DEVELOPMENT OF ANALYTICAL METHODS FOR THE IDENTIFICATION OF ORGANIC NITROGEN CHLORINATION BY-PRODUCTS IN NORTH CAROLINA SURFACE WATERS

By

James A. Jersey and J. Donald Johnson

Department of Environmental Science and Engineering
School of Public Health
University of North Carolina at Chapel Hill
Chapel Hill, North Carolina 27514

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ABSTRACT

The goal of this research was to develop an analytical method for selectively and sensitively detecting N-chloramines in dilute aqueous systems. The organic N-chloramines comprise a class of chlorination by-products which is justifiably of concern since they interfere in measurements of free chlorine and monochloramine (NH2Cl) and because of their potentially deleterious environmental and human health effects.

The method developed exploited the mild conditions of high-performance liquid chromatography (HPLC) and reductive amperometry for selective and sensitive detection of these relatively unstable oxidant compounds. Because large overpotentials were required for their direct amperometric detection, post-column reaction with iodide to produce iodine was employed. Using this technique, organic N-chloramines were measurable at concentrations down to 10^-9 M by direct injections. In addition, detector response was highly reproducible and could be calibrated over concentration ranges greater than three orders of magnitude with minimum error. This allowed quantitative measurement of oxidants separated by HPLC.

By this technique, a number of organic N-chloramines were tentatively identified in a chlorinated primary wastewater. Analyses of chlorinated surface waters and wastewater final effluents demonstrated the clear dominance of inorganic N-chloramines among the combined residual chlorine pool in these much more dilute systems. A number of small and unidentified peaks were also detected in these systems. (Key words: chlorination; surface water; wastewater; N-chloramines; cyclic voltammetry; high-performance liquid chromatography; UV detection; electrochemical detection; post-column reaction; on-line enrichment)
# TABLE OF CONTENTS

ACKNOWLEDGEMENTS .......................................................................... i

ABSTRACT .......................................................................................... iii

SUMMARY AND CONCLUSIONS .............................................................. xvii

RECOMMENDATIONS ........................................................................... xxi

INTRODUCTION .................................................................................... 1

OVERVIEW OF HEALTH CONCERNS AND NEED FOR RESEARCH .......... 1
OVERVIEW OF REACTIONS OF AQUEOUS CHLORINE ............................. 2

LITERATURE REVIEW ........................................................................... 5

REACTIONS OF AQUEOUS CHLORINE ......................................................... 5

   Inorganic Oxidations and Reactions with Organic Carbon ...................... 5
   Reactions with Nitrogenous Compounds .................................................. 7
       Kinetics of Formation of N-chloramino Compounds .............................. 7
       Hydrolysis ...................................................................................... 8
       Chlorine Transfers ......................................................................... 9
       Disproportionation ......................................................................... 9
       The Breakpoint Phenomenon ............................................................. 10

OCCURRENCE AND SIGNIFICANCE OF N-CHLORO ORGANIC
COMPOUNDS ................................................................................... 11

   Occurrence of Nitrogenous Compounds in Water ..................................... 12
       Drinking Water ............................................................................ 12
       Surface Water ............................................................................ 13
       Wastewater .................................................................................. 13
HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY ........................................... 36
Pumps and Columns ................................................................. 36
Detection .................................................................................. 38
Injection .................................................................................... 40

COLLECTION AND CHLORINATION OF WASTEWATER AND
SURFACE WATER SAMPLES .......................................................... 42

RESULTS AND DISCUSSION ......................................................... 43

ANALYSIS OF N-CHLORAMINES BY HPLC WITH UV DETECTION ........ 43

Preliminary Chromatographic Assessment ........................................ 43
Examination of N-Chloramine Mixtures .......................................... 46
Application to Breakpoint Chlorination of a Tertiary Mixture .......... 51
Conclusions of N-Chloramine Analysis by HPLC-UV ....................... 52

ELECTROCHEMICAL BEHAVIOR OF CHLORINE, IODINE AND
INORGANIC AND ORGANIC N-CHLORAMINES IN
AQUEOUS SOLUTION .................................................................. 54

Electrode Materials and Background Currents .......................... 55
Aqueous Free Chlorine ............................................................. 56
Aqueous Iodine ........................................................................ 56
Organic and Inorganic N-Chloramines ........................................ 60
Conclusions of Electrochemical Reduction Studies .................... 65

ANALYSIS OF N-CHLORAMINES BY HPLC WITH POST-COLUMN
REACTION ELECTROCHEMICAL DETECTION .............................. 66

Optimization of Post-Column Reaction Detector Design ................ 67
Chromatography ........................................................................ 67
Background Currents .................................................................. 67
Applied Potential ....................................................................... 70
Kinetic Considerations ............................................................. 70
Detector Volume ........................................................................ 72
Standard Post-Column Reaction Detector Conditions ................ 73
Post-Column Reaction Detector Evaluation Employing
Model Compounds ...................................................................... 74
Precision and Sensitivity ........................................................... 74
Calibration .................................................................................. 75
Detector Durability ..................................................................... 79
Application to Analysis of an N-Chloramine Mixture
Subjected to Breakpoint Chlorination ........................................... 79
Conclusions of HPLC with Electrochemical Detection
Method Development ................................................................. 82

ANALYSIS OF N-CHLORAMINES IN CHLORINATED
WASTEWATER AND SURFACE WATER BY
ON-LINE ENRICHMENT HPLC/EC .................................................. 82

Analysis of N-Chloramines by On-Line Enrichment ......................... 82
Applications to Model Compound Solutions .................................... 86
Application to Chlorinated Primary Wastewater ............................. 88
Application to Chlorinated Wastewater Final Effluent ...................... 93
Application to Chlorinated Surface Waters .................................... 95
Conclusions of Applications Studies .............................................. 103

LIST OF REFERENCES ............................................................... 104

LIST OF PUBLICATIONS ............................................................ 121

GLOSSARY OF TERMS AND ABBREVIATIONS ................................. 122

APPENDIX A: GERMICIDAL PROPERTIES OF N-CHLORAMINES .............. 125
APPENDIX B: INTERFERENCES IN THE MEASUREMENT OF
FREE AVAILABLE CHLORINE ....................................................... 129
APPENDIX C: TOXICITY OF INORGANIC AND ORGANIC N-CHLORAMINES . 135
APPENDIX D: HYPOCHLOROUS ACID AND N-CHLORAMINES AS
MICROBIAL TOXINS IN ISOLATED NEUTROPHILS ......................... 137
APPENDIX E: EFFECT OF POST-COLUMN REAGENT COMPOSITION
UPON DETECTION OF N-CHLORAMINES ........................................ 139
APPENDIX F: ANALYSIS OF N-CHLOROGLYCINOLGLYCINE BY HPLC
WITH POST-COLUMN REACTION DETECTION .................................. 145
APPENDIX G: RETENTION TIME SUMMARY FOR MODEL N-CHLOR-AMINES ANALYZED BY POST-COLUMN REACTION ELECTROCHEMICAL DETECTION
LIST OF FIGURES

