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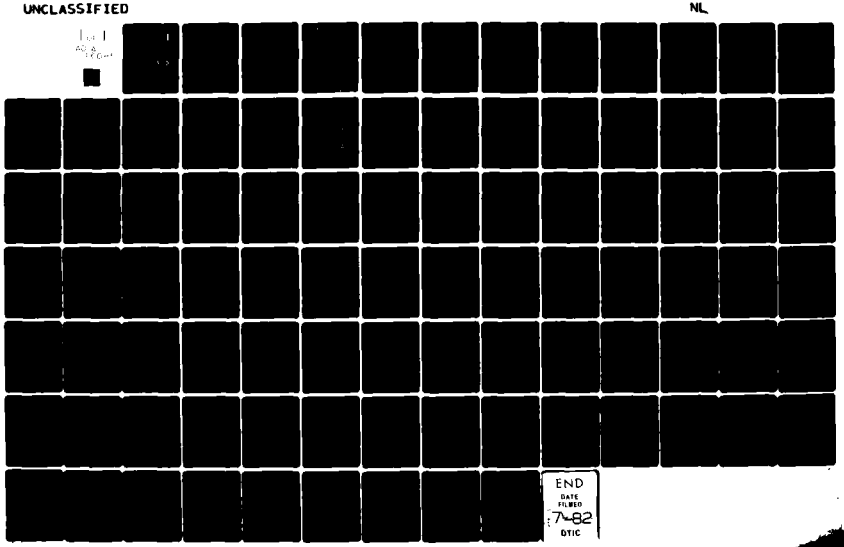

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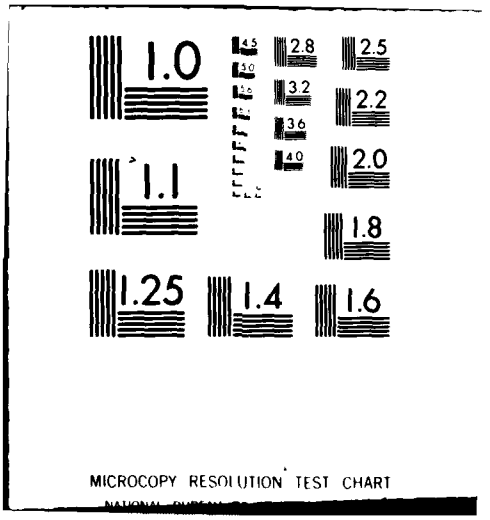
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BIOLOGICAL EVALUATION OF METHODS FOR THE DETERMINATION OF FREE AVAILABLE CHLORINE

Final Report

By

Michael C. Snead
Vincent P. Olivieri

March 1980

Supported by

US Army Medical Research and Development Command
Fort Detrick, Frederick, Maryland 21701

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The Johns Hopkins University
School of Hygiene and Public Health
Division of Environmental Health Engineering
Baltimore, Maryland 21205

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A linear, reproducible, relationship was found between the rate of f2 inactivation and free chlorine concentration at pH 6.0 and 7.0. This relationship was used as a standard curve for the determination of free chlorine concentration from the rate of f2 inactivation. Specificity of the tests for free available chlorine was determined by comparison of the level of free chlorine indicated by the test to the level indicated by the biofac procedure. A false positive result was defined as an indication of free chlorine by the test in the absence of viricidal activity. All of the methods tested yielded false positive determinations of free chlorine with one or more of the inorganic chloramines. The FACTS procedure was the most specific for free chlorine. The specificity of the DPD procedure was influenced by the form of the reagent (tablet or powder), with the powder reagent less specific. The steadifac modification of the DPD procedure reduces the magnitude and frequency of false positives obtained.

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Section 1

INTRODUCTION

The marked difference in the biocidal activity of the free and combined species of chlorine has been repeatedly noted in the literature (Butterfield and Wattie, 1946; Butterfield *et al.*, 1943; Kelly and Sanderson, 1958, 1960, Krusé *et al.*, 1970: Olivieri *et al.*, 1970). The demonstration of a free available chlorine residual provides a rapid method to evaluate the disinfection process and assess residual biocidal activity. The national interim primary drinking water regulations (1975) allow the substitution of free chlorine residual measurements for up to 75% of the samples for microbiological analysis in water systems. Thus, the free chlorine residual measurement becomes an indirect measure of microbiological quality and the reliable differentiation of free from combined available chlorine is imperative. False positive measurements of free available chlorine due to the less biocidal chlorine species provides a false assurance of safety.

Standard Methods (1975) lists five methods for determination of free chlorine in water, amperometric titration, stabilized neutral orthotolidine (SNORT), N,N-diethyl-p-phenylene diamine (DPD), titrimetric and colorimetric, leuco crystal violet, and syringaldazine (FACTS). The national interim primary drinking water regulations specify the DPD colorimetric procedure for free chlorine measurements that are to be substituted for microbiological samples. As a result of this there has been a proliferation of manufacturers marketing DPD test kits with little interest in alternate procedures. However there appears to be some controversy concerning the specificity of this and other methods for free chlorine and the applicability of one test to all situations.

Field test kits are generally limited to colorimetric methods. The amperometric titration, recognized in the United States as reliable procedure for the determination of free available chlorine (FAC) does not lend itself to field use. Cooper *et al.* (1974) and Sorber *et al.* (1975) evaluated six existing field test procedures for the determination of free available chlorine. The three most promising were syringaldazine (FACTS), N,N-diethyl-p-phenylene diamine (DPD) and stabilized neutral orthotolidine (SNORT). In a subsequent study, Palin *et al.* (1978) evaluated the specificity of the glycine modification of the DPD method and the FACTS procedure. The DPD method gave false positive readings for free available chlorine in the presence of monochloramine (NH_2Cl), dichloramine ($NHCl_2$), trichloramine (NCl_3), and chlorinated natural water known to contain only combined chlorine. The FACTS procedure did not yield false positive measurements at equivalent levels of NH_2Cl and $NHCl_2$ and in the chlorinated natural water, but did give false positive measurements with NCl_3. The DPD procedure was found to be more precise and accurate than the FACTS method.

Strupler (1978), in a study of interferences of free chlorine measurement by the DPD and FACTS procedures, found no interference in the DPD procedure at monochloramine levels up to 4 mg/l and at dichloramine levels up to 10 mg/l. FACTS gave false positives with trichloramine, the response obtained was on the order of 70% of that obtained for equivalent concentrations of free chlorine. The DPD procedure correctly distinguished free chlorine and trichloramine in a mixture of the two compounds.

Palin (1978) recently reported a DPD modification, coined DPD-Steadifac, that was more specific for free available chlorine than prior

DPD methods. The DPD-Steadifac has not been evaluated in other laboratories.

Another untested procedure, the free chlorine membrane electrode, has recently become available (Johnson, *et al.* 1978). The membrane electrode uses a microporous membrane and a positive cathode potential to obtain selectivity for HOCl. Monochloramine and dichloramine were found to produce electrode responses of 1.3 to 3.0 percent that of HOCl. while trichloramine produced a response 7.25 times that of HOCl. No interference from OCl^- was found. The authors suggested that the membrane electrode can be calibrated directly as a function of disinfection efficiency since it measures only the actively gerimicidal species, HOCl.

Since the intent of the chlorine residual determination is to reflect the biocidal activity of the solution, a biological system would provide a more meaningful procedure to evaluate the performance of existing and proposed methods for the measurement of chlorine residuals. The biological system can serve as the ultimate referee to allow a practical interpretation of the results of chlorine residual measurements by different procedures. This approach was used by Savage and Stratton (1971) to qualitatively determine the performance of the orthotolidine chlorine test and syringaldazine test strips in assaying the microbiological quality of swimming pool water. The presence of viable bacteria (*Streptococcus faecalis*, *Escherichia coli*, and *Pseudomonas aeruginosa*) was used to determine the validity of free chlorine measurement. Although this procedure was adequate for this short term qualitative test, the susceptibility of bacteria to combined chlorine makes them a poor choice for a more rigid test, specifically for free chlorine.

The purpose of this study was to develop a biological referee procedure (biofac) for the qualitative and quantitative determination of free chlorine in solutions containing compounds that may interfere with the colorimetric chemical methods and to use this procedure to compare the specificity of the DPD, FACTS, amperometric, and electrode procedures for free chlorine. The bacterial virus f2 was chosen as the test organism for the development of the biofac procedure, since f2 is resistant to inactivation by combined chlorine (Snead 1976, Richfield 1978) and sensitive to free chlorine (Olivieri 1974).

Section 2
METHODS

Chlorine

All chlorine, chloramine, and buffer solutions were prepared either in water from an acid permanganate distillation (organic free distilled water) using the method developed by Soper (1924) as described in detail by Olivieri (1968), or in double distilled deionized water. Stock hypochlorous acid solutions were prepared by washing and collecting high purity chlorine gas in organic free distilled water utilizing the method outlined by Olivieri (1974). Stock solutions contained approximately 4,000 mg/liter of free available chlorine.

Monochloramine

Stock monochloramine solutions were prepared using the method developed by Granstrom (1954) as described by Johnson and Overby (1969). Equal volumes of 0.0100 molar ammonium chloride and 0.0033 molar hypochlorite at pH 10 were mixed and allowed to react for at least one hour before use. Amperometric titration and ultraviolet absorption spectrophotometry showed that stock solutions contained about 100 mg/liter of monochloramine. Previous work (Johnson and Overby, 1969; Snead, 1976) has shown that monochloramine solutions prepared by this procedure do not contain detectable amounts of free chlorine or of other inorganic chloramines.

Dichloramine

Stock dichloramine solutions were prepared using a method employed by Chapin (1929) and modified by Richfield (1979). Equal volumes of 0.0264 molar ammonium chloride and 0.0132 molar hypochlorous acid buffered at pH 4.6 with 0.08 molar acetate were mixed and allowed to react overnight. Amperometric titration and ultraviolet absorption spectrophotometry showed the stock solutions to contain about 400 mg/

liter of dichloramine. Richfield indicated that this procedure results in a solution containing 95-97% of the available chlorine in the form of dichloramine, with the remaining chlorine as monochloramine. No free chlorine was detected.

Trichloramine

Stock trichloramine solutions were prepared using the method utilized by Saguinsin and Morris (1975). Equal volumes of 0.004 molar ammonium chloride and 0.0120 molar hypochlorous acid at pH 2.3 were mixed and allowed to react overnight. Amperometric titration and spectrophotometry showed that stock solutions contained about 300 mg/liter of trichloramine. Dilution and adjustment of pH to that of the experimental run was done immediately before the run. A 30 fold molar excesses of ammonia was also added at this time to supress free chlorine formation from the NCl_3 (Saguinsin and Morris, 1975).

Chlorine Determination

Chlorine and chloramine concentrations were measured by amperometric titration (Standard Methods, 1975) using a Sargent-Welch model XVI polarograph and ultraviolet absorption spectra were determined with a Health-Schlumberger model 707 spectrophotometer. Concentrations were determined spectrophotmetrically using the molar absorptivities determined by Galal-Gorchev and Morris (1965). Measurement of free chlorine concentration with DPD was accomplished by adding 20 ml of test solution to two standard scoops, approximately 0.2 grams, of LaMotte DPD powder or two crushed Hellige DPD tablets. The solution was mixed immediately and the absorbance at 515 nm in 1 inch cells was read within 30 seconds on a Bausch and Lomb Spectronic 20 spectrophotometer. Two drops of 10% thioacetamide were added within 30 seconds of mixing of the test solution and DPD for the DPD steadifac tests

(Palin, 1978). The measurement of free chlorine concentration by syringaldazine (FACTS) was accomplished by adding 5 ml of test solution to 0.17 ml of FACTS buffer and 1.7 ml of syringaldazine solution (Standard Methods, 1975) followed by mixing and an immediate absorbance reading at 530 nm in 1/2 inch cells. Measurement of free chlorine concentration by the membrane electrode was accomplished by exposing the electrode to approximately 100 ml of test solution with mixing. Concentration readings were taken after three minutes of exposure.

Biological Preparations and Assays

The f2 bacterial virus (ATCC #15766) was prepared by the method of Loeb and Zinder (1961) and purified by polyethelene glycol precipitation (Dennis, 1977). The purified virus produced no chlorine demand. The f2 bacterial virus was assayed by the agar overlay method described by Adams (1959) on tryptone yeast extract (TYE) media, with *E. coli* K-13 (ATCC #15766) used as host bacterium. *E. coli* B, which is not a host for f2 bacterial virus, was grown overnight under aerated conditions at 37°C in nutrient broth. Cells were harvested and washed three times by centrifugation at 5,000 x g for ten minutes followed by resuspension in 0.01 molar phosphate buffered saline. The survival of *E. coli* was determined by the pour plate procedure using TYE media (Standard Methods, 1975) and 35°C incubation temperature. Poliovirus 1 was prepared in Buffalo green monkey (BGM) cells in roller bottles in Eagle's minimum essential medium (Dahling *et al.*, 1974). The virus was harvested by three cycles of freezing and thawing followed by centrifugation to remove cell debris. The resulting virus stock was purified by sucrose density gradient centrifugation. Poliovirus 1 plaque assays were performed using BGM cells.

Experimental Methods

The reaction system shown in Figure 1 was used in the study and has been described in detail by Olivieri (1974). The system was modified by using a 500 ml baffled beaker in place of a trypsinizing flask and by using a pump-driven syringe instead of a manually operated syringe. Before the experiments stock hypochlorous acid solutions were diluted in organic free distilled water to the needed concentrations and the pH was adjusted and maintained by 0.008 molar phosphate buffer. Monochloramine stock solutions were diluted in double distilled deionized water and the pH was adjusted and maintained with 0.008 molar phosphate buffer. Dichloramine stock solutions were also diluted in double distilled deionized water and the pH was adjusted and then maintained by 0.08 molar phosphate buffer. Trichloramine stock solutions were diluted in double distilled deionized water to the needed concentration and the pH was adjusted to the experimental level and maintained by 0.17 molar phosphate buffer. A 30 fold molar excess of ammonium chloride was added to insure the absence of free chlorine just prior to experimentation. A baffled beaker was filled with chlorine or chloramine solution at the experimental concentration and pH and allowed to equilibriate to 20°C in a water bath. Samples were withdrawn for free chlorine residual analysis by amperometric titration, DPD with tablet reagents (DPDT), DPD steadifac with tablet reagents (DPDTSF), DPD with powder reagents (DPDP), DPD steadifac with powder reagents (DPDPSF) and FACTS immediately before the f2 inactivation test. Since the membrane electrode was not received until the latter part of the study, data for this procedure were obtained only for the later experimental runs. At time zero f2 virus was added, with mixing, to the chlorine or chloramine

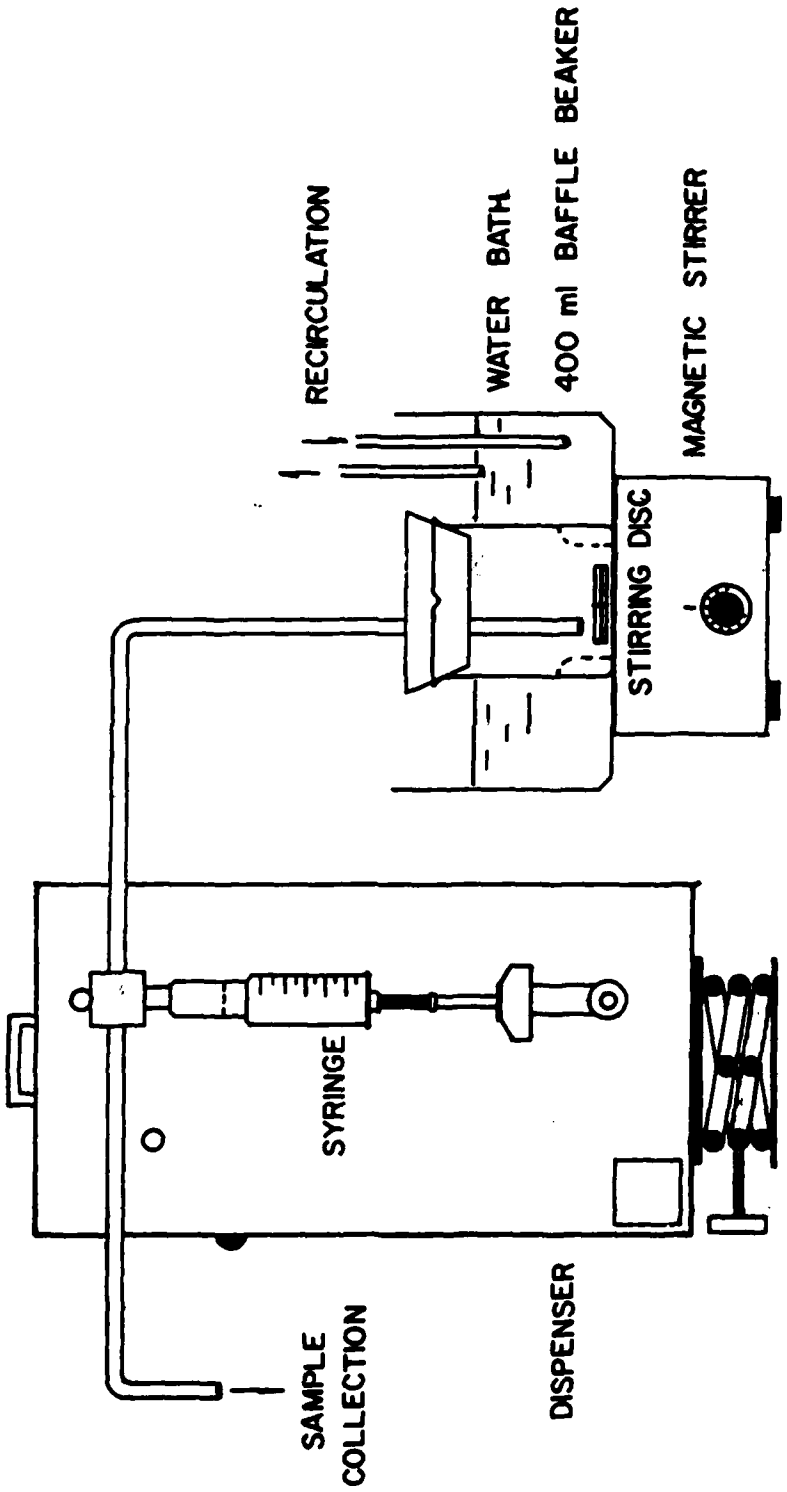


Figure 1. Reaction System used for inactivation experiments.

solution in the baffled beaker to give an f2 concentration of approximately 10^6 PFU/ml. Samples were withdrawn with time into sterile tubes containing thiosulfate. At the end of the inactivation run, samples were again taken for free chlorine residual measurement by all of the procedures. The survival of the virus was assayed by the procedure given above.

Biofac Calibration

Fair and Geyer (1954) give the following relationship for contact time and the inactivation of microorganisms with disinfectant,

$$\frac{N}{N_o} = e^{-kt}$$

where N_o is the number of microorganisms at time zero, N is the number at any time t, and k is the coefficient of proportionality or rate constant. By taking the natural logarithm

$$\text{In } \frac{N}{N_o} = -kt$$

Thus a plot of In N/N_o versus time yields a line with slope -k, the rate constant. Taking the logarithm to base 10

$$\log_{10} \frac{N}{N_o} = -k't$$

A plot of $\log_{10} N/N_o$ versus time gives a line with slope -k', the coefficient of proportionality. k' is related to k by

$$k = (k')\ (2.303)$$

k' was used in the subsequent calculations and was referred to as the rate of inactivation.

