20.73 entropy units [−86.76 J/deg-mol]) are in agreement with the values of Ginoza et al. for denaturation of RNA (12, 13). This would suggest that somehow CAT (and also chlorine) may interact with RNA of the virus rather than with protein, perhaps, as Ginoza (12) has suggested, by forming phosphotriester bonds with subsequent hydrolytic cleavage of the RNA chain.

In conclusion, these experiments indicate that (i) a clear dose-response relationship between CAT concentration and the virus inactivation rate, first order with respect to [CAT], can be demonstrated; (ii) the rate of the viral inactivation is pH dependent (faster in acid than in alkaline medium), having a fractional order with respect to both $[H^+]$ and $[OH^-]$; (iii) the kinetic reaction is first order with respect to the virus; (iv) certain anomalies in relation to virus inactivation by CAT may be associated with varying reactive species of CAT that may be formed under varying experimental conditions and relate to virus clumping and disaggregation under varying conditions; (v) the mode of attack by CAT species may be through the denaturation of the viral RNA; and (vi) CAT may be an effective viricide against poliovirus type 2 in acid rather than alkaline media. In addition, these studies suggest that CAT may be a suitable replacement for chlorine in the disinfection of slightly acidic waters.

With regard to the pH of drinking and wastewater, the waters of some rivers and lakes which receive acid rain and acid industrial and mine wastes tend to have fairly low pH's. For example, the pH of a stream of water near Johannesburg (U.K.) was found to be between 3.7 and 4.8 (23). Hence, it would be easier to adjust to pH 6 rather than a more alkaline pH before disinfection. Conventional sewage treatment has been found to be efficient in the acid range. It was shown in the United Kingdom that treatment of Bradford sewage with activated sludge gave the best results at pH 6.0 to 6.5 (24). Thus, there may be certain instances in which disinfection should be carried out in slightly acid pH. Under such circumstances, the use of CAT would be favored.

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