Figure 1. Reactions and by-products formed during disinfection by aqueous chlorine ........................................... 6
Figure 2. Breakpoint curve appearance for typical nitrogen containing water ......................................................... 11
Figure 3. Flow diagram of experimental objectives for N-chloramine analytical method development employing HPLC and amperometric detection ......................................................... 28
Figure 4. Flow diagram of experimental procedures for collection of N-chloramine electrochemical data by cyclic voltammetry ......................................................... 37
Figure 5. Schematic of instrumentation for post-column reaction/electrochemical detection of N-chloramines ......................................................... 39
Figure 6. Schematic depiction of tandem rotary injection valve on-line enrichment system used for analysis of N-chloramines by HPLC with post-column reaction detection ......................................................... 41
Figure 7. HPLC/UV chromatogram for injection of 10^{-6} M NH_{2}Cl. ......................................................... 44
Figure 8. HPLC/UV calibration curves for N-chloramines. ......................................................... 45
Figure 9. Linearity plot for calibration of N-chloramines by HPLC/UV. ......................................................... 47
Figure 10. Plot of absorption versus concentration for model N-chloramines at 270 nm ......................................................... 48
Figure 11. Experimental approach to data collection for N-chloramine mixture experiments ......................................................... 49
Figure 12. Concentrations of N-Chloramines in model solution subjected to breakpoint chlorination. ......................................................... 52
Figure 13. HPLC/UV chromatogram of tertiary solution at the lowest chlorine dose ([Cl]:[N] = 0.25). ......................................................... 53
Figure 14. Concentrations of total chlorine measured by HPLC and amperometric titration. ......................................................... 54
Figure 15. Background currents at a glassy carbon working electrode in a phosphate buffered solution at pH 2.0 ......................................................... 57
Figure 16. Cyclic voltammogram of aqueous chlorine at pH 2 and a carbon paste electrode ......................................................... 59
Figure 17. Cyclic voltammogram for N,N-dichloromethylamine at pH 2 and a glassy carbon electrode ......................................................... 64
Figure 18. Effects of post-column reactor components upon the chromatographic performance of a column test mixture analysis. ......................................................... 68
Figure 19. Background currents at thin-layer cell GC and Pt working electrodes as a function of E_{applied}. ......................................................... 69
Figure 20. Hydrodynamic voltammogram at a GC electrode of iodine produced by post-column reaction of NH_{2}Cl (17 \mu M inj.) and iodide. ......................................................... 71
Figure 21. Effect of post-column iodide concentration upon peak area measurements of N-chlorodimethylamine. ......................................................... 72
Figure 22. Effect of cell gasket thickness upon analysis of N-chlorodimethylamine standards. ........................................ 73
Figure 23. HPLC/EC chromatograms for detection of model N-chloramines at low levels ................................. 76
Figure 24. Calibration curves for analyses of model N-chloramines by HPLC/post-column reaction detection. ........................ 77
Figure 25. Linearity plots for analysis of N-chlorodimethylamine by post-column reaction detection employing simple linear (A) and nonlinear (B) calibration models. ........................ 78
Figure 26. Concentrations of individual N-chloramines (A) and total chlorine residuals (B) in a model solution chlorinated at points across the breakpoint curve. ........................................ 80
Figure 27. Analysis of an aliphatic N-chloramine mixture by on-line enrichment HPLC Post-column reaction detection. ................................. 83
Figure 28. Relative peak areas of N-chloro aliphatic amines as a function of injection volume in the on-line enrichment technique. ............... 85
Figure 29. Kinetic versus thermodynamic product distributions from chlorination of an aliphatic amine mixture. ................................. 87
Figure 30. HPLC/EC chromatogram of N-chlorarnino acid mixture at (A) 5, (B) 50, (C) 180 and (D) 360 min. following chlorination. ............... 88
Figure 31. Primary wastewater effluent breakpoint curve and HPLC/EC chromatograms obtained at chlorine dosages: (A) 0, (B) 33, (C) 65, (D) 125 and (E) 167 mg/l (pH 7, 1 hour contact time at ambient temperature in darkness). ................................. 90
Figure 32. Concentration profiles of total residual chlorine in chlorinated primary wastewater as determined by HPLC/EC and amperometric titration. ........................................ 91
Figure 33. Concentration profiles of NH₂Cl, NHCl₂, and total peak area in chlorinated primary wastewater as determined by HPLC/EC. ................................. 92
Figure 34. HPLC/EC chromatograms for primary wastewater chlorinated to the N-chloramine maximum (167 mg/l chlorine dosage). ................................. 94
Figure 35. Secondary wastewater effluent breakpoint curve and HPLC/EC chromatograms obtained at chlorine dosages: (A) 0, (B) 1.18, (C) 2.92 and (D) 4.10 mg/l (pH 7, 1 hour contact time at ambient temperature in darkness). ................................. 95
Figure 36. Concentration profiles of NH₂Cl, NHCl₂ and total peak area in chlorinated advanced secondary treated wastewater as determined by HPLC/EC. ........................................ 96
Figure 37. High Rock Lake surface water breakpoint curve and HPLC/EC chromatograms obtained at chlorine dosages: (A) 0, (B) 0.94, (C) 1.67 and (D) 2.21 mg/l (pH 7, 1 hour contact time at ambient temperature in darkness). ................................. 97
Figure 38. Concentration profiles of NH₂Cl, NHCl₂ and total peak area in chlorinated advanced secondary treated wastewater as determined by HPLC/EC. ........................................ 98

Figure 39. HPLC/EC chromatograms for High Rock Lake samples spiked by an amine mixture and chlorinated (pH 7, 1 hour contact time) at (A) 0.94 and (B) 1.67 mg/l. ........................................ 99

Figure 40. HPLC/EC chromatograms for High Rock Lake samples spiked by an amine mixture and chlorinated (pH 7, 1 hour contact time) at 0.68 mg/l. ........................................ 100

Figure 41. Breakpoint curve for Jordan Lake surface water sampled June 29, 1990. ........................................ 101

APPENDIX E:

Figure 1. Effect of post-column reagent composition upon detection of model N-chloramine compounds. ........................................ 140

APPENDIX F:

Figure 1. Concentration of N-chloroglycylglycine and total chlorine residual in solutions of glycylglycine chlorinated at various dosages. .......... 146

APPENDIX G:

Figure 1. Gradient HPLC post-column reaction detection chromatogram of N-chloramino acids and N-chloro aliphatic amines. .......... 150
## LIST OF TABLES

Table 1. Standard reduction potentials for chlorine, iodine and inorganic N-chloramines ........................................ 22
Table 2. Standard reduction potentials of organic N-chloramines ............. 23
Table 3. Summary of buffer compositions for cyclic voltammetry experiments ........................................ 36
Table 4. Species concentrations in (A) monochloramine and N-chloromethylamine (MACl) and (B) monochloramine and N-chlorodimethylamine (DMACl) mixtures as measured by UV spectroscopy and HPLC/UV ........................................ 50
Table 5. $E_{p,e}$ values for reduction of aqueous chlorine .......................... 58
Table 6. $E_{p,e}$ values for reduction of aqueous iodine ............................ 58
Table 7. $E_{p,e}$ values for monochloramine and organic N-chloramines in aqueous solution ........................................ 61
Table 8. $E_{p,e}$ values for secondary N-chloramines in aqueous solution ...... 62
Table 9. $E_{p,e}$ values for N,N-dichloramines in aqueous solution ............. 63
Table 10. Standard operating conditions for analysis of N-chloramines by post-column reaction detection ......... 74
Table 11. Percent relative standard deviation of absolute peak area measurements for several model N-chloramines ......... 75
Table 12. Percent relative standard deviations of absolute peak area and height measurements for analysis of several model N-chloramines by the on-line enrichment technique ........................................ 84
Table 13. Comparison of NH$_2$Cl concentrations measured by HPLC/EC and the DPD-FAS procedures in chlorinated Jordan Lake water .......... 102

APPENDIX A:
Table 1. Relative disinfection strengths of free and combined chlorine ...... 126

APPENDIX B:
Table 1. Interferences by monochloramine in the DPD procedure for free chlorine ........................................ 130
Table 2. Summary of interferences observed in measurement of free available chlorine by organic N-chloramines ............... 133
APPENDIX G:

Table 1.  Summary of N-chloro amino acid and aliphatic amine retention times under conditions favoring maximum chromatographic resolution

Table 2.  Summary of N-chloro amino acid and aliphatic amine retention times under conditions favoring analysis time
SUMMARY AND CONCLUSIONS

The organic N-chloramines comprise a class of chlorination by-products which warrant concern since they positively interfere in measurements of biocidally important free chlorine and monochloramine (NH₂Cl) and further because of their potentially detrimental environmental and human health effects. Studies concerning the chemistry, toxicology and environmental fate of these compounds have to date been hampered by the lack of suitable methods for their analysis.

Standard Methods for chlorine residual analyses have historically emphasized specificity and sensitivity for detection of free chlorine and to a lesser extent NH₂Cl. The organic N-chloramines are measured as their contribution to the total residual chlorine pool by these techniques with no discrimination among contributing species provided. Recent attempts to analyze organic N-chloramines by indirect derivatization techniques have suffered from low product yields and vulnerabilities to matrix effects, thereby severely restricting the usefulness of these techniques.