Rates of inactivation of f2 virus by 0 to 1 mg/l of free chlorine at pH 5.5, 6.0, 7.0, and 8.5 were determined by the procedure given above. Free chlorine solutions were prepared in double-distilled deionized water and measured by amperometric titration.

Free Chlorine Measured After Exposure to Chloramine Solutions

A series of experiments was run to compare free chlorine residuals measured by DPD, FACTS, and biofac after exposure to monochloramine, dichloramine, and trichloramine. These tests run in the same manner as the tests with free chlorine except triple distilled deionized water was used in place of organic free distilled water. Additional thioacetimide was added to the DPD steadifac test at higher levels of combined chlorine, as called for by Palin (1978).

Nitrogen Breakpoint

The addition of chlorine to an ammonia solution to the point at which an irreducible concentration of ammonia remains is referred to as breakpoint chlorination. As chlorine is added to the ammonia solution the species of residual chlorine changes. The accurate measurement of these residuals is essential if a reliable estimate of the biocidal properties of the chlorine residuals are to be obtained.

A series of experiments was set up to enable a comparison of the free chlorine residuals measured by the chlorine probe, DPD, FACTS, biofac, and amperometric titration along several stages of the breakpoint chlorination of a 2 mg/liter and 0.30 mg/l ammonia nitrogen solution. The ammonia solution was reacted with increasing doses of free chlorine at pH 7.0 and 20°C for 30 minutes. Free and combined chlorine residuals were determined by the methods given above.

Inactivation of f2 Virus, *E. coli* B, and Poliovirus

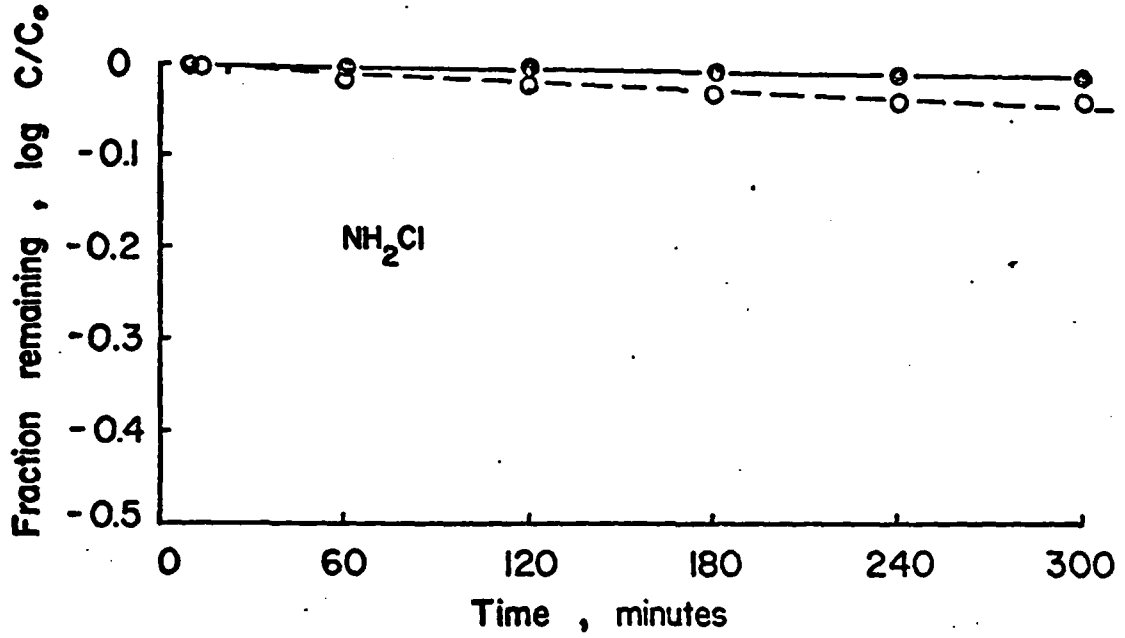
A group of experiments was run to compare the effects of free chlorine, monochloramine, dichloramine, and trichloramine on f2 virus, *E. coli* B, and poliovirus 1. The experiments were run as previously described for free chlorine and combined chlorine. The f2 virus, *E. coli* B, and poliovirus 1 were all injected into the reaction system simultaneously and were, therefore, submitted to exactly the same chemical and physical conditions. Samples for determination of microbial survival of the organisms were taken as usual and were then divided into two fractions. One fraction was mixed with one drop of chloroform to destroy the bacteria. This fraction was then used to determine virus survival. The other fraction was used to determine survival of the *E. coli* B bacterium.

SECTION 3

RESULTS

Chloramine preparation. Since the formation and stability of the inorganic chloramines is dependent on pH, the effect of the pH values used in the inactivation experiments on chloramine stability was studied.

Figure 2 (upper) shows the effect of lowering the pH of a monochloramine solution, formed at pH 10.0, to pH 6 or pH 7.0. The data are plotted as log C/C_o versus time, where C is the concentration at any given time and C_o is the time zero concentration. The results show little loss of monochloramine over 5 hours at either pH. The effect of raising the pH of a dichloramine solution from the formation pH of 4.6 to the experimental pH values of 6.0 or 7.0 is shown in Figure 2 (19234). Elevation of the pH to 6.0 had little effect on dichloramine, but the increase to pH 7.0 resulted in substantial loss of dichloramine, with a half life of 210 minutes. However, inactivation runs were always performed within 60 minutes of the pH adjustment for dichloramine. The dichloramine concentration remaining at pH 7.0 over this time period was approximately 75% of that at zero time, with 25% converted to monochloramine. No free chlorine was detected. The time for trichloramine concnetration to decrease by 50% (t 1/2) at pH 7.0 is 293 minutes for a Cl:N ratio of 3:1, as shown in Figure 3 (lower panel). However, in the inactivation experiments a 30 fold molar excess of ammonia was maintained to suppress the formation of free chlorine. This excess of ammonia decreases the t 1/2 to 17 minutes, as shown in Figure 3 (upper panel). This effect of ammonia on trichloramine stability has been previously noted and discussed in greater detail by Sanguinsin and Morris (1975).



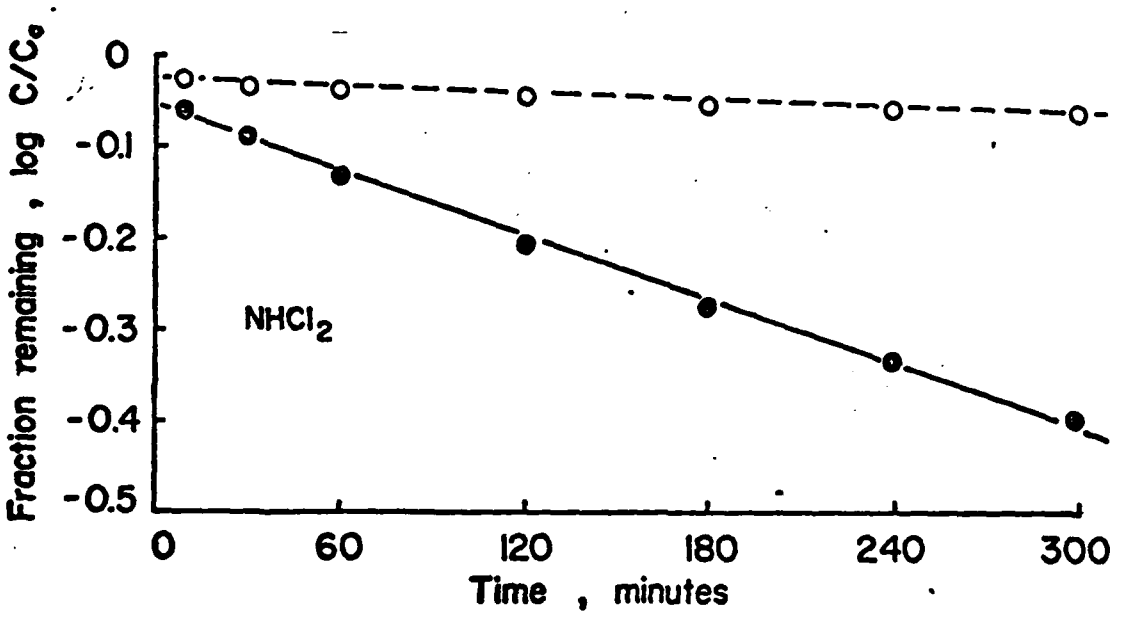
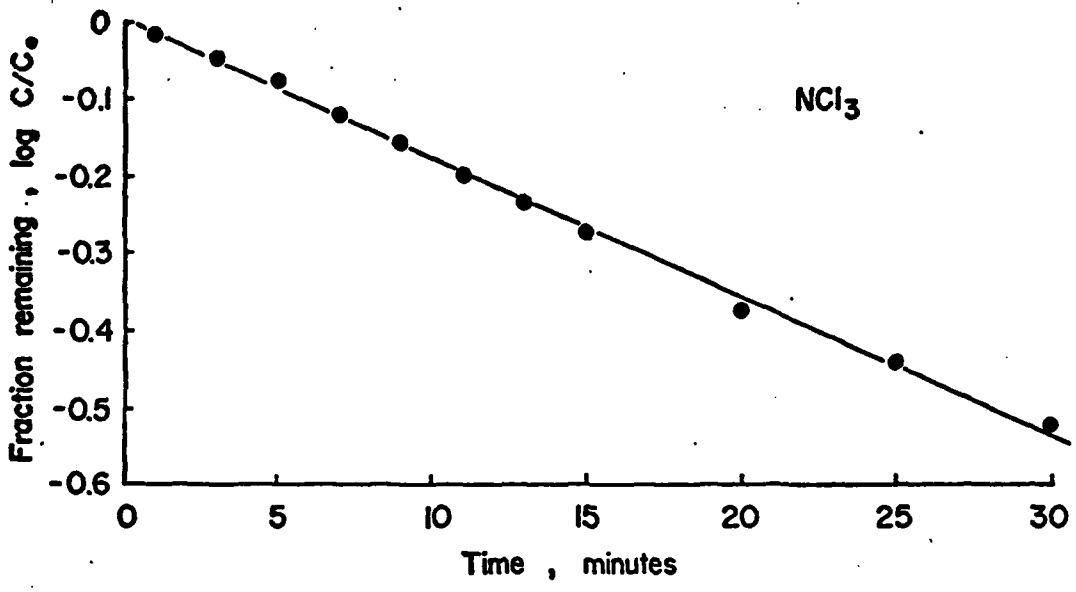


Figure 2. (upper) Stability of NH_2Cl after adjustment of pH from the formation pH of 10.0 to 7.0 (closed circles) and 6.0 (open circles).

(lower) Stability of $NHCl_2$ after adjustment of pH from the formation pH of 4.5 to pH 7.0 (closed circles) and 6.0 (open circles). Both at 20°C.



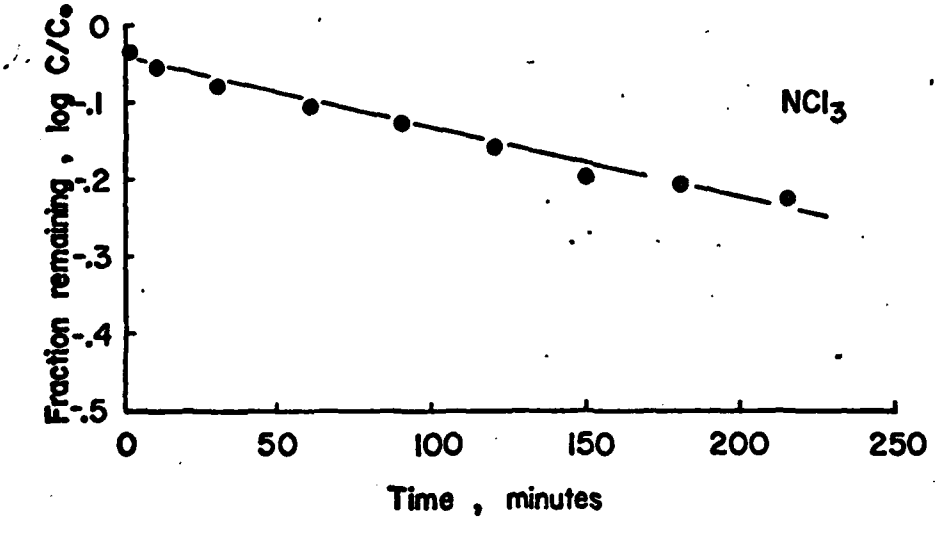
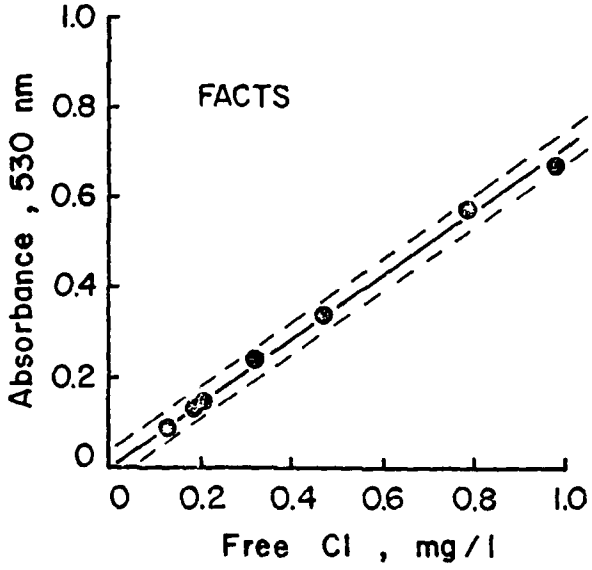


Figure 3. (upper) Stability of NCl_3 after adjustment of pH from the formation pH of 2.3 to pH 7.0, with Cl:N = 1:30, 20°C.

(lower) Stability of NCl_3 after adjustment of pH from the formation pH of 2.3 to pH 7.0, with Cl:N = 3:1, 20°C.

Colorimetric tests. Standard curves for the colorimetric tests were prepared for each reagent. When new reagent was prepared, new standard curves were run for the reagent. Amperometric titration was used to determine chlorine concentrations.

A representative standard curve for FACTS is shown in Figure 4. The slope of the line shown in this figure, along with the slopes of the lines for the other standard curves for FACTS, is also shown on this figure. A t test procedure for comparing slopes (Armitage, 1971) was used to see if the slopes differed significantly over time. For the FACTS procedure, the slopes, which are a reflection of the sensitivity, were found to be significantly ($p = .05$) different. Part of this variation may be due to variability in measuring free chlorine by the amperometric procedure in addition to the variability between batches of reagent. The slopes shown here are for reagent preparations which were satisfactory. Some batches of propanol were found to give reagents which were less sensitive to free chlorine. These reagents were not used in the testing procedure. All standard curves were plotted using linear regression procedures to force the line through the origin, since a sample with zero chlorine will have zero absorbance. Linear calibration techniques (Snedecor and Cochran, 1967) were used to determine free chlorine concentrations from any absorbance reading and to determine the 95% confidence bands around the standard curve. Of particular interest is the point where the lower 95% confidence band intersects the x axis. This point is the lowest chlorine concentration with a lower confidence limit that does not include zero. In the part of this study dealing with false positive results in the colorimetric tests, any chlorine residual value determined by the test



Date	Slope
01/15	0.64
02/16	0.71
02/26	0.77
03/23	0.71
06/01	0.67
06/25	0.73

Figure 4. (upper) Typical standard curve with 95% confidence limits for the determination of free chlorine by the FACTS procedure.

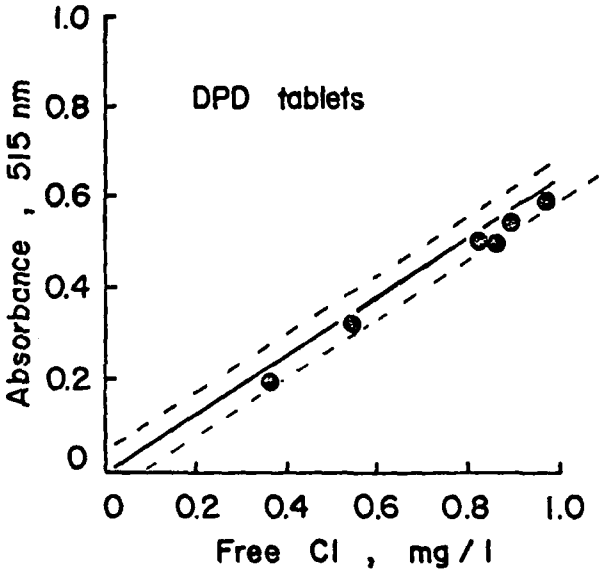
(lower) Slopes of the standard curves obtained through the time of the study.

to be greater than this point of intersection was said to be significantly ($p = .05$) greater than zero. Stated another way, this point is the quantitative limit of detection for the test. For the FACTS procedure, this limit was found to average 0.19 mg/l free available chlorine.

Standard curves for DPD and DPD-steadifac using the tablet reagents are shown in Figure 5. The slopes for standard curves are also shown in this figure. These slopes also changed significantly over time. On any given run, the DPD and DPD-steadifac procedures gave almost identical standard curves. These curves were also forced through the origin, and the lower quantitative limit of detection was found to be 0.17 mg/l free available chlorine for DPD and 0.15 mg/l free available chlorine for DPD-steadifac.

Figure 6 shows standard curves for DPD and DPD-steadifac generated using the powder reagent. As with the previously mentioned procedures, the regression lines were forced through the origin, and the slopes of the curve run for each test were found to be significantly different. The lowest chlorine residual with a 95% confidence limit that does not include zero was 0.10 mg/l free available chlorine for DPD and 0.13 mg/l for DPD-steadifac. On any given day, the addition of thioacetamide in the steadifac procedure did not change the slope of the line from that obtained with DPD alone.