The research summarized in this report demonstrates the applicability and potential utility of a method developed for direct analysis of very low concentrations of relatively unstable N-chloramino compounds. The method is based on the underlying principles of HPLC and electrochemical detection. The mild conditions inherent to HPLC avoid potentially species altering pH changes and sample handling operations, thus providing a direct means for sample analyses. Initial experiments focused upon model compound work with UV detection at 254 nm and illustrated the capability of an HPLC based method to provide accurate and precise measurements of specific N-chloramino compounds free of interferences. For model compounds examined in these initial studies, limits of detection were on the order of 10⁻⁶ M. Linearity of detector response observed at dilute N-chloramine concentrations supported the premise that chlorine demand originating and exerted within the separation media was negligible at these concentrations.

Data from experiments examining binary mixtures provided evidence that distributions and concentrations of N-chloramines remain unaffected by the chromatographic process prior to their detection. Application of the HPLC/UV method to analysis of model compound solutions chlorinated at points along the breakpoint curve demonstrated the method’s ability to resolve concentration profiles for individual components from the composite breakpoint curve. This information is unattainable through use of current analytical methods for combined residual chlorine.

While weak absorbance in the UV region allows for selective detection of some N-chloramines, detection in this manner is neither selective nor sensitive enough for their determination at trace levels in complex matrices such as chlorinated wastewaters and natural waters. The fact that the N-chloramines are strong oxidants, however, provides a
property or tag by which they can be detected with high selectivity and sensitivity, namely through use of electrochemical methods.

During initial investigations of N-chloramine detection by this approach we examined the electrochemical behavior of aqueous free chlorine and model N-chloramines by cyclic voltammetry. A variety of working electrode and solution conditions were evaluated. In general, our findings were in agreement with those reported by Scully et al. (1984a), though for most compounds examined we observed reductions at slightly more negative potentials. For all compounds, working electrodes, and solution conditions examined, reductions were highly irreversible, required large negative overpotentials and proceeded more favorably under acidic conditions. Overall, the performance of glassy carbon was superior to platinum and carbon paste due to proximity of reduction waves at the latter near regions of high background current.

Reductions of aqueous N-chloro primary alkylamines at pH 2 were observed with $E_{p_c}$ values of approximately 0.400 and -0.600 to -0.700 V vs Ag/AgCl for platinum (Pt) and glassy carbon (GC) working electrodes, respectively. For each working electrode material, reductions of N-chloro secondary alkylamines were observed at slightly more positive potentials under similar conditions. Inorganic NH$_2$Cl was found to undergo reductions at potentials similar to the N-chloro primary alkylamines.

Based upon the results described above, development of an analytical method employing direct detection of N-chloramines by reductive amperometry is problematic since these compounds do not undergo rapid heterogeneous electron transfer at, or near their thermodynamic potential values. This situation consequently necessitates application of large overpotentials for their direct detection which in turn compromises detection selectivity. Similarly, sensitivity is degraded by the resulting high background currents caused by reductions of dissolved oxygen at these negative applied potentials. Noise associated with reduction of dissolved oxygen can be reduced but this requires experimentally cumbersome deoxygenation procedures.

Due to the problems described above, we investigated conditions for a general N-chloramine detection scheme based upon post-column reaction with iodide. The H$^+$ catalyzed oxidation of I$^-$ to I$_2$ by inorganic and organic N-chloramines is used in nearly all standard methods for combined chlorine residual analysis (APHA et al., 1985). The iodide/iodine couple was found to readily undergo redox reactions at both GC and Pt electrodes at pH 2 and 7. Reductions at pH 7 and GC and Pt electrodes were observed with $E_{p_c}$ at 0.05 and 0.150 V vs Ag/AgCl, respectively. Unlike aqueous chlorine and the N-chloramines, reduction of iodine exhibited a degree of electrochemical reversibility and did not require applications of large negative overpotentials.

The faster kinetics of homogeneous electron transfer reactions between N-chloramines and iodide, relative to that of the heterogeneous electron transfer between N-chloramines and the electrode surface, provide an important advantage for post-column
reaction detection by lowering the potential required for detection. The most fruitful approach to pursue concerning N-chloramine method development was, therefore, employment of post-column reaction between HPLC eluate and iodide followed by detection of the iodine product by reductive amperometry. Detection in this manner offered potential sensitivity enhancements (lower noise levels), maintained detection selectivity (oxidant analysis) and eliminated the need for sample and mobile phase deoxygenation.

Work next undertaken concerned selection and optimization of the post-column reactor design. Because we wished to apply the method to the analyses of complex environmental samples, it was essential that the post-column reactor not degrade the chromatographic resolution provided by the analytical column. Following an examination of the literature, a knitted open tubular reactor (KOT) was selected as the best design for this application. Preliminary work with a reactor of this design proved it to be experimentally simple and easy to work with. It provided the needed delay time and enhanced mixing while minimizing degradation of the resolution provided by the analytical column.

Solutions of model N-chloramines were analyzed directly by HPLC with post-column reaction detection at concentrations as low as $10^{-8}$ M without sample concentrations or pretreatment procedures. The post-column reaction detector possessed an absolute detection limit on the order of 100 picograms of injected material. Combined with an on-line enrichment technique used in later experiments, detection limits for specific compounds were further lowered to $10^{-9}$ M. In addition to its excellent sensitivity characteristics, the kinetics of the post-column reactor were sufficiently fast to allow detector calibrations over concentration ranges in excess of 3 orders of magnitude with minimum error for the model N-chloro alkylamines examined. Furthermore, since the N-chloramines are detected as their iodine product, detector response for individual compounds is similar and solely a function of their active chlorine content. Reproducibility of detector response over time was excellent with electrode polishings required very infrequently.

Application of the optimized method to the analysis of chlorinated primary wastewaters revealed an abundance of products. The high levels of monochloramine and dichloramine formed in these samples, however, hampered the chromatographic resolution of closely eluting compounds. Analyses performed on a chlorinated wastewater receiving advanced secondary treatment demonstrated the effectiveness of treatment processes in removing organic N-chloramine precursor materials. Analyses of chlorinated surface waters from Jordan Reservoir and High Rock Lake, collected during late spring and early summer of 1990, showed the clear dominance of the inorganic N-chloramines among the combined residual chlorine pool in these much more organically dilute systems. Though a few minor peaks were observed, results from these analyses demonstrated that part per trillion sensitivity will be required to detect organic N-chloramines in these very dilute systems.
Though we were disappointed by results obtained from limited applications to the analysis of chlorinated surface waters, the method developed for analysis of N-chloramines in this work provides a very significant contribution to tools available for investigating the formation, environmental fate and health consequences of these compounds. This work reports the first method capable of directly detecting these relatively unstable compounds with high sensitivity and selectivity. The method is capable of providing analyses of individual N-chloramines at levels tenfold lower than existing Standard Methods (APHA et al., 1985) for total combined chlorine that provide no selectivity. This level of sensitivity and selectivity is attainable from a relatively simple analytical system and directly from unaltered samples.

In terms of the rather disappointing results obtained from analyses of chlorinated surface water, we strongly believe that applications to a broader range of surface waters with a greater temporal sampling frequency would yield more significant and positive results. In addition, there are many improvements which can be made to the method in its current state. Similarly, information from additional applications could shed insight into aspects of the chemistry and health effects associated with N-chloramines which heretofore have been inaccessible by means of current analytical techniques. Some of these areas are discussed in the section which follows.
RECOMMENDATIONS

This research has contributed to the development of a new analytical tool for investigating the formation, environmental fate and potential health consequences of organic N-chloramines. Opportunities for future research are many-fold and can be broadly categorized as falling into three areas: improvements in method sensitivity; increases in chromatographic resolution; and specific application work. Each of these areas will be discussed in sequence below.

SENSITIVITY

Through a combination of factors we believe it is possible to readily improve the absolute detection limits (quantity of material injected) of the technique reported here by greater than an order of magnitude. Specific areas to examine, in order of preference, which may prove beneficial are listed below:

- Improved solvent and post-column reagent delivery (e.g. pulseless flow via syringe pumps);
- Increased purity of reagents and HPLC solvents;
- Less dilution by post-column reagent addition;
- Improved passivation of the HPLC system;
- Increased temperature of post-column reaction - more favorable kinetics and a higher diffusion rate to the working electrode surface.