Since the DPD-steadifac procedure was relatively untested, some effort was directed toward chemical evaluation of this procedure, with emphasis on determining the importance of time of addition of the thioacetamide reagent. Figure 7 shows the development of color with time for DPD with monochloramine and dichloramine solutions in the absence of free chlorine. DPD was found to produce substantial color



Date	Slope
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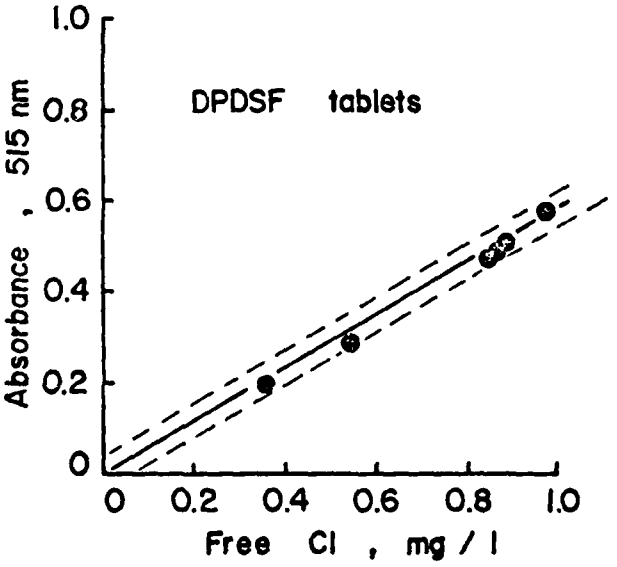
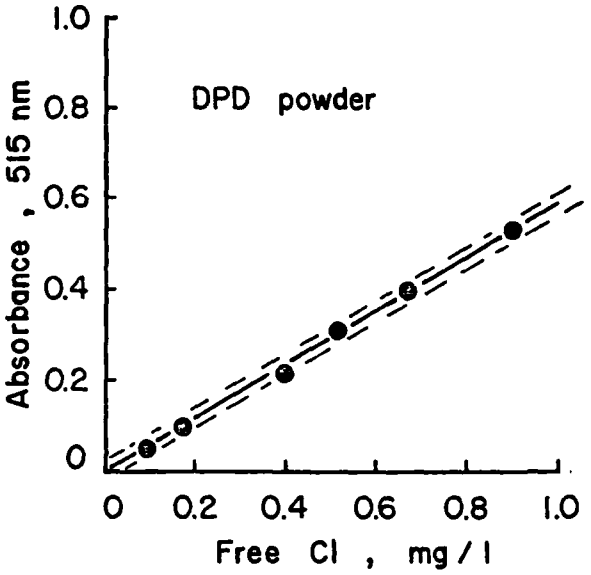
02 / 26	0.61
06/01	0.62

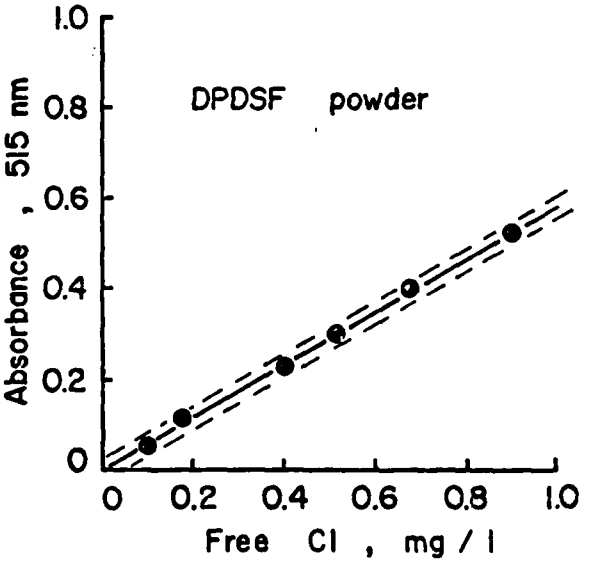


Date	Slope
01 / 16	0.49
02 / 26	0.59
06/01	0.62

Figure 5. Typical standard curves for determination of free chlorine by the DPD and DPD-steadifac procedures with the tablet reagent and the slopes of the standard curves.



Date	Slope
02/02	0.58
06/01	0.67



Date	Slope
02/02	0.57
06/01	0.65

Figure 6. Typical standard curves for determination of free chlorine by the DPD and DPD-steadifac procedures with the powder reagent and the slopes of the standard curves.

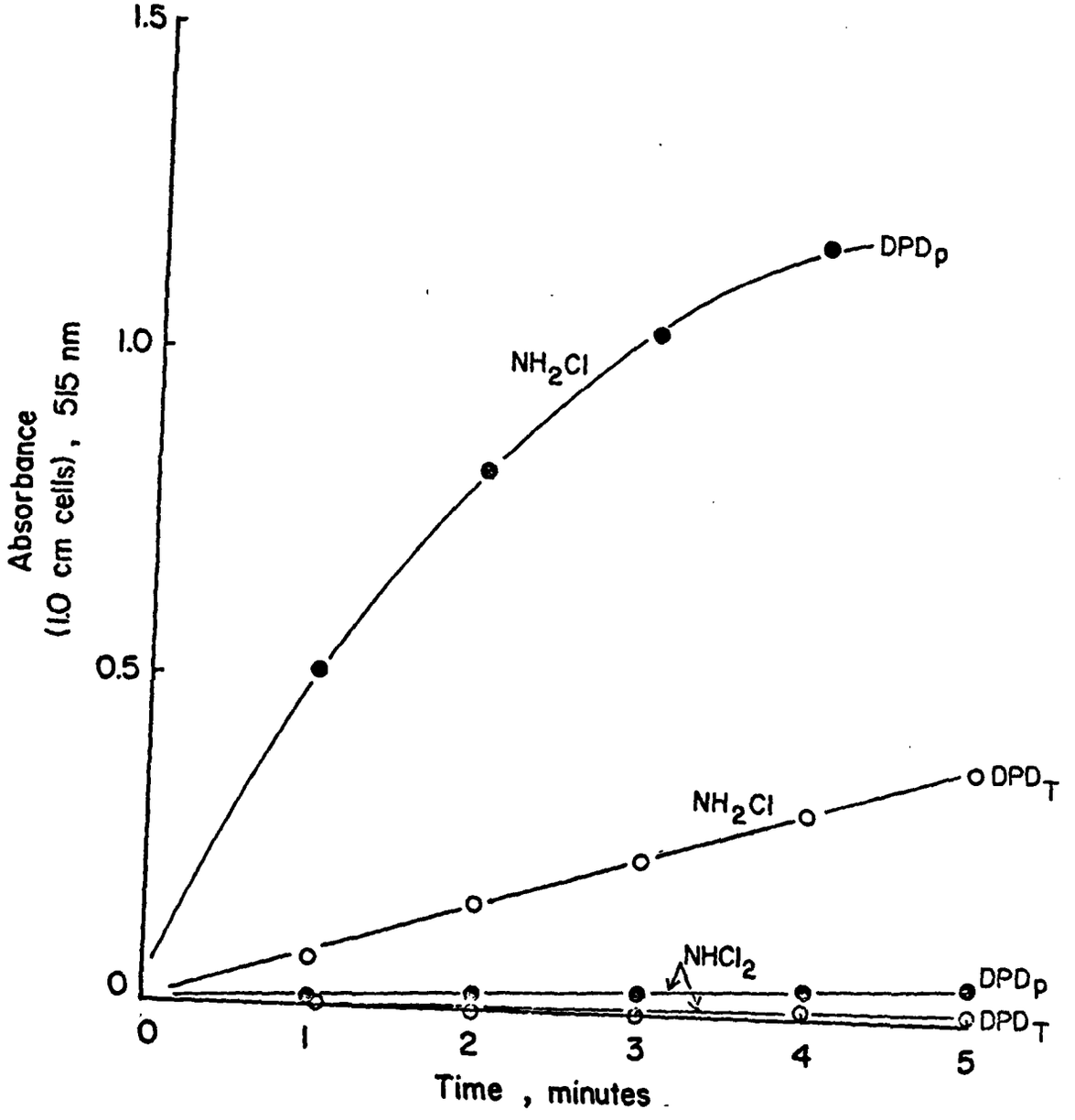


Figure 7. Time course of color development for the DPD reagents with 10.0 mg/ℓ monochloramine and 10.0 mg/ℓ dichloramine at 20°C.

with monochloramine, with the color increasing over the 5 minute period observed. A noticeable difference was observed for the different commercially available DPD reagent preparation. The DPD powder reagent produced an absorbance at 515 nm of greater than 1.0 for 10 mg/l NH_2Cl while the DPD tablet yielded only an absorbance of 515 nm of 0.2 for the same NH_2Cl preparation. The DPD reagent, both tablet and powder, was relatively insensitive to dichloramine. In a subsequent experiment, monochloramine color development with DPDT was allowed to proceed for varying times. At the end of the time period, the sample was removed from the spectrophotometer and the appropriate amount of thioacetamide was added. The sample was returned to the spectrophotometer and the absorbance at 515 nm was determined. The results of this experiment are shown in Figure 8 (upper panel) for times of 1 to 5 minutes. The dashed lines represent the time when the sample was out of the spectrophotometer. The addition of thioacetamide resulted in a decrease in the absorbance in all cases. A similar experiment with free chlorine is shown in Figure 8 (lower panel). In this case, thioacetamide addition had no effect on the developed color with free chlorine.

The apparent decolorization of the DPD color obtained with monochloramine and thioacetamide is shown in Figure 9. Figure 9A is a scan from 400 to 600 nm of the DPDT reagent with 1.80 mg/l free chlorine while Figure 9B shows the same chlorine-DPDT mixture with the addition of thioacetamide. The addition of thioacetamide had no effect on the shape of the absorbance curve or on the magnitude of the absorbance. Figure 9C and D shows a similar experiment using monochloramine. In Figure 9C, 10.3 mg/l NH_2Cl was mixed with DPDT, the color was allowed to develop for 2 minutes, and the solution was scanned from 400-600 nm. The shape of the absorbance curve is similar to that observed for free

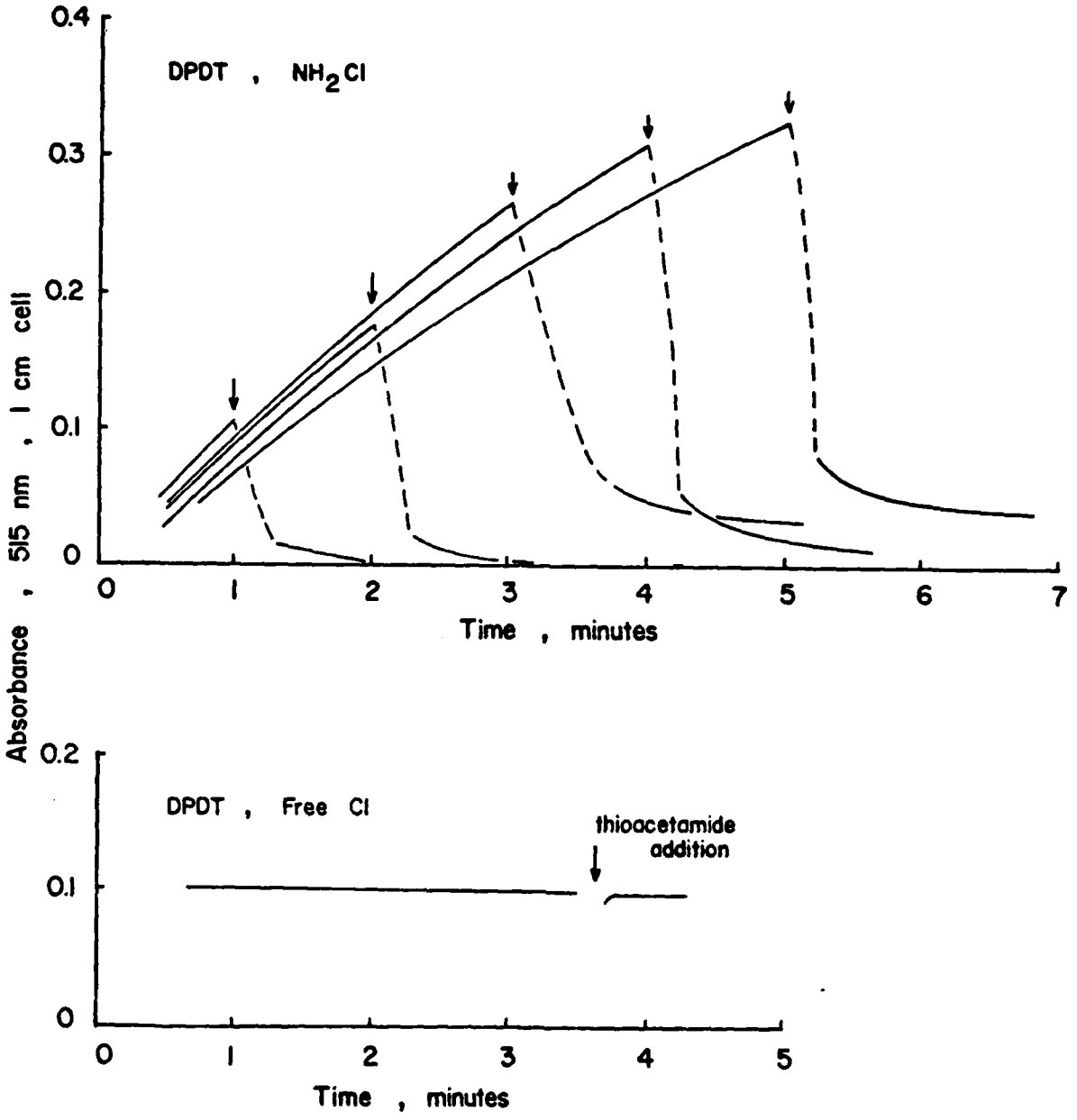


Figure 8. Effect of thioacetamide addition on the color developed by DPD, tablet reagent, with monochloramine (upper) and with free chlorine (lower). Arrows indicate time of addition of thioacetamide.

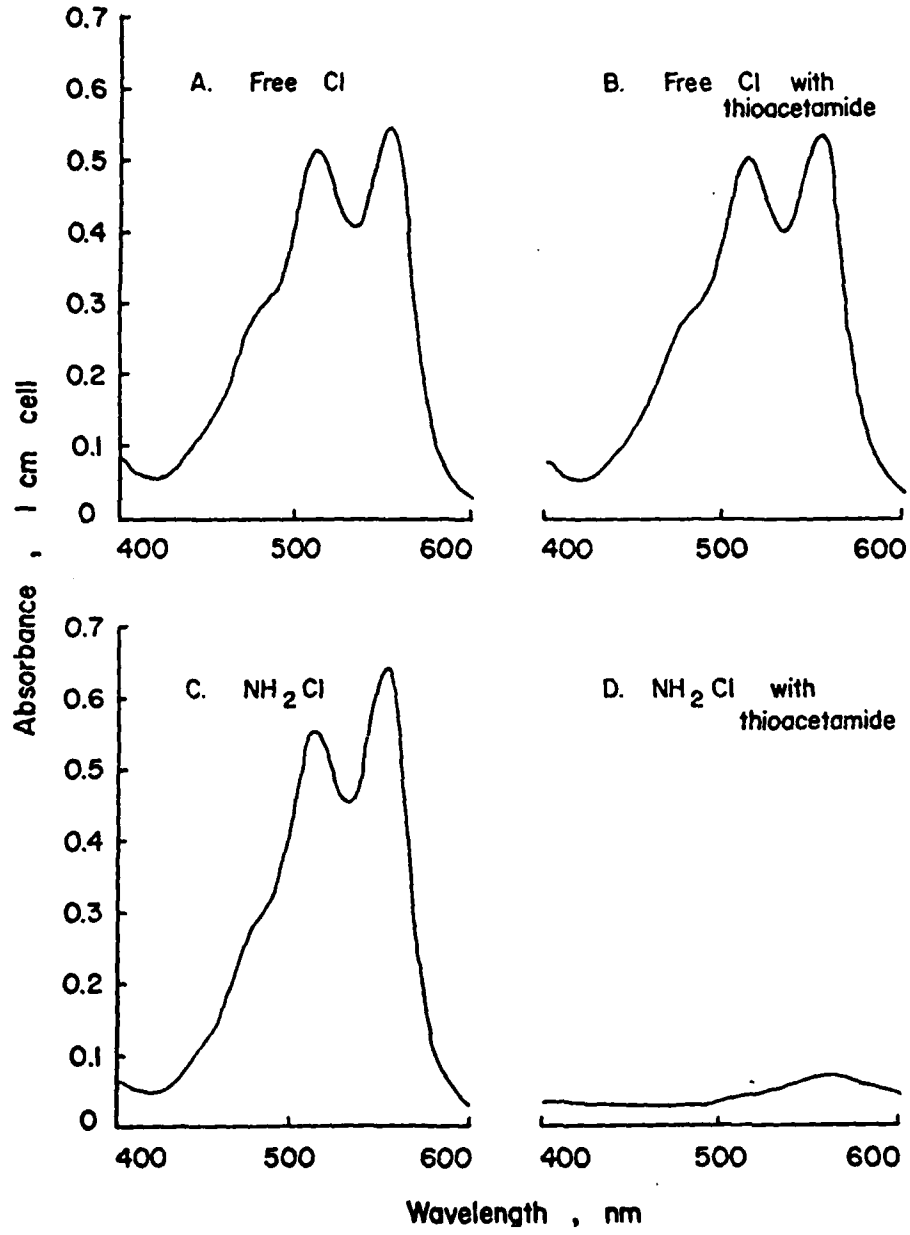


Figure 9. A. Absorption spectra of the colored product produced by 1.0 mg/ℓ free chlorine with DPD.

B. Effect of thioacetamide addition on the absorption spectrum shown in A.

C. Absorption spectra of the colored product produced by 10 mg/ℓ monochloramine with DPD.

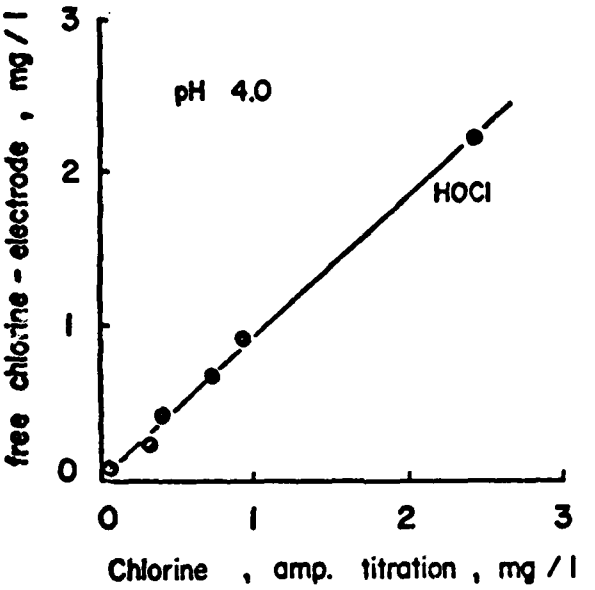
D. Effect of thioacetamide addition on the absorption spectra shown in C.

chlorine in Figure 9A. Figure 9D shows the results obtained when 10.3 mg/l NH_2Cl was mixed with DPDT, the color allowed to develop for 2 minutes, and then thioacetamide was added. The thioacetamide addition resulted in reduction of absorbance in the 400-600 nm region to less than 0.05 units.

Chlorine membrane electrode. The chlorine membrane electrode was calibrated daily or twice daily according to the manual provided (Orion Research Inc., 1979). The response of the electrode to a solution containing only HOCl is shown in Figure 10 (upper panel). The response was linear over the range observed with a slope of 0.93. Figure 10 (lower panel) gives the response for a slution containing HOCl and OCl^-. The slope of this line was 0.63, while the slope for a line drawn from the calculated HOCl concentration at this pH, labeled HOCl, had a slope of 0.88. Thus, the calibration curve for the electrode can be calibrated in terms of HOCl, the species to which the electrode is said to respond (Johnson, 1978).

The response of the electrode, given as apparent HOCl concentration, to solutions of monochloramine and dichloramine containing no free chlorine is shown in Figure 11. The meter reading was 2% of the actual NH_2Cl concentration over the range 5-25 mg/l (Figure 11 upper panel). The response to dichloramine was 18% of the dichloramine concentration over the range 2-15 mg/l $NHCl_2$. The electrode was found to be very sensitive to NCl_3, with a 0.59 mg/l NCl_3 solution yielding a meter reading of 2.7 mg/l HOCl. False positive readings increased as the number of chlorine atoms on the inorganic chloramine increased.