Sensitivity may also be enhanced by monitoring the iodine post-column reaction product by spectrophotometric methods. Buchberger (1988) reported a method for iodide determination in serum, food and water which utilized post-column reaction between iodide, chloramine-T and 4,4'-bis(dimethylamino)diphenylmethane. At a detection wavelength of 600 nm, the limit of detection for iodide was ca 20 pg. In a similar fashion, post-column reacting N-chloramines, iodide and DPD with detection at 515 nm may prove an effective means of detection. When analyzing very dilute and complex environmental samples by this approach, however, selectivity and compatibility with gradient elution may be issues warranting concern.
Sensitivity for analysis of N-chloramino acids can be improved significantly by performing on-line sample enrichments with anion exchange resins. This approach would provide the additional benefit of removing neutral N-chloramines such as monochloramine and dichloramine. These neutral compounds can obscure the presence of trace level N-chloro amino acids and other N-chloramines simply due to the magnitude of their levels within a chromatographic run, as was observed during our analyses of chlorinated wastewaters and surface waters.

APPLICATIONS

There are many areas in which application of the method reported here could provide interesting and useful information. Some of these are listed below:

- Application to a wider variety of wastewaters and surface waters, including an examination of the following parameters or variables:
  - Greater diversity of surface waters both spatially and temporally;
  - Determining product distributions as a function of time following chlorination;
  - Determining the significance of proteinaceous and other macromolecular organic nitrogen in water chlorination and N-chloramine chemistry. This could potentially be examined by application of size exclusion HPLC/EC;
  - Development of an on-line enrichment (anion exchange resins) procedure for analysis of very dilute acidic N-chloramines such as the N-chloramino acids;
  - More complete examination of the susceptibility of current methods of NH$_2$Cl analysis to interferences by organic N-chloramines;
  - Application to the direct analysis of stomach fluids and saliva following ingestion of chlorinated drinking water using microdialysis techniques;
  - Application to stimulated systems of isolated neutrophils, a major component of the human immune system. Evidence exists that supports the role of N-chloramines in the functioning of this system.
We feel strongly that significant new information could be derived from applications of the method described in this report to a greater diversity of chlorinated surface waters and wastewaters. Due to time constraints, the sampling during this work was necessarily limited in terms of spatial and temporal distributions. Sampling at a higher frequency and more locations would greatly increase the likelihood of obtaining samples with greater organic nitrogen content, and thus N-chloramine product formation. Applying the method to samples examined at more time intervals following chlorination would shed insight into product distributions as a function of time, i.e. stable versus non-stable products. Longer time interval sampling would have the additional benefit of reducing the relative dominance of inorganic N-chloramines, due to their instability, at points past the N-chloramine maximum.

Development of an HPLC/EC method employing size exclusion chromatography would generate useful information regarding the extent to which proteinaceous and other macromolecular organic nitrogen participates in the water chlorination process. This may be of particular significance because these forms measure largely as NH₂Cl by current Standard Methods. Development of a sample enrichment scheme based upon on-line enrichment with anion exchange resins would significantly increase sensitivity for N-chloramino acids and other acidic N-chloramines.

Perhaps one of the most exciting applications of the analytical technique described here would be that of analyzing stomach fluids following ingestion of chlorinated drinking water. This system is well suited for study since concentrations of free amino compounds are relatively high (10⁻⁶ M). Current techniques of microdialysis could be coupled to the HPLC/EC technique to provide real-time in vivo analyses of N-chloramines formed upon ingestion of chlorinated water in living test systems. In this manner potential experimental artifacts could be kept to an absolute minimum. In addition, the dialysis membrane itself serves a cleanup function since larger molecules (e.g. proteinaceous materials) can not cross it. Consequently, formation of only small molecular weight N-chloramines could be followed. Such experiments could greatly aid investigations of the toxicological significance of the N-chloramines.

RESOLUTION

Improving upon the chromatographic resolution observed in this work would notably improve the strength of this technique. Though the system was carefully designed to minimize loss of resolution provided by the analytical column during post-column reaction, an inherent drawback of liquid chromatographic based methods is their limited number of theoretical plates. Use of microbore systems or more efficient analytical columns may provide some increase in resolution, but unfortunately the extent to which this may occur is rather limited. Perhaps the most fruitful avenue to pursue along these
lines would be the development of a capillary electrophoretic method since this technique is capable of providing much higher numbers of theoretical plates. With respect to separations of acidic N-chloramines, such as the N-chloramino acids, use of ion-pair chromatography may also result in significant resolution improvements.
INTRODUCTION

OVERVIEW OF HEALTH CONCERNS AND NEED FOR RESEARCH

Chlorine is a highly versatile substance having many uses, with some applications resulting in potentially toxic discharges. It was chlorine's power as an oxidant that led to its widespread adoption as a disinfecting agent for potable and treated wastewaters and as an agent for biofouling control within the power industry. By recent estimates, chlorine use in treatment of municipal water supplies and wastewater amounts to 2-7 percent of annual production or about 1 million tons per year. Similarly, use for cooling water biofouling control accounts for approximately 0.1 percent of annual production (Pierce 1978 and White 1986). Chlorine is also widely used in many industrial process waters, including the food industry. It can be seen, therefore, that chlorine production is a major industry and further that a significant fraction of that which is manufactured may ultimately be discharged to the aquatic environment in a chemical form where its oxidant characteristic is retained. These discharges in turn give rise to potential environmental and public health concerns.

Inorganic and organic N-chloramines warrant concern as potential risks to the environment and public health for several reasons. First, while they are acknowledged to form, little is known about the specifics of their formation upon chlorination of drinking water and wastewaters. Consideration of combined oxidant residuals in these systems is tantamount to a discussion of N-chloramines due to the rapidity and completeness of amino compound reactions with aqueous chlorine.

Organic N-chloramines are additionally of concern due to the potential hazards they present to human health as a result of ingestion of chlorinated drinking water. In a recent report on drinking water and disinfection by-products, the National Research Council (1987) concluded that the N-chloramines could present deleterious health effects but that progress in evaluating these considerations was hampered by the lack of suitable analytical methods for their analysis. To the extent these compounds are formed, there is a real need to consider their formation in the design of practical water treatment processes. If they are proven detrimental to human health, effective treatment may consist of designing the disinfection process to minimize their formation or to remove their precursors.

A third and final reason to develop an analytical method for N-chloramines is their potential to interfere in the measurement of free chlorine. Organic N-chloramines are much weaker disinfectants than free chlorine (Feng 1966). The U.S. EPA currently allows substitution of free chlorine residuals exceeding 0.2 mg/l for microbiological assessments of the hygienic status of treated drinking waters (USEPA 1975). The potential of these compounds to interfere in free chlorine measurements could thus
hinder correct assessment of whether adequate disinfection has occurred under this regulatory procedure. There is still a poor understanding of specific compounds causing free chlorine interferences and their significance under actual treatment conditions.

With stricter disinfection by-product regulations soon to be implemented, there is a growing trend within the drinking water treatment community towards the use of alternative disinfectants. Use of monochloramine as the primary disinfectant figures prominently in this scenario since it is quite stable and widely perceived to produce fewer and lower levels of chlorination by-products, particularly trihalomethanes (USEPA 1983; Fleischacker and Randtke 1983; Jensen 1983). Thus, methods will need to be developed to selectively and sensitively measure monochloramine in order to properly assess the microbiological status of water disinfected by it. By current methods, significant interferences in the measurement of monochloramine by organic N-chloramines are highly probable. This is an area in which there is a clear need for research since so little is known regarding the occurrence and extent of this problem.

OVERVIEW OF REACTIONS OF AQUEOUS CHLORINE

When added to water, chlorine gas rapidly hydrolyzes to form molecular chlorine, hypochlorous acid and hypochlorite. Together these forms constitute free available chlorine (FAC), the distribution among species being a function of pH and temperature (Palin 1975 and Morris 1978). For conditions typical of those encountered environmentally, hypochlorous acid and hypochlorite are the most important forms (Morris 1978). In the presence of nitrogenous compounds possessing one or more exchangeable protons, chlorine reacts quickly to produce inorganic and organic N-chloramines (Moms 1967 and Margerum et al., 1978). These nitrogenous chlorine substitution products are commonly referred to as combined residual chlorine (CRC) and unlike FAC are considerably weaker oxidants and disinfectants (Feng 1966; Palin 1975; and Wolfe et al., 1984).

An additional reaction pathway available to aqueous chlorine involves reactions with inorganic reducing agents. These reactions proceed very quickly and terminate in production of chloride and oxidized substrates. Through reactions with organic carbon, aqueous chlorine can produce organic halides by oxidation, substitution or addition reactions. The relative importance of these reaction mechanisms is dependent upon the nature of the organic carbon and reaction conditions. Research on the reactions of aqueous chlorine with organic carbon has been extensive, and literally hundreds of disinfection by-products have been identified (National Research Council (NRC) 1987). Knowledge in this area has progressed to the point where regulations have been promulgated to control the level of some chlorination by-products in finished drinking water.
While research concerning the reactions of chlorine and organic carbon has been ongoing and extensive, comparatively little is known of the chemistry and by-products formed by reaction of aqueous chlorine with organic nitrogenous substrates. To a large extent the gaps in our knowledge regarding the chemistry of these compounds results from the unavailability of sensitive and specific analytical methods for their analysis. Consequently, it is difficult to assess their environmental fate and potential toxicity. At present, standard analytical methods attempt to discriminate only FAC and inorganic N-chloramines from compounds comprising the total residual chlorine pool (APHA et al., 1985). Organic N-chloramines are measured as a part of each of these fractions and as their contribution to the total residual chlorine pool (Helz 1981; Jolley and Carpenter 1983b; and Jensen and Johnson 1989b).

To further our knowledge of the chemistry, environmental fate and potential deleterious human and environmental health effects of organic N-chloramines, analytical methods capable of providing sensitive and interference free analyses of specific N-chloramino compounds must be developed. The goal of the research presented here was to develop such a method and to apply it to the analysis of chlorinated surface waters and wastewaters.
LITERATURE REVIEW

REACTIONS OF AQUEOUS CHLORINE

Inorganic Oxidations and Reactions with Organic Carbon

When added to water, chlorine can react in several manners as depicted in Figure 1. At environmentally significant pH values (near neutral), free chlorine exists almost entirely as HOCl and OCl⁻, the distribution being dependent upon pH and temperature (pKₐ = 7.54 at 25°C) (Morris 1967 and 1978). Chlorine in this form displays the characteristics of a strong oxidant, the standard reduction potentials being 1.49 and 0.90 V for HOCl and OCl⁻, respectively (White 1986). Consequently, an important reaction of aqueous chlorine in some systems may involve oxidation of inorganic ions which may be present. Ions whose oxidations are most often significant in terms of chlorine consumption include Mn²⁺, Fe⁺², NO₃⁻, S²⁻, and in saline waters Br⁻. In general, the kinetics of these oxidation reactions are very rapid and a function of pH (Helz 1981 and Jolley and Carpenter, 1983a). For some waters, the chlorine demand arising from these and similar reactions may be significant. The capacity of free chlorine to oxidize inorganic ions is oftentimes used to advantage in water treatment processes. Examples include removal of unwanted substances such as S²⁻ and CN⁻ and in-situ generation of desirable species such as the coagulating agent Fe⁺³ from Fe⁺².

Research conducted in the early nineteen seventies began to shed insight onto reactions involving aqueous chlorine other than disinfection, specifically reactions with organic carbon, that were of sufficient magnitude to justify concerns regarding its use in water treatment processes (Rook 1976). Typically, surface waters receiving chlorinated discharges contain several mg/l of organic carbon. Similarly, surface and subsurface waters used for municipal water supplies may contain concentrations of organic carbon at comparable or higher levels. Since chlorination is by far the predominant method practiced for disinfection of drinking water and wastewaters in the United States, concerns involving these unwanted side reactions fostered a large and continuing research effort focused upon the chemistry of disinfection and disinfection by-products.

A large portion of the early research in this area focused upon the trihalomethanes (THMs) which were found to be produced in essentially all water supplies disinfected by chlorine. Ensuing studies of their toxicology resulted in promulgation of drinking water regulations for their control. Under present law, the level of THMs in finished drinking water are not to exceed 100 µg/l (National Research Council, 1987).

Since this early work, additional studies of disinfection by-products have resulted in identification of literally hundreds of compounds. Some by-products are chlorinated while
Figure 1. Reactions and by-products formed during disinfection by aqueous chlorine
others are not (Christman et al., 1980, 1983; Johnson et al., 1982; Norwood et al., 1983; de Leer et al., 1985). To date, only a small fraction of the mutagenic activity associated with water disinfection by chlorination has been identified. More recent research has identified the exceptionally mutagenic compound 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) in chlorinated pulp effluents and drinking water. Even at the extremely low levels (parts per trillion) detected, this compound can account for a significant percentage of the observed mutagenicity (Holbom et al., 1984; and Kronberg et al., 1988). The source of much of the mutagenic activity produced by disinfection with chlorine, however, remains to be identified.

Knowledge we have acquired thus far has led to the promulgation of regulations for control of some chlorine disinfection by-products, probable future regulations for many others and much debate regarding the potential hazard they pose to human and environmental health (NRC 1980, 1987). Research continues on identification of additional chlorination by-products and means to control those compounds believed to pose significant threats to human and/or environmental health.

Reactions with Nitrogenous Compounds

In terms of disinfection efficacy and environmental fate, the most important reaction involving aqueous chlorine is its reaction with nitrogenous substrates. Nitrogenous organic compounds occur ubiquitously in surface waters, originating from both natural and man-made sources (Hunter 1971; Ram and Morris 1980; Le Cloirec et al., 1983 a,b,c; Jolley et al., 1983; and Thurman 1985). Any nitrogenous compounds possessing one or more exchangeable protons will react rapidly and completely with aqueous chlorine through substitution reactions to form N-chloramino products. Thus, under many conditions (Cl:N molar ratios of less than 1, neutral or higher pH) the environmental consequences of aqueous chlorinations are determined by the distribution, fate and transport of N-chloramino products. Moreover, these N-chloramino compounds are important with respect to disinfection efficacy since the organic N-chloramines are much weaker disinfectants than free chlorine and inorganic monochloramine (Feng 1966). The following text reviews the chemistry of reactions between aqueous chlorine and nitrogenous substrates.

Kinetics of Formation of N-chloramino Compounds. Inorganic and organic N-chloramino compounds are known to form quickly under typical water treatment conditions. (Weil and Morris 1949a; Morris 1967; and Margerum et al., 1978). Evidence suggests that the rate determining step involves nucleophilic attack by the amino-N on the chlorine atom of HOCl. Rates of chlorination of amines by HOCl have been found, in fact, to be highly correlated with amine basicity, thus lending support to the above mechanism (Morris 1967 and Margerum et al., 1978). The higher the precursor amine
basicity, the more rapid the chlorination reaction. Available data suggests that the amide nitrogen of the peptide bond common to proteins, which possibly comprises the majority of dissolved organic nitrogen, is unreactive towards chlorine under conditions typically encountered in water treatment processes (Morris 1967 and Ayotte et al., 1985).

The rapidity of reaction of aqueous chlorine with free amino nitrogen can perhaps be best appreciated by citing an example. Isaac and Morris (1983b) estimated that chlorination of non-nitrified wastewater results in instantaneous formation of N-chloramines and further that reaction is complete within 0.5 seconds of chlorine addition. Thorough reviews of the kinetics of chlorination of nitrogenous substrates are available elsewhere (Morris 1967; Margerum et al., 1978; Helz 1981; and Jensen 1988).

When chlorinations are performed at chlorine-to-nitrogen molar ratios of greater than 1, N,N-dichloramines can be formed by reactions of aqueous chlorine with nitrogenous compounds possessing two exchangeable protons. Rates of their formation by direct chlorination of the monochloro precursors are, however, considerably slower than the initial chlorination. Margerum et al. (1978) found that substitution of chlorine for a proton in ammonia resulted in an over four orders of magnitude decrease in the rate of chlorination by HOCl at 25°C. The greatly reduced rate of formation can be attributed to the much lower nucleophilicity of the chlorine substituted nitrogen (Margerum et al., 1978 and Morris 1967). Weil and Morris (1949b) estimated that substitution of a proton by chlorine decreased the basicities of methylamine and dimethylamine by factors of $2 \times 10^6$ and $1.3 \times 10^6$, respectively.