Biofac calibration. Figures 12 and 13 show the inactivation of the f2 virus by free chlorine at pH 5.5, 6.0, 7.0 and 8.5. Virus survival was plotted as log N/N_o versus time where N_o is the number of micro-



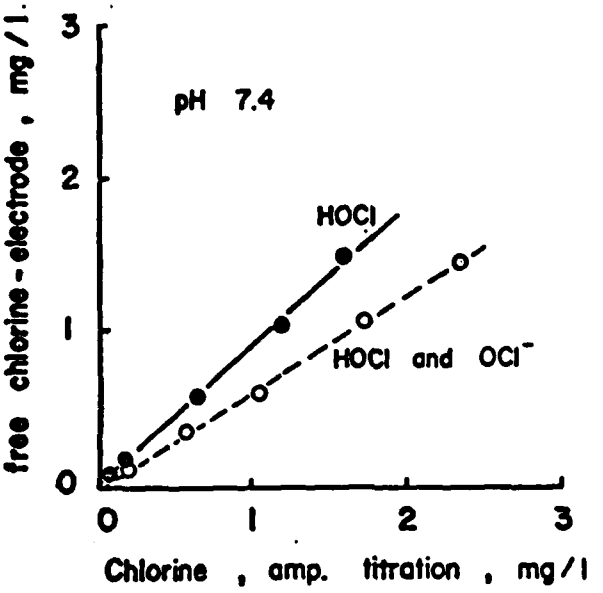
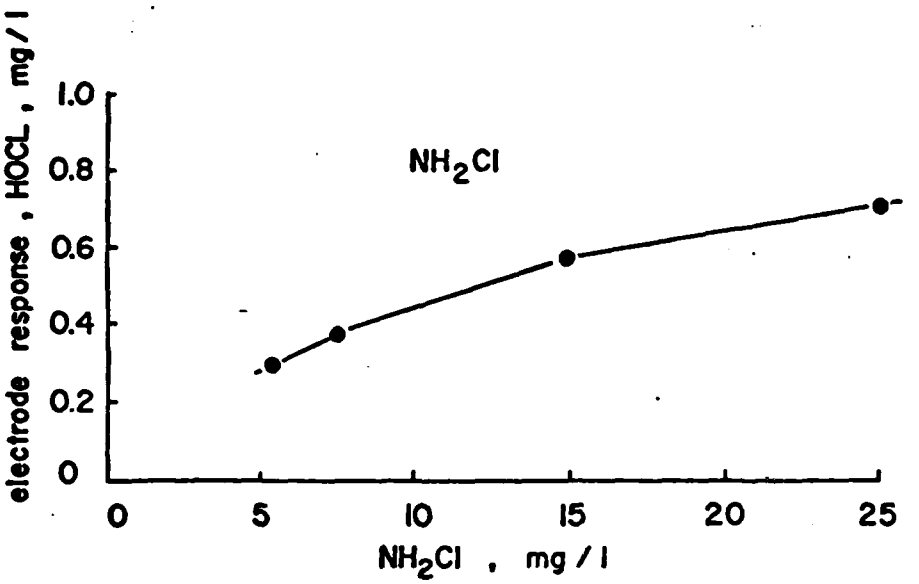


Figure 10. (upper) Response of the chlorine membrane electrode to free chlorine at pH 4.0, 20°C.

(lower) Response of the chlorine membrane electrode to total free chlorine and the HOCl fraction of the free chlorine residual at pH 7.4, 20°C.



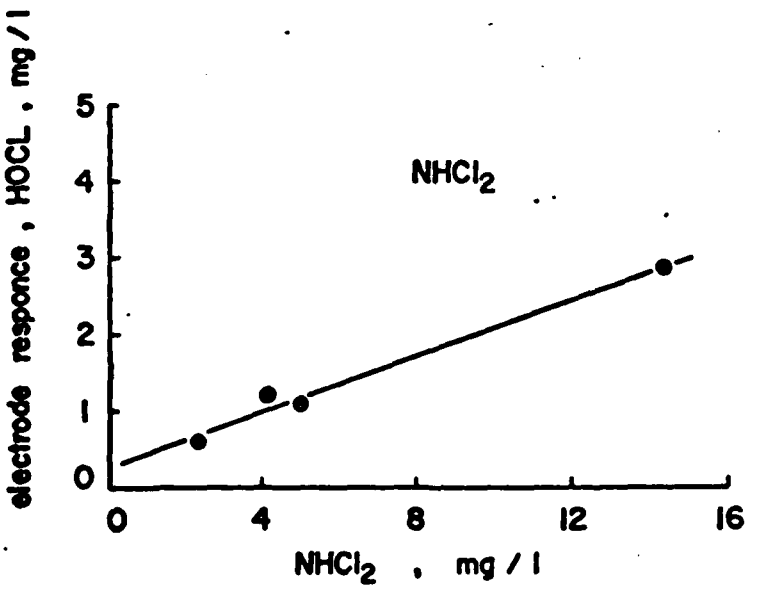
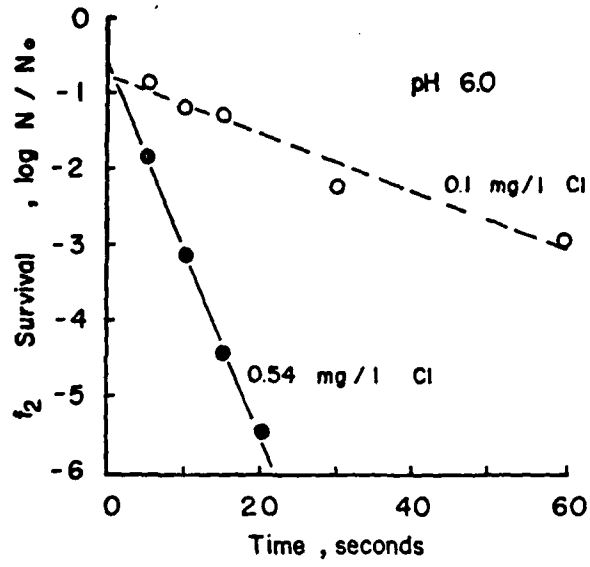


Figure 11. (upper) Apparent HOCl concentration measured by the membrane electrode as a function of NH_2Cl concentration at 20°C, pH 7.0.

(lower) Apparent HOCl concentration measured by the membrane electrode as a function of $NHCl_2$ concentration at 20°C, pH 7.0.



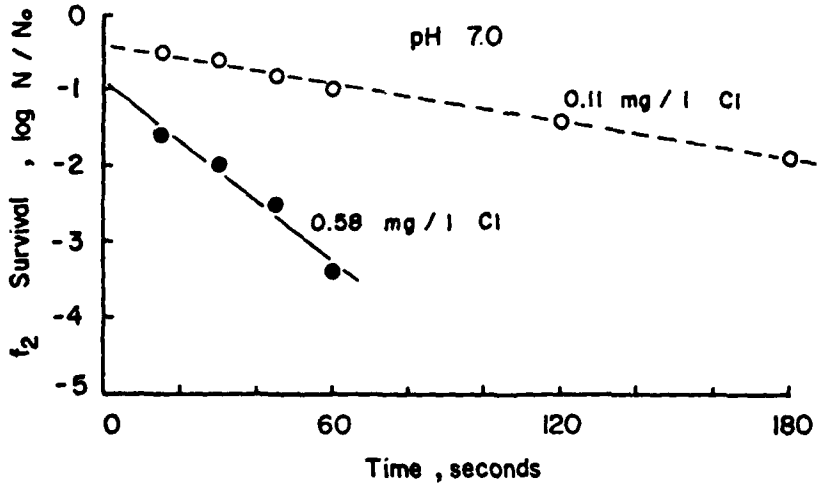
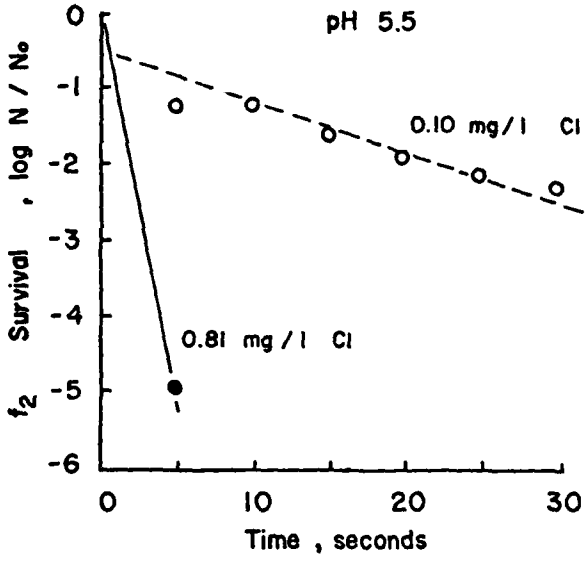


Figure 12. Inactivation of f2 bacterial virus by free chlorine at pH 6.0 and 7.0, 20°C.



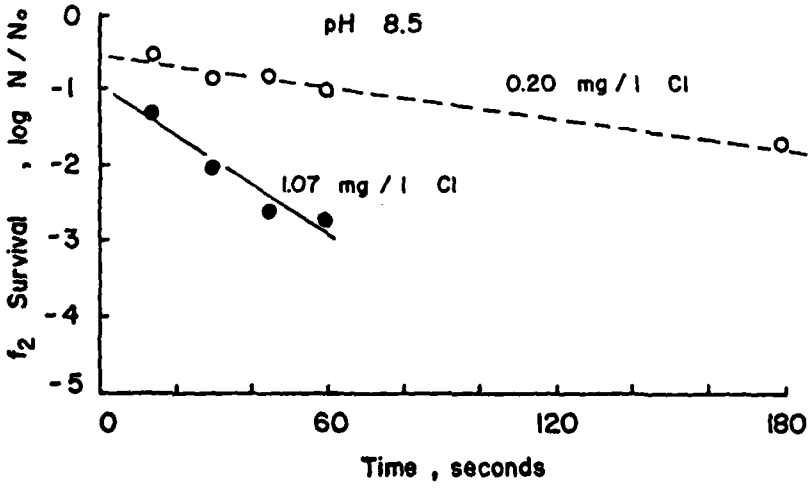


Figure 13. Inactivation of f2 bacterial virus by free chlorine at pH 5.5 and 8.5, 20°C.

organisms at time zero and N is the number at any time t. A series of similar curves were generated at each pH for free chlorine concentrations of 0 to 1.0 mg/l. Complete data are given in Appendix A. At each pH, the degree of inactivation increased as the chlorine concentration increased. The rate of inactivation k' was determined as the slope of the regression line drawn through the points, as shown in these figures. The regression lines were not forced through the origin.

Figure 14 shows the inactivation of f2 virus by monochloramine and dichloramine at pH 7.0. Little inactivation of the virus was observed over the 5 minute test period.

Figure 15 (upper panel) shows the inactivation rates, taken from the log N/N_o versus time plots, plotted against the free chlorine concentration at pH 6.0. Although there was some scatter, the relationship appears to be sufficiently linear (R = .949, slope = 29.8) for a quantitative determination of free chlorine concentration to be made from the inactivation rate. This figure also gives the inactivation rate of f2 by NH_2Cl, $NHCl_2$, and NCl_3 at pH 6.0. The great disparity between the rates of inactivation of f2 by the chloramines and by free chlorine forms the basis of the biofac calibration procedure. For free chlorine at pH 6.0, the rates of inactivation ranged from 2.4 min^{-1} for 0.1 mg/l to 20.0 min^{-1} for 0.8 mg/l. For combined chlorine, the rate of inactivation was 0.4 min^{-1} for 0.8 mg/l. The biofac calibration procedure can be used to quantitatively determine free chlorine levels, and to qualitatively determine the presence or absence of free chlorine for unknown solutions.

The results for inactivation experiments done at pH 7.0 are given in Figure 15 (lower panel) and the results for pH 5.5 and 8.5 are shown in Figure 16. The pH 7.0 results were also linear with an R value of 9.32, slope 3.9, and were used as a calibration curve for the determina-

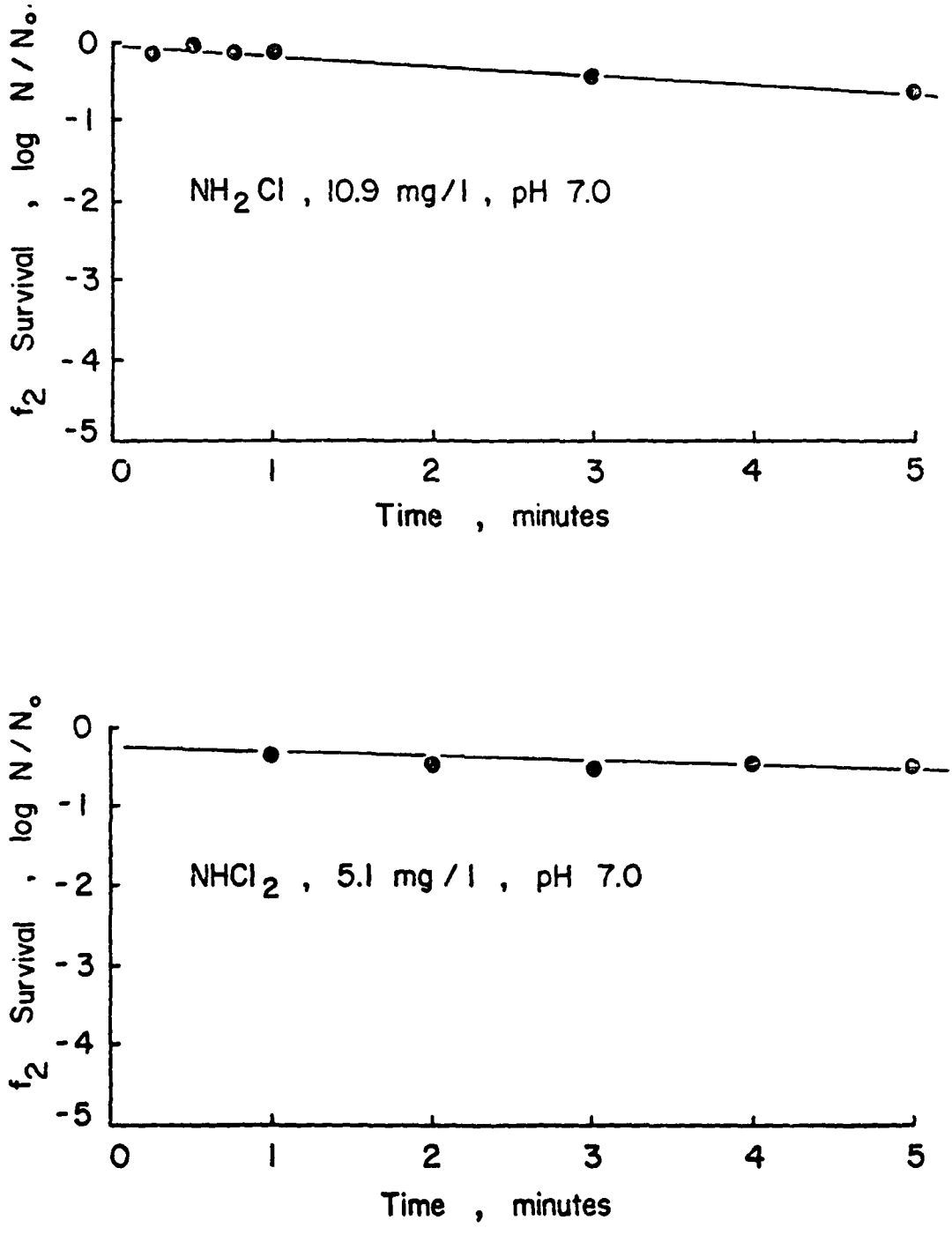


Figure 14. Inactivation of f2 bacterial virus by 10.9 mg/ℓ monochloramine and by 5.1 mg/ℓ dichloramine at pH 7.0, 20°C.

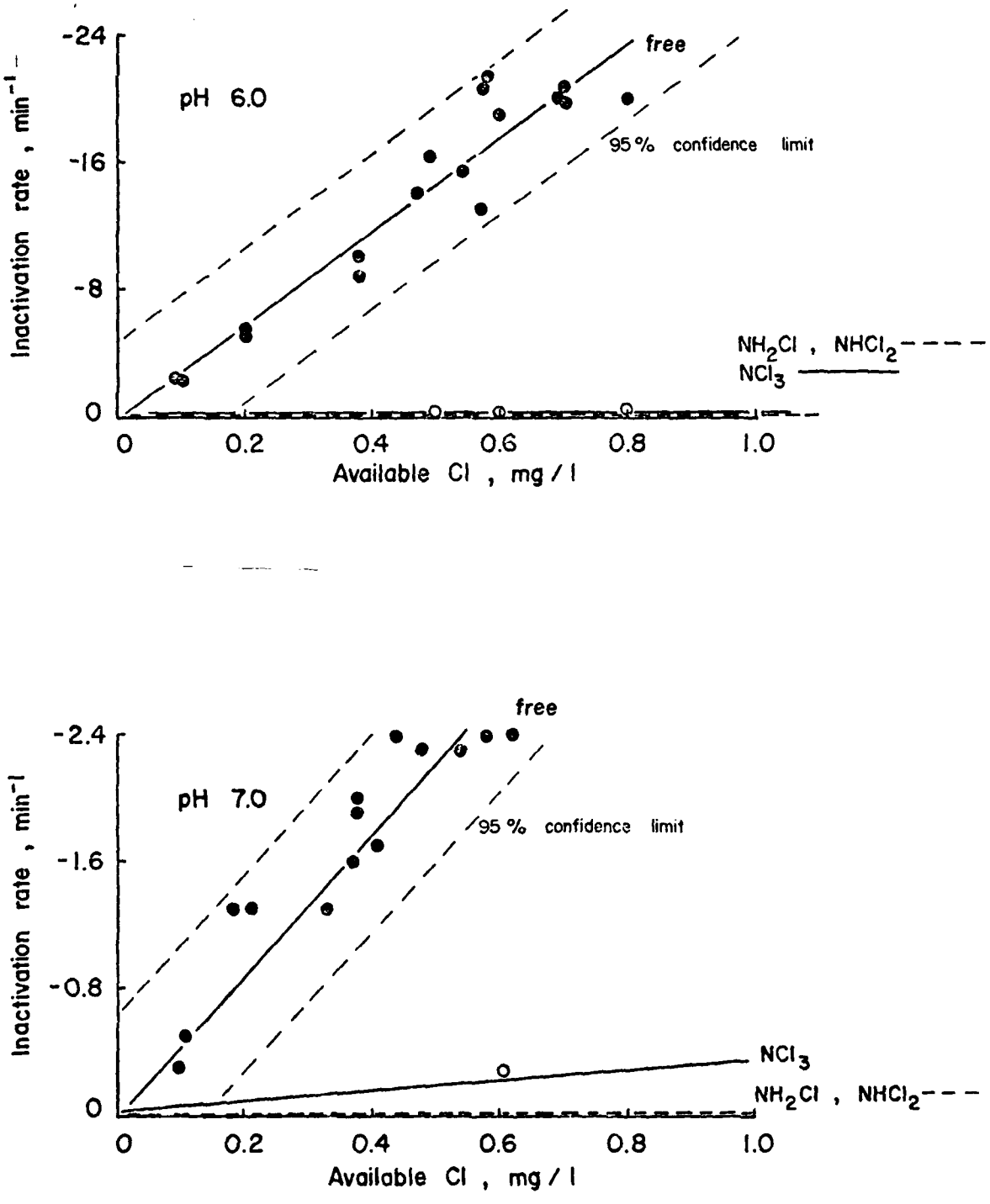


Figure 15. Rate of inactivation (K') of f2 by varying concentrations of free and combined chlorine at pH 6.0 and 7.0, 20°C.