**Hydrolysis.** Under thermodynamically controlled conditions, N-chloramines exist in equilibrium with free chlorine. A general chemical equation depicting the hydrolysis reaction is provided below:

$$\text{RR'NCl} + \text{HOH} \rightleftharpoons \text{RR'NH} + \text{HOCI}; K_h$$

For the strongly basic amines, the equilibrium lies strongly in favor of the N-chloramine. Typical values of the equilibrium constant ($K_h$) for monochloramine and N-chloroalkyl amines are on the order of $10^{-12}$ to $10^{-15}$. For the less basic nitrogen compounds, however, $K_h$ values can be large and thus equilibrium concentrations of free chlorine can be significant. Values of the hydrolysis equilibrium constant of N-chloroamides can be as large as $10^4$ (see Jensen, 1988 for a review of hydrolysis constants). The favorable free chlorine equilibrium chemistry of the N-chloro amides is used to advantage in the manufacture of chemicals for disinfection of swimming pool waters.
**Chlorine Transfers.** Due to the fast kinetics of reaction, initial N-chloramine concentrations in a chlorinated water are kinetically controlled (Jolley and Carpenter 1983a). Initial distributions will be dependent upon concentrations, pH and the relative rates of chlorination as well as other variables. Assuming 20 mg/l NH$_3$-N, 2 mg/l Org.-N and relative specific rates of reaction of 8.5 (Org.-N to NH$_3$), Isaac and Morris (1983b) estimated the N-chloramine distribution to be 54 percent monochloramine and 46 percent organic N-chloramine within 0.3 s of chlorine addition at pH 7 and 25°C. With the passage of time, however, product distributions initially determined by kinetic factors will progress towards distributions best described by equilibrium chemistry. One reaction by which such redistributions can occur, and thus possibly of environmental consequence, involves the transfer of chlorine among nitrogenous compounds.

Two mechanisms for transfer of chlorine among nitrogenous compounds exist. An indirect mechanism is possible wherein the N-chloramine undergoes hydrolysis to form free chlorine which in turn reacts with a second reactive amino compound. By this mechanism, the rate of chlorine transfer would be anticipated to be equal to or less than the rate of N-chloramine hydrolysis. The second mechanism involves transfer of chlorine directly by interaction of the N-chloramine and an amino compound, the rate being proportional to the basicity of the receptor amine (Hussain et al., 1972; Higuchi and Hasegawa 1965; Isaac and Morris 1983a and 1985; and Snyder and Margerum 1982).

Examples of situations where transfer of chlorine among nitrogenous substrates may be important include the discharge of chlorinated wastewaters and maintenance of monochloramine residuals in drinking waters for disinfection purposes. In each case the transfer of chlorine may affect the potential toxicity of the chlorinated water by causing the composition of the N-chloramine pool to change with time. In the drinking water example, disinfection efficacy may also be negatively affected by transfers of chlorine to much less germicidal organic N-chloramines. At the relatively high nitrogen concentrations encountered in wastewater (10$^{-4}$ M), available data suggests that the direct mechanism of chlorine transfer is clearly more important. At the lower concentrations of amino-N (10$^{-5}$ M or less) found in lake water and drinking waters, both mechanisms of chlorine transfer are probably important (Isaac and Morris 1983a).

**Disproportionation.** N-chloramine distributions are strongly a function of pH, reflecting the desire of these systems to approach equilibrium controlled conditions. For the simple inorganic N-chloramines, the dominant species are nitrogen trichloride, dichloramine and monochloramine for the pH ranges <3, 3-5 and >8, respectively (Drago, 1957; Gray et al., 1978; and Sisler 1983). At neutral pH values it is not possible to have solutions containing only monochloramine or dichloramine. Organic primary N-chloramine distributions are similarly a strong function of pH (Gray et al., 1978). Under some conditions, pH induced changes in distributions of inorganic and organic N-chloramines can be complete in seconds. The lability of N-chloramine distributions as a function of
pH has strong implications regarding the results generated by procedures requiring pH adjustments prior to performing analytical measurements. Interconversions of N-chloramines to N,N-dichloramino compounds can occur by means of disproportionation reactions as shown in the equation below:

\[
2 \text{RNHCl} + \text{H}^+ \rightarrow \text{RNCl}_2 + \text{RNH}_3^+ ; K_{\text{dis}}
\]

Equilibrium constants lie strongly in favor of the N,N-dichloro compounds. For a primary N-chloramine prepared in basic media, a pH jump to more acidic values results in rapid conversion of a portion of the N-chloro compound to the N,N-dichloro derivative and its unchlorinated precursor. The degree of conversion is dependent upon the change in pH and the N-chloramine and reaction rates are strongly pH dependent. Gray et al. (1978) reported rate constants for disproportionation of monochloramine and N-chloromethylamine of 60 and 980 M\(^{-1}\) s\(^{-1}\), respectively. These reactions proceed with a net retention of the residual oxidant capacity.

**The Breakpoint Phenomenon.** For natural waters and wastewaters, the breakpoint phenomenon has been the subject of extensive study (White 1986; Palin 1975; and Saunier and Selleck 1979). The general shape of the breakpoint curve is attributable to the chlorination of ammonia and other nitrogenous compounds (see Figure 2). Initial increases in measured chlorine residual with chlorine dose result from formation of N-chloro compounds which retain the oxidative capacity of Cl\(^+\). Subsequent decreases in measured residuals at higher dosages result from oxidations or degradations of these compounds by chlorine additions past a chlorine to free reactive amino-N molar ratio of one.

Inorganic dichloramine, for example, has been shown to be unstable in the presence of free chlorine (Hand and Margerum 1983). Similarly, N-chloro alpha amino acids have been shown to be quite unstable with half lives on the order of hours or less under typical environmental conditions (Hand et al., 1983; Stanbro and Smith 1979; Isaac and Morris 1983b and LeCloirec and Martin 1985). Further additions of chlorine past that required to satisfy the water's chlorine demand result in the appearance of free chlorine residuals.

The position of the N-chloramine maximum is among other things a function of the free reactive amino-N content of the sample (Le Cloirec et al. 1985 and 1988 and Jensen 1988). The non-zero breakpoint minimum occurs somewhere near a Cl:reactive-N molar ratio of 1.5 (Helz 1981 and Palin 1975), the exact location being dependent upon the
particular water and conditions examined. In general, as the predominance of NH3-N within the reactive N pool decreases, the position of the breakpoint shifts towards higher molar Cl:reactive-N ratios.

OCCURRENCE AND SIGNIFICANCE OF N-CHLORO ORGANIC COMPOUNDS

The preceding section has reviewed possible reactions of aqueous chlorine with reactive nitrogenous substrates. These nucleophilic compounds occur ubiquitously and arise from natural and anthropogenic sources. Concentrations for individual species can vary widely among and within sources. In the section immediately following, a brief review of the occurrence and levels of nitrogenous compounds found in natural waters and wastewaters is presented. Next, a review of N-chloramine compounds identified in model systems and chlorinated wastewaters is presented. And finally, the role played by organic and inorganic nitrogen as it affects the practice of water chlorination is reviewed. The germicidal properties and potential toxicity of organic and inorganic N-chloramines are reviewed in Appendices A and B, respectively. The suggested involvement of N-chloroamino compounds in the human immunological response is reviewed in Appendix C.
Occurrence of Nitrogenous Compounds in Water

This review is not intended to cover completely the full spectrum of occurrence and distribution of organic nitrogenous compounds in water. Rather, it is intended to briefly summarize general trends which have been found for various waters and to discuss selected references. Several excellent, more detailed reviews of this topic are available elsewhere for a variety of waters (Hunter 1971; Johnson 1979; Morris et al., 1980; Ram and Morris 1980).

To date, the great majority of compounds identified and quantified in natural waters and wastewaters have been those which are amenable to separation methods applicable to chemically stable, nonpolar and volatile materials. Compounds such as these can be readily separated from the matrix of interest by use of hydrophobic resins and nonpolar solvent extractions. Traditionally, analyses of the resulting extracts have been performed by gas chromatography (GC) employing specific detection methods. Organic nitrogen compounds unfortunately do not fall within this rather broad class of substances which can be easily detected and measured. Consequently, the compounds comprising the organic nitrogen fraction of water remain rather poorly characterized. These compounds are, for the most part, highly polar and nonvolatile. It is interesting that the very properties rendering their analyses difficult by conventional methods, namely high nucleophilicity and polarity, also cause them to be extremely reactive towards aqueous chlorine.

The necessity of nitrogen for biological activity results in concentrations and distributions of organic and inorganic nitrogen species being highly seasonally dependent in unpolluted waters and subject to rapid turnover. In highly polluted waters, on the other hand, temporal variations lessen and levels and distributions become more spatially dependent. These caveats notwithstanding, several broad generalizations regarding the occurrence of organic nitrogen compounds in various waters can be made. First, levels of organic-N typically range from 1 to 10 times NH$_3$-N concentrations. Secondly, concentrations of free dissolved amino acids are very low, with totals on the order of a few µg/l. And finally, total hydrolyzable amino acid content can be much higher, with concentrations oftentimes at several hundred µg/l. Concentrations of nitrogenous compounds in a specific water may, of course, differ considerably from these generalized values. The remainder of this section reviews selected literature pertaining to organic-N compounds found in freshwater, wastewater and drinking waters.

**Drinking Water.** Canelli (1980) reported that drinking water concentrations of ammonia were normally less than 50 µg/l and that total organic-N was present at concentrations several-fold higher. In other studies, free amino acids were found to occur at concentrations of 1-40 µg/l as N while total hydrolyzable amino acid content was several hundred µg/l as N (Sidle 1967; Kasiske et al., 1978; Le Cloirec and Martin 1985). For selected finished drinking waters in northwest France, Le Cloirec (1984) reported concentrations...
for total organic-N of up to 2 mg/l. The alkylamines piperidine and propylamine have been found in finished drinking water by the U.S. EPA (1978).

**Surface Water.** In studies of two lake waters, Tuschall and Brezonik (1980) found that 23-30 percent of dissolved organic nitrogen could be accounted for by proteinaceous material and that most of the organic-N was associated with materials having an apparent molecular weight between 10,000 and 50,000 daltons. Lytle and Purdue (1981), in an Oregon watershed study, found total amino acid content to be correlated with total organic carbon. Concentrations of total amino acid were found to range between 2 and 16 μM for the two year period of study. Relative distributions of the amino acids, in terms of mole percent, were found to be: glycine > aspartic acid > alanine > serine > glutamic acid.

In studies employing high performance ion-exchange liquid chromatography, purine and pyrimidine bases of nucleic acids were detected in municipal surface water supplies at concentrations ranging from 20 to 860 μg/l (Ram and Morris 1980; Morris et al., 1980). Koga et al. (1982) reported finding methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, dimethyl, and diethyl amines in three riverine samples at concentrations ranging from 1 to 10 μg/l. Derivatized compounds were identified and quantified by GC-chemical ionization mass spectrometry (GC/CI-MS). Crathorne et al. (1984) identified a diversity of man-made nitrogenous compounds in surface waters, including pesticide and pharmaceutical compounds.

**Wastewater.** Concentrations of NH$_3$-N and organic-N in raw domestic wastewater commonly range between 12-50 and 8-35 mg/l, respectively (Metcalf and Eddy 1979). Isaac and Morris (1983a), citing unpublished data, reported median organic-N to NH$_3$-N ratios of 0.59 and 0.92 for secondary and nitrified wastewaters, respectively. Koga et al. (1982) reported finding methylamine and isopropylamine in untreated sewage at 15.5 and 10.5 μg/l, respectively. Dimethylamine, diethylamine, piperidine and morpholine were detected at concentrations less than 5 μg/l. Scully et al. (1988) identified and quantified 5 volatile amines and amino acids in primary and secondary wastewaters. Detected amines, including piperidine, were found at low ppb levels. These concentrations were approximately equal to those found for some free amino acids. Pitt et al. (1975) and Jolley et al. (1983) reported finding amino acids and purine and pyrimidine bases in primary and secondary treated wastewaters at ppb levels. Burleson et al. (1980) found concentrations ranging from less than 10 to greater than 1000 μg/l of individual free amino acids in raw sewage.
Due to limitations of current analytical methods, only a handful of N-chloroamino compounds have been positively identified in chlorinated waters or solutions of model compounds. By means of a derivatization based method, the monochloramines of ammonia, methylamine, dimethylamine and several alpha amino acids have been identified and confirmed in chlorinated primary wastewaters (Scully et al., 1984b; Choshen et al., 1990). Limitations inherent to this analytical approach, however, preclude accurate quantifications of the levels of each compound detected (Jersey et al., 1990).

Amino acids, proteins and nucleic acids comprise a large portion of the naturally occurring dissolved organic-N commonly found in water. Consequently, some of the more nucleophilic members of these compound classes have been used as models to study aqueous chlorination reactions and to identify the resulting N-chloro by-products. Free amino acids are known to react rapidly with HOCl to form N-chloroamino acids (Morris 1967). In chlorination studies of several amino acids, Pereira et al. (1973) found only aldehyde and nitrile oxidation products. Stable N-chloroamino acid products were not identified. These findings are consistent with the known instability of chlorinated alpha-amino acids (Stanbro and Smith 1979; Hand et al., 1983; Isaac and Morris 1983b; and Le Cloirec and Martin 1985).

In contrast to free amino acids, chlorination of dipeptides has been found to produce stable N,N-dichloro products. Pereira et al. (1973) reported finding N,N-dichloro derivatives of several model dipeptides. Identities were confirmed mass spectrometrically by performing accurate mass measurements and acquiring characteristic mass spectra. These workers also isolated and identified N-chlorimines produced by treatment of N,N-dichloro peptides with ethanolic base. N-chlorimines have been implicated as intermediates in mechanisms proposed by several researchers for decomposition of chlorinated alpha amino acids and peptides (Stelmaszynska and Zgliczynski 1978; Stanbro and Smith 1979; Le Cloirec and Martin 1985; and Stanbro and Lenkovitch 1985). In more recent work Newke and Scully (1989) confirmed the production of a N-chlorimine product in chlorinated isoleucine solutions. Peters et al. (1990) have recently reported detection of various N-chloroamides produced by chlorination of cyanoethanoic acid at pH 10. The N-chloroamides were detected as N-chloroimidates following their derivatization with diazomethane and analysis by GC/MS. Under typical water treatment conditions (neutral pH, low chlorine dosages) the amide-N of peptide bonds is not, however, believed to react with chlorine to a significant extent (Morris 1967 and Ayotte and Gray 1985).

The majority of model compound work conducted thus far has been focused upon examining the reactions of aqueous chlorine with the purine and pyrimidine bases of nucleic acids at high chlorine to substrate ratios. Thin layer chromatography and mass
spectrometry were the primary analytical tools employed for product isolation and identification in this research. In general, the purine bases have been found to be less reactive towards chlorine than the pyrimidine bases. The high chlorine to substrate ratios employed in these studies resulted in oftentimes degradative scale reactions. The net effect being the production of a wide variety of oxidation end products which in turn made product isolation and identification difficult. In studies performed using this experimental approach, Hoyano et al. (1973) identified N-chloro derivatives of the pyrimidine bases thymine and uracil and some of their alkylated derivatives. For adenine and guanine, two purine bases, little reactivity towards added chlorine was observed and no N-chloro products were identified. Dennis et al. (1978 and 1979) subjected uracil and the nucleotides of RNA to degradative scale chlorinations (15:1 and 40:1 chlorine to substrate molar ratios, respectively). Not surprisingly, under these conditions all compounds were found to undergo rapid reaction with ring cleavage. Nitrogen trichloride, trichloroacetic acid and carbon dioxide were identified products. Bacon et al. (1972) identified 4-N-chlorocytosine as a product when cytosine chlorination was performed at a substrate to chlorine molar ratio of 1.