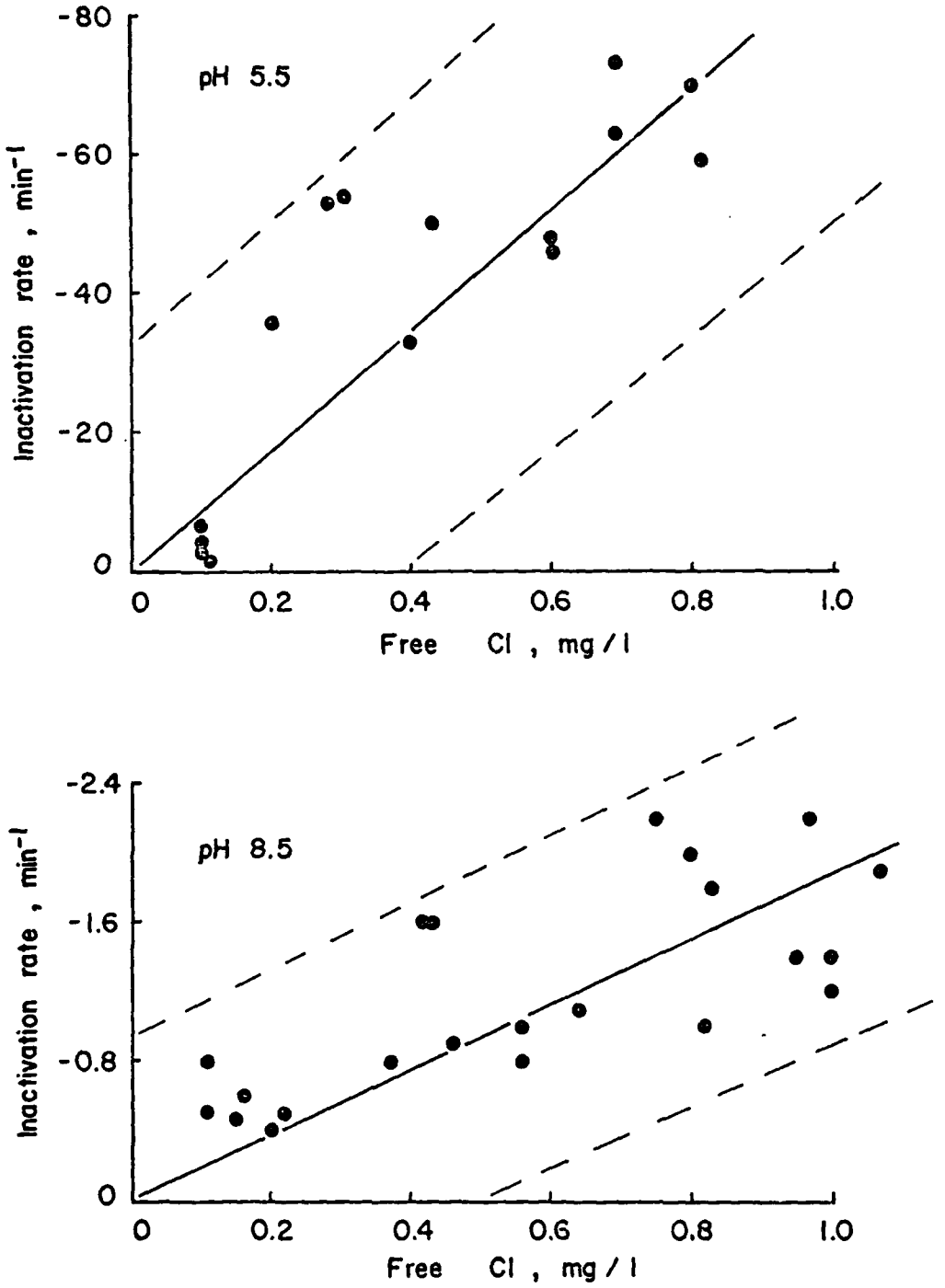


Figure 16. Rate of inactivation (K') of f2 by varying concentrations of free chlorine at pH 5.5 and 8.5, 20°C.

tion of free chlorine from the inactivation rate in unknown solutions. Although the inactivation rates at pH 5.5 and 8.5 did increase with chlorine concentration, the degree of scatter was too great for reliable quantitative use, with R values of .810 and .744 respectively, so subsequent experiments were confined to pH 6.0 and 7.0.

White (1972) states that hypochlorite (OCl^-) is 1/80 to 1/300 as effective a biocide as HOCl. The percent HOCl in the free chlorine solutions at each experimental pH used in this study were 99% at pH 5.5, 98% at pH 6.0, 80% at pH 7.0 and 11% at pH 8.5. The observed inactivation of f2 should therefore be attributable to HOCl alone, particularly at pH 5.5, 6.0 and 7.0 because of the preponderance of the more active species. Figure 17 was constructed by assuming the contribution to the inactivation of the virus by OCl^- was negligible and shows the inactivation rate of f2 as a function of the calculated HOCl concentration at each pH. There appears to be some effect of pH on the virus itself, since greater inactivation was observed at lower pH values for equivalent HOCl concentrations. The dependence of inactivation rate on HOCl concentration decreases from pH 5.5 to 7.0. The slope of the line for pH 8.5 is similar to that for pH 7.0.

Specificity of the colorimetric tests. The specificity of the colorimetric tests was determined by comparing the values of apparent free chlorine obtained by the test to the value of free chlorine obtained by the biofac procedure for chemically defined chloramine solutions. In these experiments, chloramine solutions were added to the reaction vessel, f2 was added at time zero, and colorimetric tests for free chlorine and the f2 inactivation rate was determined. Levels of free chlorine were determined from the standard curves for the colorimetric

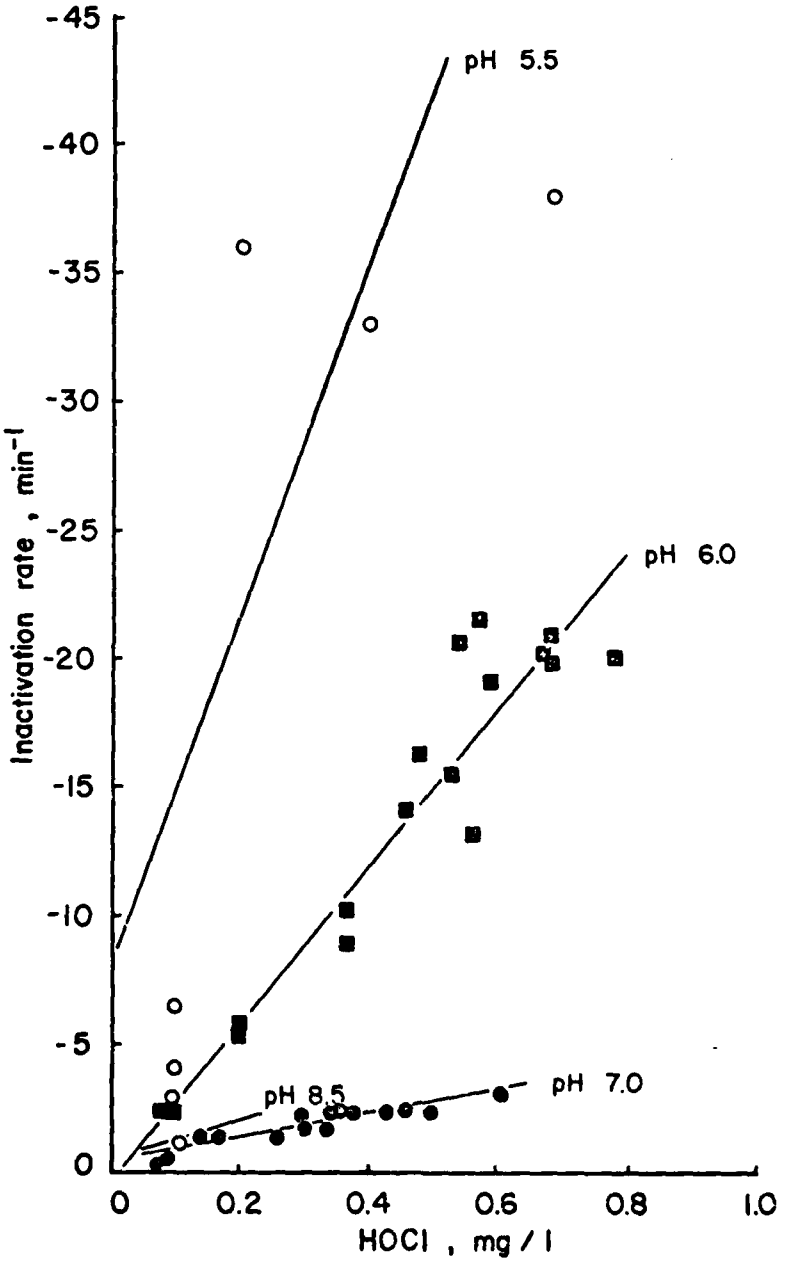


Figure 17. Inactivation rate of f2 bacterial virus as a function of the calculated HOCl concentration at pH 5.5, 6.0, 7.0, and 8.5, all at 20°C.

tests and from the appropriate biofac calibration curve. A false positive for the colorimetric test is defined as a significant (absorbance $\neq 0$, $p = .05$) indication of free chlorine by the test where the biofac procedure indicates no free chlorine. Levels of significance for DPD and FACTS were given in a preceeding section. Table 1 gives the monochloramine concentrations used in this set of experiments, determined amperometrically and spectrophotometrically, and the apparent free chlorine level measured by the other tests. The FACTS procedure was the most specific, with no false positives occurring at monochloramine concentrations of up to 22.3 mg/1, the highest level tested. The DPD-steadifac procedure using the tablet reagent gave marginally significant false positive readings at 9.7 mg/1, with higher levels required for greater false positive results. The DPD test with powder reagent was the least specific test for free chlorine, showing false positive indications of free chlorine at monochloramine levels as low as 0.93 mg/1. The addition of thioacetamide was effective in reducing, but not eliminating, the false positives obtained with DPD. A similar order of specificity was obtained at pH 7.0 as shown in Table 2, with the FACTS procedure being the most specific and the DPD powder procedure the least specific.

All of the tests performed well with dichloramine as shown by the results in Table 3 (pH 6.0) and Table 4 (pH 7.0). It should be noted that the pH values used, 6 and 7, are those pH's at which the f2 inactivation was determined. The colorimetric procedures all incorporate a buffer to maintain the optimum pH for the test. No false positives were obtained with FACTS, DPDT-steadifac, and DPDT at dichloramine concentrations of up to 18.89 mg/1. False positives were given by the DPD and DPD-steadifac procedure with the powder reagent at

TABLE 1. Apparent free chlorine concentration measured by the DPD procedures, FACTS, and biofac at varying levels of monochloramine at pH 6.0, 20°C.

Chlorine, mg/l			Free Chlorine, mg/l					
Amperometric		Spectrophotometric	Colorimetric					
NH_2Cl	Total	NH_2Cl	FACTS	DPDP	DPDPSF	DPDT	DPDTSF	Biofac
1.05	1.05	--	.01	.17*	.07	.04	0	0
.93	.93	--	0	.17*	.08	.06	.02	0
5.10	5.10	5.90	.01	.95*	.11	.49*	0	0
4.80	4.80	5.30	.01	.77*	.19*	.24*	0	0
9.70	9.70	11.20	.09	2.24*	.46*	.37*	.11	0
9.70	9.70	10.20	.04	2.62*	.57*	.36*	.16*	0
21.40	21.40	18.90	.06	$\geq$3.44*	2.96*	.65*	.85*	0
22.30	22.30	19.6	.02	2.92*	2.65*	.65*	.71*	0

* significant (p = .05) false positive

TABLE 2. Apparent free chlorine concentration measured by the DPD procedures, FACTS, and biofac at varying levels of monochloramine at pH 7.0, 20°C.

Chlorine, mg/l			Free Chlorine, mg/l					
Amperometric		Spectrophotometric	Colorimetric					
NH_2Cl	Total	NH_2Cl	FACTS	DPDP	DPDPSF	DPDT	DPDTSF	BioFAC
1.0			0	.26*	.05	.07	.03	.03
1.01			0	.17*	.12	.02	0	.02
5.01			0	.77*	.17*	.24*	.09	.02
5.04			0	1.20*	.10	.32*	.04	.04
9.10	9.10	9.70	.03	1.38*	.19*	.37*	0	.03
10.93			.04	$\geq$3.44*	.23*			.03
11.10	11.10	9.70	.04	1.46*	.12	.26*	.02	.02
11.24			.03	1.93*	.82*			.04
19.46		20.31	.03	$\geq$3.44*	1.17*	.81*	.75*	.02
20.03		18.75	.06	$\geq$3.44*	2.26*	1.04*	1.12*	.02

* significant (p = .05) false positive

TABLE 3. Apparent free chlorine concentration measured by the DPD procedures, FACTS, and biofac at varying levels of dichloramine at pH 6.0, 20°C.

$NHCl_2$		Free Chlorine, mg/l					
		Colorimetric					
Amperometric	Spectrophotometric	FACTS	DPDP	DPDPSF	DPDT	DPDTSF	BioFAC
1.10		.01	.03	.03	.03	.03	0
1.19		.01	.03	.03	.02	.03	0
4.78		.01	.03	.03	.02	.03	0
4.89		.03	.03	.03	.02	.03	0
9.39	9.45	0	.17*	.11	.07	.11	0
9.62	9.45	0	.33*	.13*	.10	.09	0
18.33	16.50	.04	.38*	.23*	.10	.09	0
18.89	17.00	.01	.38*	.21*	.10	.10	0

* significant (p = .05) false positive

TABLE 4. Apparent free chlorine concentration measured by the DPD procedures, FACTS, and biofac at varying levels of dichloramine at pH 7.0, 20°C.

$NHCl_2$, mg/l spectrophotometer	total Cl amperometric	Free Chlorine, mg/l						
		Colorimetric						
		FACTS	DPDP	DPDPSF	DPDT	DPDTSF	Electrode	BioFAC
5.10		0	.03	.02	.03	.02	1.7	0
5.10		.03	.03	.02	.02	.02	1.0	0
8.85	10.40	.06	.09	.09	.04	.08	--	0
9.00	10.63	.06	.06	.08	.04	.08	--	.01
17.24	18.40	.06	.19*	.13*	.07	.12	--	.01
17.58	19.38	.06	.14*	.14*	.04	.12	--	.02

* significant (p = .05) false positive

$NHCl_2$ levels of 9.62 mg/l at pH 6.0 and 17.24 mg/l at pH 7.0.

Trichloramine was found to produce false positives in all the tests at relatively low levels (Tables 5 and 6). Indications of free chlorine levels of approximately 0.2 mg/l were seen for all tests except DPDT at trichloramine concentration of 0.6 mg/l. The results for DPDT at this level were not significantly greater than zero, although close in magnitude to the values obtained by the other tests, which accounts for the higher level of NCl_3 required to produce a false positive given in Tables 5 and 6.

The biofac procedure showed significant free chlorine levels at trichloramine concentrations of 3.2 mg/l at pH 7.0, but not at pH 6.0. Trichloramine was more effective in inactivating f2 than NH_2Cl and $NHCl_2$, which resulted in this indication of free chlorine.

Nitrogen Breakpoint. Figure 18 (top panel) shows the breakpoint curve for a 2.0 mg/l NH_3-N solution for 30 minutes reaction time. The breakpoint of this solution was 17 mg/l chlorine. Free chlorine residuals were determined at points along this curve by the DPD, FACTS, free chlorine electrode, amperometric, and biofac procedures. The shape of the breakpoint curve indicates that no free chlorine should be present below 17.0 mg/l and this was found to be the case as shown by the biofac procedure (Figure 18, middle panel). The FACTS and DPDTSF procedures were found to be specific for free chlorine under these conditions with no significant levels of free chlorine measured until after the breakpoint. The amperometric procedure and the free chlorine electrode gave significant false positives for samples below the breakpoint at chlorine residuals of approximately 2 to 8 mg/l.

TABLE 5. Apparent free chlorine concentration measured by the DPD procedures, FACTS, and biofac at varying levels of trichloramine at pH 6.0, 20°C.

NCl_3, mg/l spectrophotometric	total Cl amperometric	Free Chlorine, mg/l Colorimetric FACTS	DPDP	DPDPSF	DPDT	DPDTSF	Electrode	BioFAC
.50	.51	.36*	.22*	.19*	.14	.14		.01
.60	.64	.36*	.22*	.19*	.16	.17*		.01
.84	1.28	.54*	.36*	.35*	.30*	.29*		.01
.84	1.35	.51*	.34*	.28*	.28*	.27*		.01
2.13	3.07	1.07*	.54*	.47*	.36*	.37*		.02
2.40	3.20	1.05*	.64*	.47*	.41*	.37*		.02
3.32	--	1.64*	.96*	.66*	.62*	.48*		.02
3.37	--	1.74*	.98*	.70*	.66*	.34*		.02
3.70	5.40	1.74*	.91*	.73*	.56*	.53*		.03
3.70	5.80	1.64*	.84*	.75*	.58*	.55*		.03
7.10	13.90	2.16*	1.29*	.92*	1.05*	.77*	>5.0	.06
7.20	10.24	1.85*	1.27*	.82*	.87*	.63*	>5.0	.06
9.20	13.49	>2.84*	1.72*	1.50*	1.23*	.87*		.10
9.50	13.20	>2.84*	1.89*	1.43*	1.23*	.95*		.09
19.70	23.90	>2.84*	2.40*	1.74*	1.69*	1.29*	>5.0	.14
21.30	21.10	>2.84*	2.62*	1.48*	1.87*	1.28*		.12

* significant (p = .05) false positive

TABLE 6. Apparent free chlorine concentration measured by the DPD procedures, FACTS, and biofac at varying levels of trichloramine at pH 7.0, 20°C.

NCl_3, mg/l spectrophotometric	total Cl amperometric	Free Chlorine, mg/l						
		Colorimetric						
		FACTS	DPDP	DPDPSF	DPDT	DPDTSF	Electrode	BioFAC
.58		.28*	.19*	.20*	.15	.16*		.06
.59		.31*	.20*	.20*	.16	.17*		.06
3.16		1.56*	.77*	.64*	.53*	.52*		.25
3.24		1.60*	.76*	.63*	.53*	.54*		.25
3.16	6.69	1.85*	.93*	.75*	.58*	.51*		.57
3.16	7.03	1.91*	.91*	.68*	.60*	.47*		.57
8.90	9.10	1.85*	.96*	.77*	.70*	.53*	>5.0	.48
9.01	16.60	>2.84*	1.14*	1.03*	.81*	.82*		.86
16.54	16.70	>2.84*	1.72*	1.15*	1.23*	1.09*		1.29

* significant (p = 05\ false positive

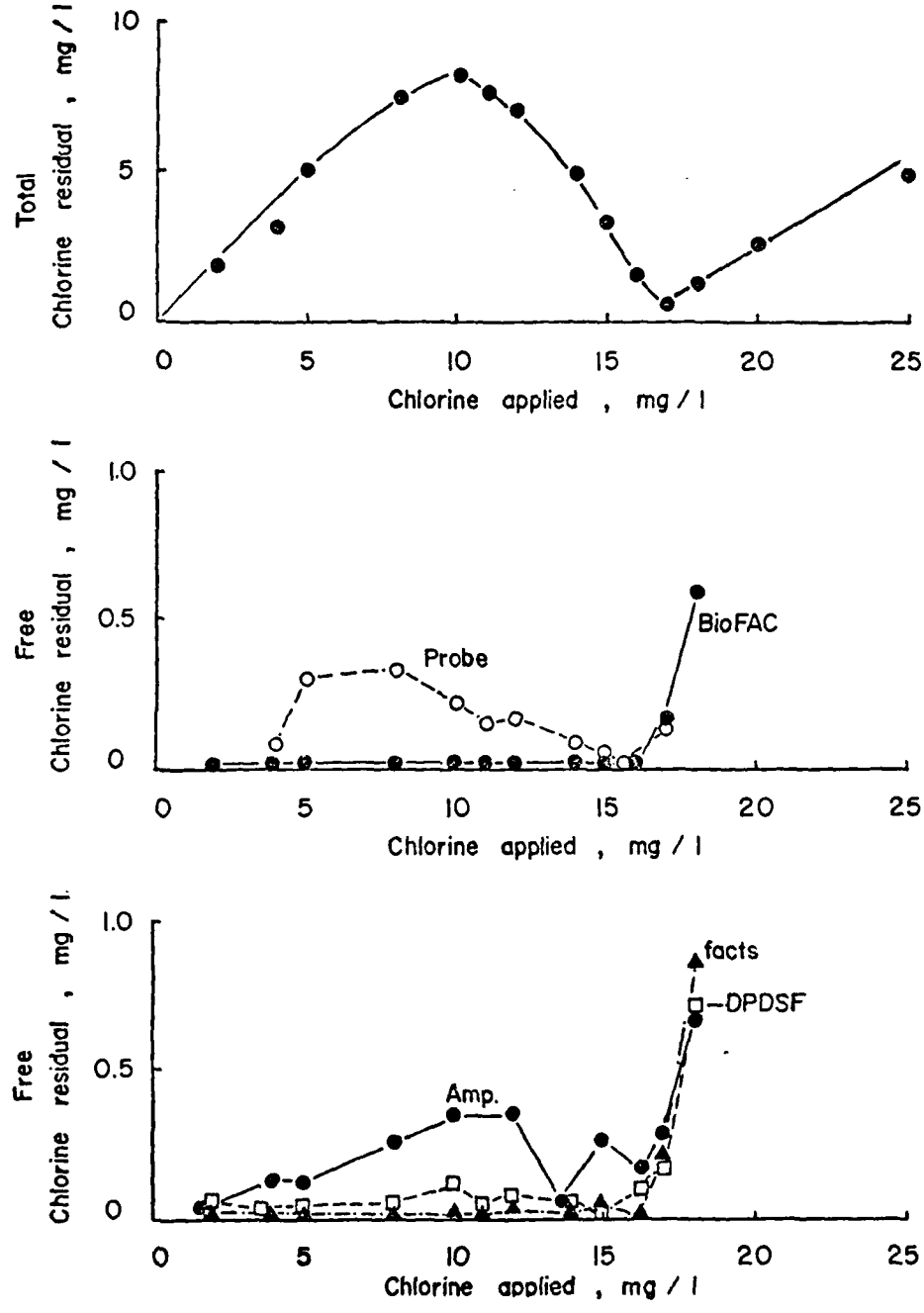


Figure 18. (upper) Breakpoint curve for a 2.0 mg/ℓ NH_3-N solution

(middle) Free chlorine measured by the electrode and biofac procedures at points along the breakpoint curve.

(lower) Free chlorine measured by the FACTS, amperometric titration, and DPD-steadifac (tablet) procedures at points along the breakpoint curve.

Figure 19 gives a comparison of the DPD methods for points along the same breakpoint curve. While false positives were observed for DPDP and DPDT, the addition of thioacetamide resulted in a reduction in the magnitude of the false positive and gave results comparable to the FACTS and biofac procedure. Figure 20 shows the results of a similar experiment performed at a lower nitrogen concentration of 0.3 mg/l. The breakpoint at 2.8 mg/l available chlorine was analogous to that obtained with many surface waters and reflects concentrations that might be encountered at a water treatment plant. No free chlorine was detected by the FACTS, DPD-steadifac, and biofac procedures below the breakpoint. A slight color was developed by the DPD procedure, but the absorbance was below that required for the sample to be designated as significantly false positive. The amperometric titration procedure was found to be the least specific in the experiment, giving false positive indications of free chlorine of approximately 0.1 mg/l before the breakpoint dose was reached. Figure 21 gives a comparison of the four DPD procedures for the same experiment. Again, the tablet reagent was more specific than the powder and the addition of thioacetamide resulted in an improvement in specificity.

Comparison of inactivation of f2, *E. coli* and polio 1. The inactivation of polio 1, f2, and *E. coli* B by 0.50 mg/l free chlorine at pH 7.0 and 20°C is shown in Figure 22. The poliovirus stock was purified by sucrose density gradient centrifugation but still had some chlorine demand, resulting in the loss of free chlorine observed over the first minute. Since all three microorganisms were mixed in the same reaction vessel and therefore exposed to identical conditions, valid comparisons of resistance can be made. Polio 1 and f2 were inactivated at about th same rate, with 2.5 logs reduction in 10 seconds before

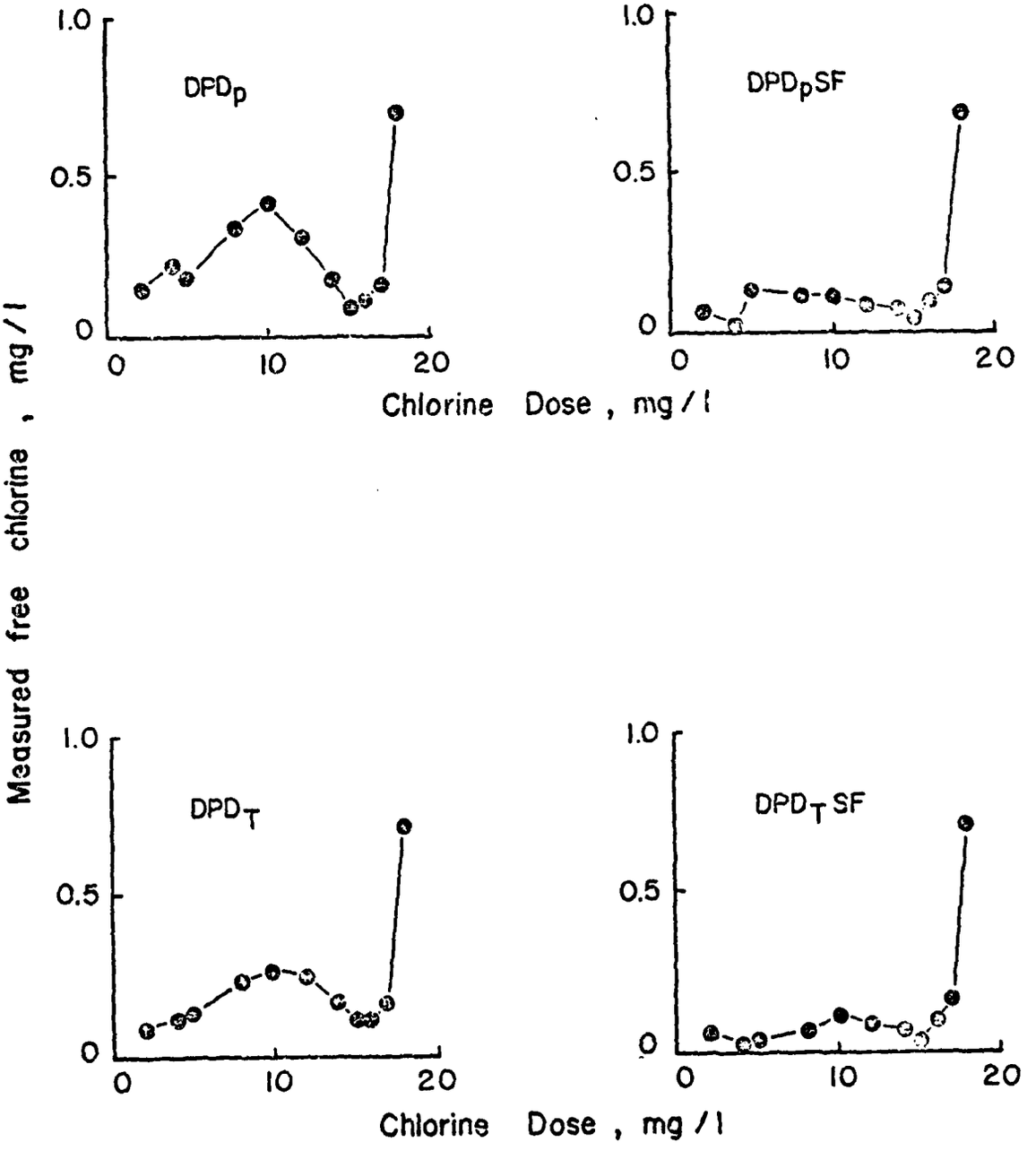


Figure 19. Free chlorine measured by the DPD procedures at points along the breakpoint curve of a 2.0 mg/ℓ NH_3-N solution.

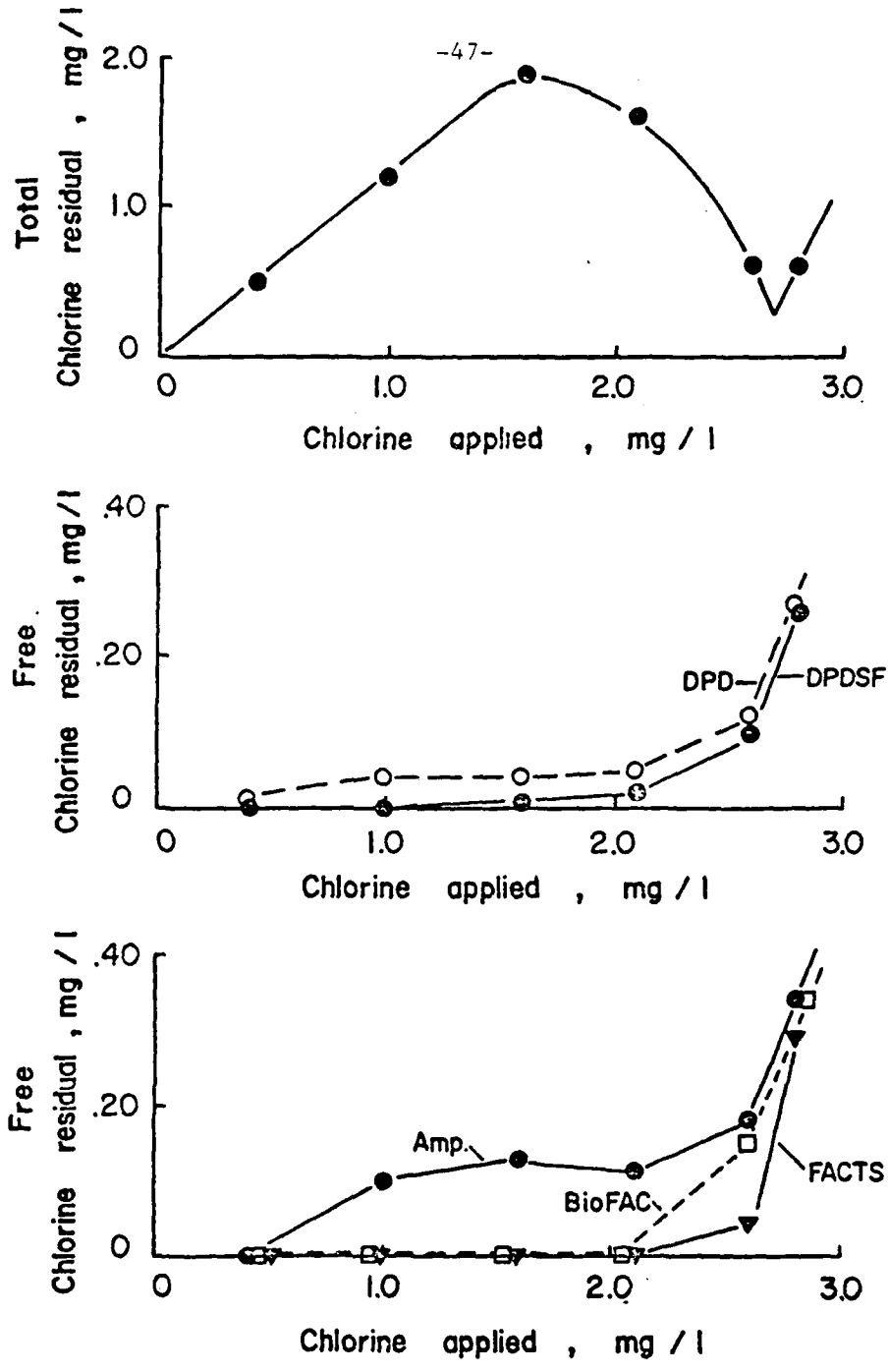


Figure 20. (upper) Breakpoint curve for a 0.30 mg/ℓ NH_3-N solution.

(middle) Free chlorine measured by the DPD and DPD-steadifac (tablet) procedures at points along the breakpoint curve.

(lower) Free chlorine measured by the amperometric titration, FACTS and biofac procedures at points along the breakpoint curve.

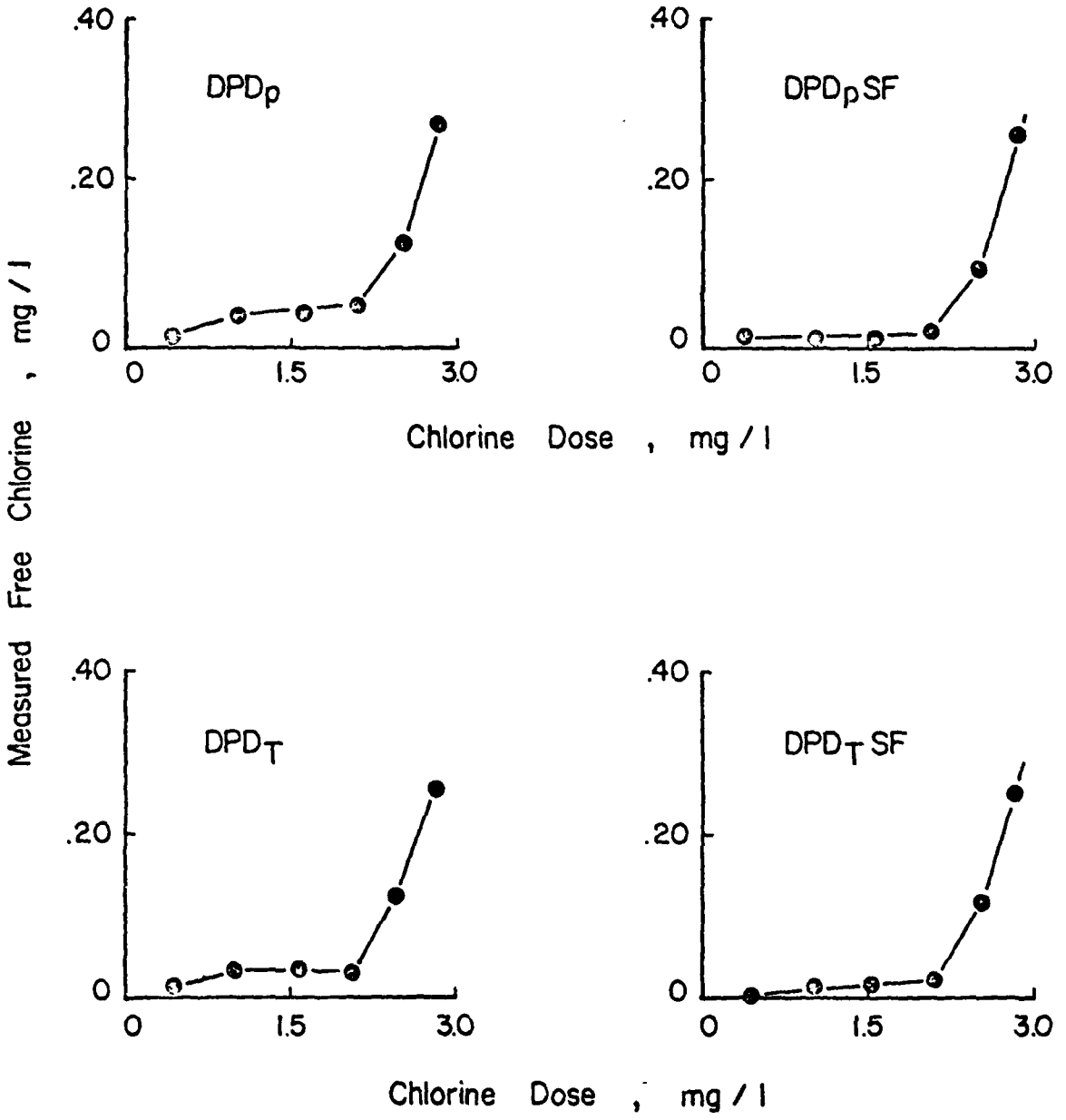


Figure 21. Free chlorine measured by the DPD procedures at points along the breakpoint curve of a 0.30 mg/ℓ NH_3-N solution.

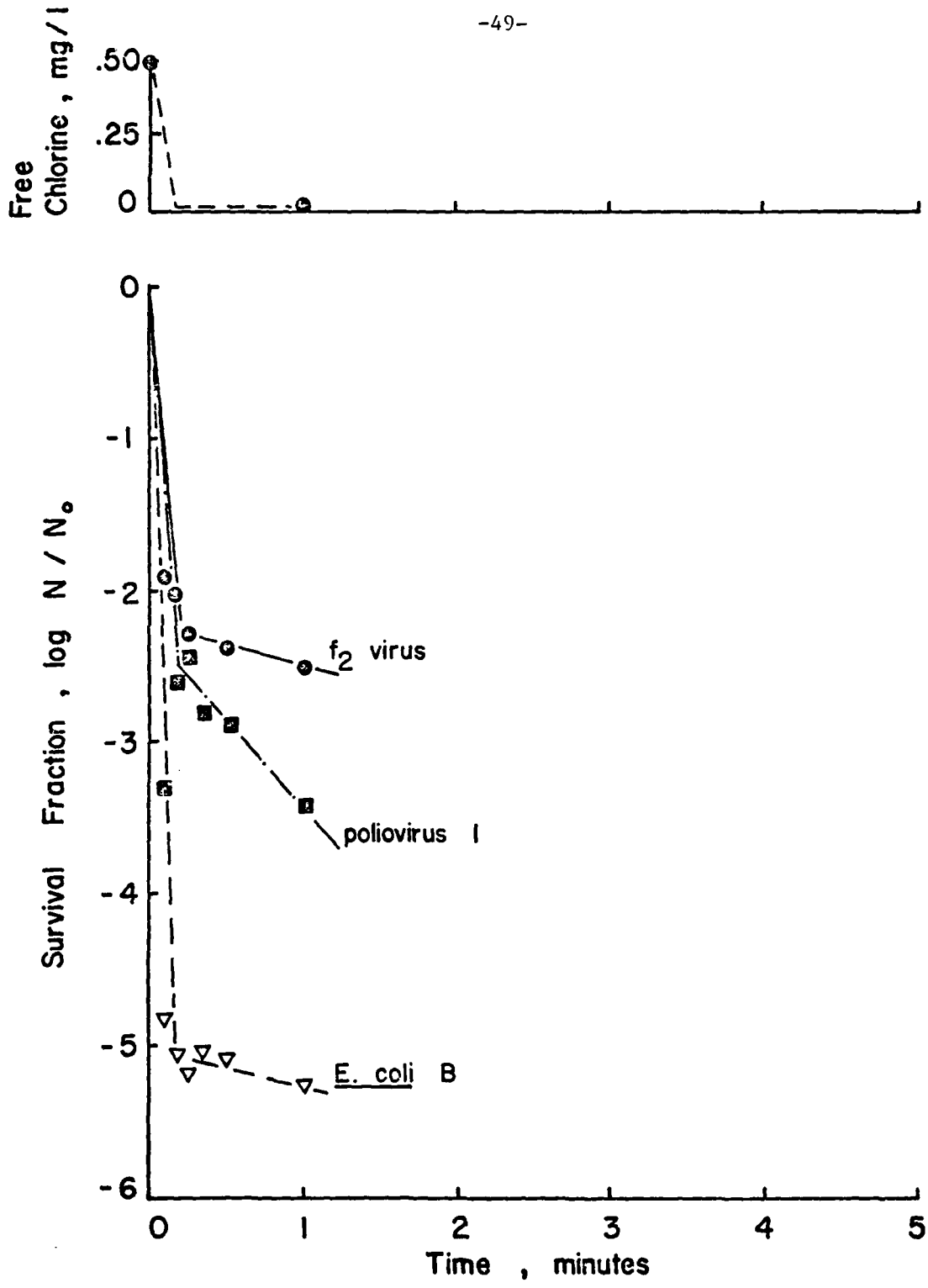


Figure 22. Inactivation of f2, poliovirus 1 and *E. coli* B by free chlorine at pH 7.0, 20°C.

the free chlorine was lost. *E. coli* B was reduced by 5.0 logs over the same time period.

The inactivation of the three microorganisms by monochloramine and dichloramine is shown in Figures 23 and 24. Both compounds were poor bactericides and viricides when compared to free chlorine. Little difference between biocidal activity of NH_2Cl and $NHCl_2$ was observed at pH 6.0. The order of resistance of the microorganism to NH_2Cl and $NHCl_2$ was found to be f2 > polio 1 > *E. coli* B.

Figure 25 shows the inactivation of f2, polio 1 and *E. coli* B by 5 mg/l NCl_3 at pH 7.0, 20°C. A 30 fold molar excess of ammonia was added to the NCl_3 solution to eliminate free chlorine. *E. coli* B was found to be very susceptible to NCl_3, with 5.5 logs inactivation in 5 seconds. As a viricide, NCl_3 was found to be intermediate between the poor viricides NH_2Cl and $NHCl_2$ and the effective viricide, free chlorine (HOCl). Polio 1 was more resistant to NCl_3 than f2.

DISCUSSION

Biofac. One of the primary goals of this study was to develop a biological test for the quantitative determination of free chlorine. The results obtained indicate that a system using the rate of inactivation of the bacterial virus f2 as an indicator of free chlorine concentration yields quantitative calibration for pH values of 6.0 and 7.0. In this pH range, the inactivation rate increases linearly with free chlorine concentration. Satisfactory calibration curves were not obtained at pH 5.5, since the inactivation rate is too rapid for biological samples to be taken with time. A short time sampling system has been described by Sharp *et al*, (1976) and would be suitable for use at the lower pH range. At pH 8.5, where the less viricidal OCl^- predominates, the results showed a great deal of scatter. Inactiva-

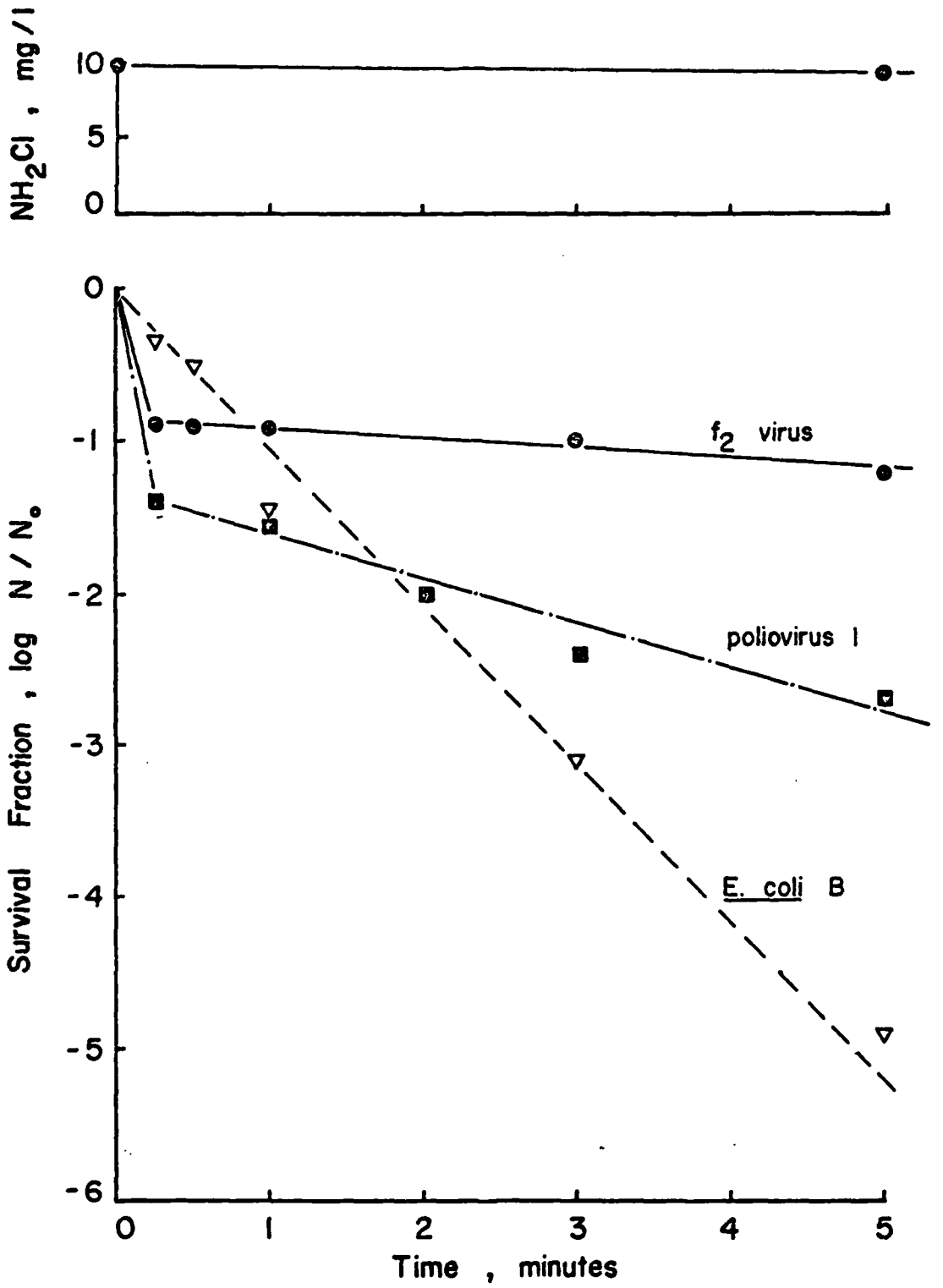


Figure 23. Inactivation of f2, poliovirus 1 and *E. coli* B monochloramine at pH 7.0, 20°C.

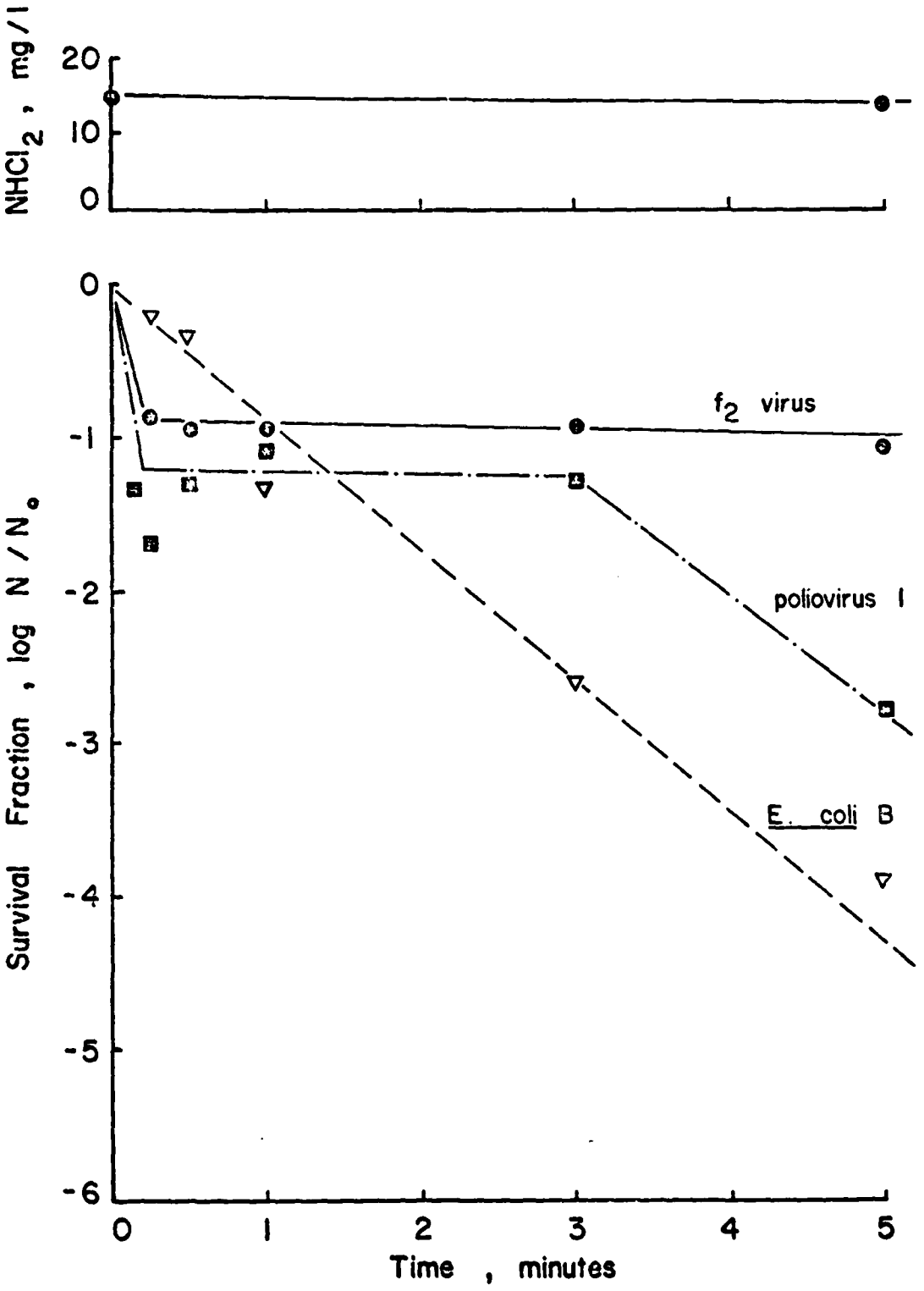


Figure 24. Inactivation of f2, poliovirus 1, and *E. coli* B by dichloramine at pH 7.0, 20°C.

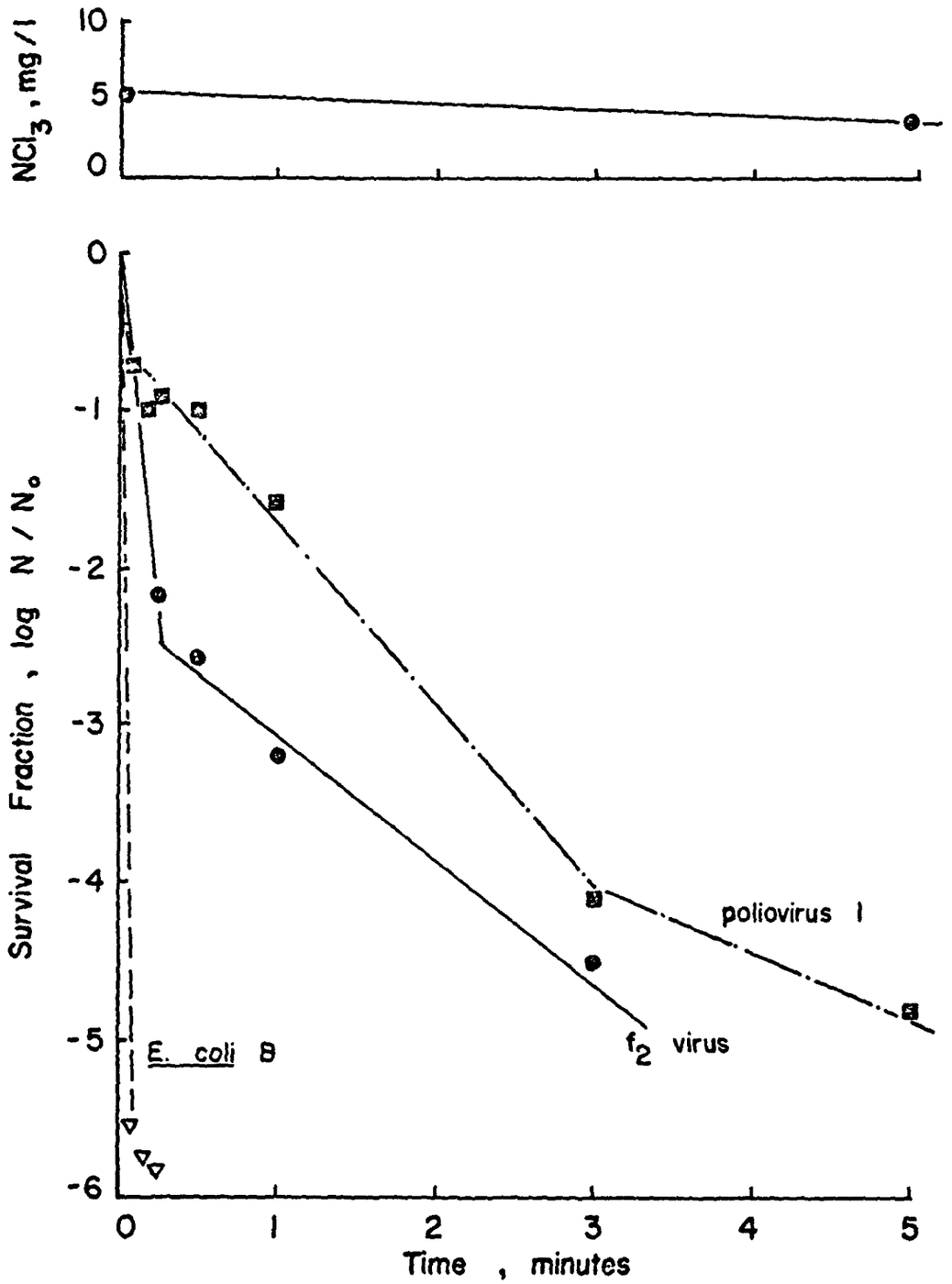


Figure 25. Inactivation of f2, poliovirus 1, and *E. coli* B by trichloramine at pH 7.0, 20°C.

tion studies with f2 generally yield biphasic inactivation curves, with a rapid initial inactivation and a slower secondary rate. The secondary rate was used for the biofac calibration curves since a better correlation was obtained. The pH 6.0 and 7.0 data were used for comparison to the colorimetric tests.

A biological test system of this type provides the ideal referee technique for evaluation of test specificity, since there is no interference from monochloramine and dichloramine (Figure 15) and since the results obtained from the chlorine residual tests are intended to reflect the biocidal activity of the solution. An extension of this biological system to the measurement of other actively germicidal compounds (O_3, ClO_2, UV) should be possible. The procedure is suggested for evaluation of other tests only, not for routine use or monitoring, since there is a one day delay in obtaining results.

Chloramines. The preparation of chemically defined chloramine solutions was essential for evaluation of the tests for chlorine. Previous work with monochloramine (Johnson and Overby 1964, and Snead 1976) and dichloramine (Richfield 1978) has shown that solutions of these chloramines can be prepared with free chlorine absent. Monochloramine and dichloramine were sufficiently stable at the pH values and time periods used so that the studies could be performed without appreciable loss of either compound. The absence of free chlorine in the preparations was confirmed by the lack of inactivation of f2 by these solutions. Much less work has been done with trichloramine, primarily because of the lack of stability of the compound and because trichloramine is not

thought to be as prevalent as monochloramine and dichloramine under conditions normally encountered in the field. A 30 fold molar excess of ammonia was used in the trichloramine preparations in this study to suppress free chlorine. Since the viricidal activity of NCl_3 was evaluated at pH 6.0 and 7.0 and the reaction between free chlorine and ammonia was rapid at higher pH values, any free chlorine formed by the decomposition of NCl_3 would be consumed. Chemical tests for determining the presence of free chlorine in trichloramine solutions are not adequate, since the tests respond to NCl_3 in much the same manner as to free chlorine. Spectrophotometric methods are not sensitive enough to detect small quantities of free chlorine in trichloramine solutions. The biological procedure also gives equivocal results. Trichloramine was found to be a better biocide than monochloramine or dichloramine, but not as potent as free chlorine. This intermediate result may reflect the true biocidal efficacy of NCl_3, or it may be due to the presence of trace quantitites of free chlorine in the NCl_3 preparation. Biofac gave free chlorine levels of 8-18% of the NCl_3 level at pH 7.0 and levels of 1.0% of the NCl_3 concentration at pH 6.0. This is contrary to that expected since the reaction rate of free chlorine with ammonia increases from pH 6.0 to 7.0 (Weil and Morris 1949, 1978), and is due to the fact that f2 is more rapidly inactivated at pH 6.0 than at pH 7.0. That is, the difference in the inactivation of f2 at pH 6.0 by the NCl_3 solution and an equivalent free chlorine solution is greater than the difference obtained at pH 7.0. The inactivation by NCl_3 remains relatively constant when going from pH 7.0 to 6.0, while the inactivation by free chlorine increases. The biofac results obtained at pH 6.0 are probably more indicative of the true levels of free chlorine, if any is present, in the NCl_3 preparation.

DPD and DPD-steadifac. The specificity of the DPD test was found to vary with the reagent (tablet or powder) used and with the species of chloramine. Monochloramine produced false positives with DPD powder at lower levels than with DPD tablets. At equivalent monochloramine concentrations, the powder reagent consistantly gave higher false positive readings than the tablet reagent. The DPD reagents are now commercially available in liquid. The liquid reagent should be evaluated, since the results show that the form of the reagent may influence the specificity. The thioacetamide modification to the DPD procedure was found to be effective in reducing false positive readings. Palin (1978) states that the thioacetamide "provides immediate dechlorination without having any effect on a previously developed DPD color from free chlorine". This implies that the time of addition of the thioacetamide is important. However, the results of this study indicate that thioacetamide selectively decolorizes the colored product resulting from the DPD-chloramine reaction, and that the time of addition is not critical. The color produced by the DPD-free chlorine reaction was not affected by thioacetamide.

Dichloramine was not found to be a significant interference in the DPD tests since the levels required to produce a false positive was greater than that which would be encountered in normal practice. The breakpoint experiments show the behavior of the test in the presence of mixtures of chloramine species. For the 2.0 mg/l NH_3-N breakpoint studies where the maximum total residual was approximately 8.0 mg/l, the DPDT-steadifac procedure accurately measured the concentration of the active species. The other DPD procedures were not as specific and showed false positive free chlorine measurements.

All of the DPD procedures gave false positives with trichloramine. However, the significance of these false positives is debatable, since NCl_3 appears to be a better biocide than NH_2Cl and $NHCl_2$ and since NCl_3 is rarely found in the absence of free chlorine. Thus a false positive with NCl_3 may lead to an overestimation of the free chlorine residual, if both are present. A procedure is available for the estimation of NCl_3 by DPD, but it is doubtful if it is routinely used by field personnel.

FACTS. The FACTS procedure was found to be specific for free chlorine at all levels of monochloramine up to 22 mg/l and dichloramine up to 19 mg/l tested. Additionally, FACTS accurately paralleled the biofac procedure in measurements along the 2.0 mg/l NH_3-N breakpoint curve. False positive measurements were obtained with NCl_3. The significance of this interference is debatable, as indicated above. The greatest problem with the FACTS procedure was with the reagent itself. Some preparations of the FACTS indicator were relatively insensitive to free chlorine. The problem appears to be associated with the quality of the propanol used in the reagent. Different lots of propanol yielded reagents with different sensitivity. Although this does not pose a great problem in the laboratory, since reagent can be calibrated, a standard indicator is necessary where a color comparator is to be used.

Chlorine Membrane Electrode. The electrode was not received until late in the study and therefore complete results with the individual chloramine solutions were not available. The electrode was included in the breakpoint experiments with 2.0 mg/l NH_3-N. False positive indications of free chlorine were obtained below the breakpoint. The response was 2-5% of the total chlorine level. Johnson et al. (1978)

reported an interference of 3.0% and 1.3% from monochloramine and dichloramine respectively. Under the pH conditions of the breakpoint studies, monochloramine and dichloramine were the predominant combined chlorine species present before the breakpoint. The electrode responded as expected from the results given by the above authors. The instrument used was a prototype model and the manufacturer indicated that the specificity can be improved by adjustment of the applied voltage.

Johnson *et al.* (1978) has suggested that since the electrode measures only HOCl, the active germicidal species in free chlorine solutions, the electrode can be calibrated as a direct function of disinfection efficiency. However, it appears that there is some virus effect influencing the inactivation at various pH levels. This effect would not be accounted for in a biocidal calibration of the electrode unless the calibration was applied only as a narrow pH range.

Amperometric titration. The amperometric titration procedure has long been used in the United States as a referee technique for evaluation of other methods of chlorine residual measurement. The results obtained in this study show that the method is not absolutely specific for free chlorine. False positive measurements by the amperometric procedure were similar in magnitude to those obtained with the membrane electrode and the DPD procedures without thioacetamide. Although Standard Methods (1975) states that the amperometric titration method is a "...standard of comparison for the determination of free or combined chlorine...", it is also noted that "monochloramine can intrude into the free chlorine fraction". Nicholson (1965) reported 5 to 7% loss of free chlorine due to the high speed stirrers employed on commercially available amperometric titrators and expressed some doubts as to the completion of the reaction between free chlorine and

phenylarsenoxide. At the phenylarsenoxide endpoint, positive colorimetric reactions were consistantly observed.

A summary comparison of the specificity of DPD and FACTS is shown in Tables 7 and 8. The FACTS procedure was the most specific for free chlorine. However, the DPD-steadifac method with tablet reagents approached FACTS in specificity.

The data reported are shown as significant false positives, with significance determined from the 95% confidence bands around the standard curves. In actual field practice, any color is probably interpreted by field personnel as positive for free chlorine. In cases where free chlorine levels are low (<.2 mg/l), a measurement of total chlorine should be taken. If the total chlorine residual is high, the validity of the free chlorine measurement may be in question.

The breakpoint studies with 0.3 mg/l ammonia nitrogen solutions show that the colorimetric tests reflect the biocidal activity of the solution in the absence of high levels of chloramines. The levels of combined chlorine found in this experiment reflect those likely to be encountered in water treatment.

TABLE 7. Lowest concentration of monochloramine, dichloramine and trichloramine yielding significant false positive indications of free chlorine in the colorimetric tests at pH 6.0, 20°C.

Lowest Cl level for false positive, mg/l			
	Chloramine Species		
Test	NH_2Cl	$NHCl_2$	NCl_3
DPDT	4.8	b	0.8
DPDTSF	4.8	b	0.6
DPDP	1.1	9.4	0.5
DPDPSF	4.8	9.6	0.5
FACTS	a	b	0.5

a - no false positive observed at levels up to 22.3 mg/l

b - no false positive observed at levels up to 18.9 mg/l

TABLE 8. Lowest concentration of monochloramine, dichloramine and trichloramine yielding significant false positive indications of free chlorine in the colorimetric tests at pH 7.0, 20°C.

Lowest Cl level for false positive, mg/l			
	Chloramine Species		
Test	NH_2Cl	$NHCl_2$	NCl_3
DPDT	5.0	b	3.2
DPDTSF	19.5	b	0.6
DPDP	1.0	17.2	0.6
DPDPSF	5.0	17.2	0.6
FACTS	a	b	0.6

a - no false positive observed at levels up to 20.0 mg/l

b - no false positive observed at levels up to 17.6 mg/l

CONCLUSIONS

All of the methods tested for measurement of free chlorine residuals, DPD, FACTS, amperometric titration and membrane electrode, yield false positive determinations with one or more of the inorganic chloramines. A fundamental understanding of chlorine chemistry and the particular test procedure employed is an absolute necessity.

The steadifac modification of the DPD procedure reduces the frequency and magnitude of false positives obtained. The thioacetamide appears to selectivity decolorize the product of the DPD-monochloramine reaction.

The biological calibration procedure, biofac, clearly differentiates and quantitates free chlorine and may have possible extension for other disinfectants.

The FACTS procedure was the most specific for free chlorine.

Variability of reagent preparations of FACTS limits the quantitative use of this test, in its present form, to the laboratory.

Specificity of the DPD reagent was found to vary with the form of the reagent, with the tablet reagent consistantly yielding fewer false positives than the powder reagent.

The membrane electrode compares favorably with the other procedures for the measurement of free chlorine in the absence of combined chlorine, but was not as specific as DPD and FACTS in the presence of combined chlorine.

RECOMMENDATIONS

A total chlorine residual measurement should be taken along with a free chlorine measurement to aid in the interpretation of the free residual measurement.

Effort should be directed towards further development of the FACTS procedure to make the reagent more consistant.

The steadifac modification of the DPD procedure appears to improve the specificity of the free chlorine measurement. The method should be evaluated by other laboratories before wide-spread application for field use.

The commercially available DPD reagents and preparations should be further evaluated for free chlorine specificity. Significant differences were observed for tablet and powder reagent in this study.

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APPENDIX A - f2 inactivation rate (k') at varying free chlorine levels used for construction of biofac calibration curves.

Table A-1 f2 inactivation rate (k') at pH 7.0, 20°C

pH	free chlorine mg/l	time seconds	f2 inactivation log N/N_o	f2 inactivation rate min^{-1}
7.0	0.11	15	-0.5	-0.5
		30	-0.6	
		45	-0.8	
		60	-1.0	
		120	-1.4	
		180	-1.9	
7.0	0.10	15	-0.3	-0.3
		30	-0.6	
		45	-0.7	
		60	-0.7	
		120	-1.1	
		180	-1.2	
7.0	0.18	15	-1.3	-1.3
		30	-1.3	
		60	-1.9	
		180	LSL*	
		300	LSL	
7.0	0.21	15	-1.2	-1.3
		30	-1.5	
		45	-1.5	
		60	-2.10	
		180	LSL	
		300	LSL	

* LSL - lower sensitivity limit of viral assay

APPENDIX A (cont.)

Table A-2 f2 inactivation rate (k') at pH 7.0, 20°C

pH	free chlorine mg/l	time seconds	f2 inactivation log N/N_o	f2 inactivation rate min^{-1}
7.0	0.37	15	-1.4	-1.6
		30	-2.0	
		45	-2.1	
		60	-2.7	
		120	LSL	
7.0	0.38	15	-0.9	-1.9
		30	-1.6	
		45	-1.9	
		60	-2.4	
		120	LSL	
7.0	0.38	15	-2.4	-2.0
		30	-2.9	
		45	-3.2	
		60	-3.7	
		75	-4.5	
7.0	0.41	15	-2.3	-1.7
		30	-2.6	
		45	-3.1	
		60	-3.4	
		75	-4.0	
7.0	0.44	15	-2.2	-2.4
		30	-2.8	
		45	-3.5	
		60	-4.1	
		75	-4.5	

APPENDIX A (cont.)

Table A-3 f2 inactivation rate (k') at pH 7.0, 20°C

pH	free chlorine mg/l	time seconds	f2 inactivation log N/N_o	f2 inactivation rate min^{-1}
7.0	0.47	15	-1.8	-2.4
		30	-2.7	
		45	-3.0	
		60	LSL	
7.0	0.48	15	-1.0	-2.3
		30	-1.4	
		45	-2.2	
		60	-2.6	
		180	LSL	
7.0	0.54	15	-1.0	-2.3
		30	-1.5	
		45	-2.1	
		60	-2.7	
		180	LSL	
7.0	0.58	15	-1.6	-2.4
		30	-2.0	
		45	-2.5	
		60	-3.4	
		120	LSL	
7.0	0.62	15	-1.4	-2.4
		30	-2.0	
		45	-2.6	
		60	-3.2	
		120	LSL	

APPENDIX A (cont.)

Table A-4 f2 inactivation rate (k') at pH 7.0, 20°C

pH	free chlorine mg/l	time seconds	f2 inactivation log N/N_o	f2 inactivation rate min^{-1}
7.0	0.76	15	-1.7	-3.1
		30	-2.1	
		45	-2.7	
		60	-4.1	
		120	LSL	

APPENDIX A (cont.)

Table A-5 f2 inactivation rate (k') at pH 6.0, 20°C

pH	free chlorine mg/l	time seconds	f2 inactivation log N/N_o	f2 inactivation rate min^{-1}
6.0	0.09	5	-1.1	-2.4
		10	-2.0	
		15	-1.8	
		30	-2.7	
		45	-2.9	
		60	-3.6	
6.0	0.10	5	-0.8	-2.3
		10	-1.2	
		15	-1.4	
		30	-2.3	
		60	-2.9	
6.0	0.20	5	-1.3	-5.6
		10	-1.8	
		15	-2.5	
		30	-4.1	
		45	-4.9	
		60	LSL	
6.0	0.20	5	-1.6	-5.5
		10	-2.3	
		15	-3.2	
		30	-5.0	
		45	-5.1	
		60	LSL	

APPENDIX A (cont.)

Table A-6 f2 inactivation rate (k') at pH 6.0, 20°C

pH	free chlorine mg/l	time seconds	f2 inactivation log N/N_o	f2 inactivation rate min^{-1}
6.0	0.38	5	-3.8	-8.9
		10	-4.0	
		15	-5.3	
		30	LSL	
6.0	0.38	5	-3.9	-10.1
		10	-4.6	
		15	-5.6	
		30	LSL	
6.0	0.47	5	-1.9	-14.1
		10	-3.2	
		15	-4.3	
		30	LSL	
6.0	0.49	5	-2.7	-16.3
		10	-4.3	
		15	-5.5	
		30	LSL	
6.0	0.54	5	-2.7	-15.5
		10	-4.3	
		15	-5.5	
		20	LSL	
6.0	0.57	5	-2.3	-13.1
		10	-3.4	
		15	LSL	
6.0	0.57	5	-4.0	-20.7
		10	-5.6	
		15	-7.5	
		20	LSL	

APPENDIX A (con.)

Table A-7 f2 inactivation rate (k') at pH 6.0, 20°C

pH	free chlorine mg/l	time seconds	f2 inactivation log N/N_o	f2 inactivation rate min^{-1}
6.0	0.58	5	-2.6	-21.5
		10	-4.2	
		15	-6.2	
		30	LSL	
6.0	0.60	5	-2.9	-19.1
		10	-4.9	
		15	-6.1	
		30	LSL	
6.0	0.69	5	-1.2	-20.2
		10	-3.3	
		15	-4.6	
		30	LSL	
6.0	0.70	5	-1.3	-20.9
		10	-3.5	
		15	-4.8	
6.0	0.70	5	-2.2	-19.9
		10	-3.8	
		15	LSL	
6.0	0.80	5	-3.1	-20.0
		10	-5.3	
		15	-6.4	
		20	LSL	

APPENDIX A (cont.)

Table A-8 f2 inactivation rate (k') at pH 8.5, 20°C

pH	free chlorine mg/l	time seconds	f2 inactivation log N/N_o	f2 inactivation rate min^{-1}
8.5	0.11	15	-0.6	-0.8
		30	-0.8	
		45	-1.0	
		60	-1.4	
8.5	0.11	15	-0.3	-0.5
		30	-0.6	
		45	-0.6	
		60	-0.8	
		180	-1.7	
		300	-2.3	
8.5	0.15	15	-0.7	-0.5
		30	-1.0	
		45	-1.0	
		60	-1.2	
		180	-2.3	
		300	-3.0	
8.5	0.16	15	-0.4	-0.6
		30	-0.8	
		45	-1.0	
		60	-1.4	
		180	-2.6	
		300	-3.4	
8.5	0.20	15	-0.5	-0.4
		30	-0.8	
		45	-0.8	
		60	-1.0	
		180	-1.7	
		300	-3.3	

APPENDIX A (cont.)

Table A-9 f2 inactivation rate (k') at pH 8.5, 20°C

pH	free chlorine mg/l	time seconds	f2 inactivation log N/N_o	f2 inactivation rate min^{-1}
8.5	0.22	15	-0.5	-0.5
		30	-0.6	
		45	-0.9	
		60	-1.1	
		180	-1.9	
		300	-3.0	
8.5	0.37	15	-0.6	-0.8
		30	-0.9	
		45	-1.4	
		60	-1.5	
		180	-3.0	
		300	LSL	
8.5	0.42	15	-1.0	-1.6
		30	-1.6	
		45	-1.8	
		60	-2.3	
		180	LSL	
8.5	0.43	15	-1.0	-1.6
		30	-1.4	
		45	-1.7	
		60	-2.2	
		180	LSL	
8.5	0.46	15	-0.5	-0.9
		30	-0.9	
		45	-1.3	
		60	-1.6	
		180	-3.1	
		300	LSL	

APPENDIX A (cont.)

Table A-10 f2 inactivation rate (k') at pH 8.5, 20°C

pH	free seconds mg/l	time seconds	f2 inactivation log N/N_o	f2 inactivation rate min^{-1}
8.5	0.56	15	-0.8	-1.0
		30	-1.2	
		45	-1.3	
		60	-1.7	
		180	LSL	
8.5	0.56	15	-0.9	-0.8
		30	-1.1	
		45	-1.6	
		60	-1.8	
		180	LSL	
8.5	0.64	15	-0.7	-1.1
		30	-1.4	
		45	-1.4	
		60	-1.6	
		180	LSL	
8.5	0.75	15	-0.7	-2.2
		30	-1.9	
		45	-2.0	
		60	-2.5	
		180	LSL	
8.5	0.80	15	-1.0	-2.0
		30	-1.9	
		45	-2.2	
		60	-2.6	
		180	LSL	

APPENDIX A (cont.)

Table A-11 f2 inactivation rate (k') at pH 8.5, 20°C

pH	free seconds mg/1	time seconds	f2 inactivation log N/N_o	f2 inactivation rate min^{-1}
8.5	0.82	15	-1.3	-1.0
		30	-1.5	
		45	-2.0	
		60	-2.0	
		180	LSL	
8.5	0.83	15	-1.3	-1.8
		30	-1.6	
		45	-2.2	
		60	-2.6	
		180	LSL	
8.5	0.95	15	-1.3	-1.4
		30	-1.8	
		45	-2.2	
		60	-2.3	
		180	LSL	
8.5	0.97	15	-2.0	-2.2
		30	-2.8	
		45	-3.1	
		60	LSL	
8.5	1.00	15	-1.3	-1.4
		30	-1.9	
		45	-2.1	
		60	-2.4	
		180	LSL	

APPENDIX A (cont.)

Table A-12 f2 inactivation rate (k') at pH 8.5, 20°C

pH	free seconds mg/l	time seconds	f2 inactivation log N/N_o	f2 inactivation rate min^{-1}
8.5	1.00	15	-1.6	-1.2
		30	-1.8	
		45	-2.2	
		60	-2.4	
		180	LSL	
8.5	1.07	15	-1.3	-1.9
		30	-2.0	
		45	-2.6	
		60	-2.7	
		180	LSL	

APPENDIX A (cont.)

Table A-13 f2 inactivation rate (k') at pH 5.5, 20°C

pH	free seconds mg/l	time seconds	f2 inactivation log N/N_o	f2 inactivation rate min^{-1}
5.5	0.10	15	-3.4	-4.0
		30	-4.4	
		45	LSL	
5.5	0.10	15	-2.9	-6.4
		30	-4.5	
		45	LSL	
5.5	0.10	5	-1.2	-2.9
		10	-1.2	
		15	-1.6	
		20	-1.9	
		25	-2.1	
		30	-2.3	
5.5	0.11	5	-.8	-1.2
		10	-.9	
		15	-1.2	
		20	-1.3	
		25	-1.2	
		30	-1.3	
5.5	0.20	5	-3.0	-36.0
		10	LSL	
5.5	0.28	5	-4.4	-53.0
		10	LSL	
5.5	0.30	5	-4.5	-54.0
		10	LSL	

APPENDIX A (cont.)

Table A-14 f2 inactivation rate (k') at pH 5.5, 20°C

pH	free seconds mg/l	time seconds	f2 inactivation log N/N_o	f2 inactivation rate min^{-1}
5.5	0.40	5	-4.2	-33.0
		10	-5.5	
		15	LSL	
5.5	0.43	5	-4.2	-50.0
		10	LSL	
5.5	0.60	5	-4.0	-48.0
		10	LSL	
5.5	0.60	5	-3.8	-46.0
		10	LSL	

APPENDIX A (cont.)

Table A-15 f2 inactivation rate (k') at pH 5.5, 20°C

pH	free seconds mg/l	time seconds	f2 inactivation log N/N_o	f2 inactivation rate min^{-1}
5.5	0.69	5	-5.3	-38.0
		10	-6.3	
5.5	0.69	5	-6.1	-73.0
		10	LSL	
5.5	0.80	5	-5.8	-70.0
		10	LSL	
5.5	0.81	5	-4.9	-59.0
		10	LSL	

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