Gould et al. (1984a,b) studied the reactions of the pyrimidine bases uracil, cytosine and 5-methyl cytosine with aqueous chlorine. An N-chloro derivative of cytosine was identified and decay of combined chlorine residuals was found to be independent of H⁺ concentration over the pH range 5-9. Degradation of combined chlorine followed first order kinetics with an estimated half-life of 3.9 days. Also identified in this work was the product 3,5-dichloro uracil, a N-chloro derivative of uracil. The results of Gould and coworkers (1984a) have been confirmed in research conducted more recently by Reynolds et al. (1988). These workers identified 1-chloro, 3-chloro and 3,5-dichloro cytosine as N-chloro by-products of cytosine chlorinations at low doses (chlorine to substrate ratio of 3) and neutral pH.

**Significance of Organic Nitrogen in Water Chlorination**

Among the major differences between chlorination of NH₃-N and organic-N are the amount and time dependency of nitrogen loss and the formation of complex N-chloramine residuals that are not distinguished by currently available analytical methods. Researchers have acknowledged the role of organic nitrogen in determining the chlorine demand exerted by many waters. In some of the earliest work on this subject, Taras (1950 and 1953) found that consumption of organic-N could be described by three distinct temporal patterns following chlorination. First, for chlorinations sufficient to maintain free residuals of 0.3 to 0.5 mg/l after several hours, NH₃-N was found to be completely consumed within 1 hour. Secondly, reactive amino-N of proteins and free amino acids was found to require several hours for consumption of nitrogen to be complete. And finally, proteinaceous-N consumption was found to be very slow with negligible losses over periods of days. Correlations between nitrogen content and
chlorine demand were highest in systems dominated by NH$_3$-N and worsened as levels of organic-N increased.

Several research groups have examined chlorine demand kinetics, residual decays and breakpoint curve morphologies for natural and cooling waters to determine the role of nitrogen in water chlorination. Qualls and Johnson (1985), in studies of chlorine demand kinetics for three riverine waters, estimated that approximately 10 percent of dissolved organic-N reacted with chlorine to form N-chloramines. In studies examining total chlorine produced oxidant decay in estuarine cooling waters, Helz et al. (1978, 1983 and 1984) found residual oxidant losses to occur by two temporally distinct processes: one operating very fast -- on the order of minutes, the other, typically requiring hours. Levels of organic-N in intake water in these studies were found to be approximately 10 times NH$_3$-N. The slowly decaying component of total chlorine produced oxidant was measured analytically as combined chlorine, and its loss followed first order decay kinetics with half-lives ranging from 0.5 to 4.6 hours. The relatively slow decay kinetics in conjunction with the analytical behavior of the residual oxidant implicated the role of organic-N as determining the fate of residual chlorine in these waters. Protein bound organic-N was not believed to be a large source of the observed chlorine demand. Interestingly, for 9 of 10 periods sampled, levels of NH$_3$-N in discharged cooling water were found to have actually increased significantly over levels found in intake water of the same day. The authors postulated that perhaps oxidative deamination or lysis of plankton and bacterial cells were responsible for the observed increases.

In his dissertation research, Jensen (1988) examined the morphology of breakpoint chlorination curves for four cooling waters to determine what role if any organic-N had in the chlorination of these waters. His results demonstrated clearly that the observed breakpoint curve morphologies could not be adequately described by consideration of only NH$_3$-N chlorination. Four features of the breakpoint curves were found to consistently demonstrate the importance of organic-N in describing chlorinations of these waters: the locations and magnitudes of both the chloramine maximum and the breakpoint. It was estimated that 70-80 percent of reduced-N chlorinated and that 20 percent was present at the breakpoint in the form of a stable chlorine residual. A stable chlorine residual existing at the breakpoint has been reported elsewhere (Le Cloirec et al., 1988).

**CURRENT ANALYTICAL METHODS FOR CHLORINE AND CHLORINE PRODUCED OXIDANTS**

**Standard Methods**

Since the widespread adoption of chlorination for disinfection, evolution of analytical methods for measurement of chlorine residuals and insights gained into chlorination
chemistry have been closely intertwined. Refinements made to the practice of chlorination have traditionally resulted in increasingly more difficult demands being placed upon methods for chlorine and chlorine produced oxidant analyses. Already in many states maximum chlorine residual concentrations are considered as part of the wastewater discharge permitting procedure. Similarly, federal regulations allow measurement of free chlorine residuals to be substituted for microbiological assays of treated drinking waters to insure a hygienic water supply.

Because of the emphasis upon disinfection, analytical research has traditionally been focused on the analysis of free available chlorine (FAC) with efforts primarily in two areas: (1) development of methods specific for FAC and (2) improving method detection limits. Over the years there have been many methods proposed for the specific analysis of free chlorine. These methods, based upon assorted principles, have achieved varying degrees of success. By careful control of reaction conditions some of these methods are used for measuring inorganic chloramines as well as total combined chlorine residuals. The underlying principles of these methods will be briefly reviewed in the following text. More comprehensive reviews of this subject are available elsewhere (Nicolson 1965; Palin 1975; Johnson 1978; Helz 1981; and Jolley and Carpenter 1983b) for the interested reader.

Standard Methods (APHA et al., 1985) for free chlorine analysis can be broadly classified as falling within two groups based upon the type of measurement performed: spectrophotometric and titrimetric. The spectrophotometric (or colorimetric) methods are based upon reaction of free available chlorine with a color producing reagent. The degree of color development being proportional to the free chlorine concentration. Freedom from interferences is achieved by the slower reaction rates between interfering substances, including combined chlorine, and the color producing reagents. Considerations important to this analytical approach include the shelf life of reagents and the vulnerability of the methods to turbidity and other spectrophotometric interferences. Also, because specificity is achieved by differences in the kinetics of reaction of oxidants with color producing reagents, interferences are generally found to increase as the time required for analysis increases.

Colorimetric methods in Standard Methods (APHA et al., 1985) include the FACTS procedure for free chlorine and the Leuco Crystal Violet (LCV) method which provides for discrimination of FAC and total residual chlorine. Probably the most commonly employed standard method for analysis of chlorine in drinking waters is the DPD-colorimetric procedure first introduced by Palin (1957). This method allows for resolution of total chlorine residuals into the following components by careful control of reaction conditions: FAC, NH₂Cl, NHCl₂, NCl₃ and total residual chlorine. Later modifications to the method (DPD-steadifac) were reported to have minimized its largest problem, breakthrough of monochloramine into the FAC fraction (Palin 1980).
The other major class of methods appearing in Standard Methods is based upon the principles of titrimetry. Iodometric titrimetry is the method of choice for standardizing chlorine solutions of higher concentration. This method analyzes total residual chlorine by titration of liberated iodine with standardized thiosulfate using a starch endpoint indicator. Palin's DPD indicator has also been adapted into a titrimetric procedure using ferrous ammonium sulfate as the titrant. As in the colorimetric procedure, control of reaction conditions provides for a degree of resolution of components of the total chlorine residual pool. A third and very important titrimetric method is the amperometric titration procedure, first introduced by Marks and Glass (1942). Later refinements made to the method resulted in the ability to distinguish FAC, NH₂Cl, NHCl₂, NC₁₇, and total residual chlorine (Marks et al., 1951). This procedure uses amperometry to detect the titrimetric endpoint and is commonly acknowledged as the standard method against which others are judged.

In the above methods, the total residual chlorine pool can be partially resolved into individual components through the use of iodide additions with careful control of pH. A serious drawback inherent to this approach, however, is the non-specificity introduced by use of iodide. Iodine will be produced by reaction with any oxidant of sufficient oxidizing strength. To detect weaker N-chloramine oxidants, further additions of acid and iodide are made in the above methods. This increases the rate of iodide oxidation by an oxidant which in turn further decreases the specificity of the measurement technique. In addition, changes of sample pH prior to analysis can introduce errors by changing the equilibria among components of the total residual chlorine pool and between chlorine and chlorine demand substances present in the sample at the time of collection. An ideal method of analysis would thus not require alteration of sample pH.

Johnson et al. (1978) introduced an additional method for free chlorine analysis that was reported to be specific for the most important disinfectant species, HOCl. The measurement technique used a fluoropolymer membrane to select for HOCl which subsequently was measured by electrode amperometry. Detection in this manner achieved specificity by both a physical separation process and the applied potential used for detection. The method showed high precision and selectivity, insensitivity to ionic strength and a moderate temperature dependence. The technique has not, however, been adopted as a standard method at this time.

Reports in the literature clearly demonstrate the vulnerability of current methods for free chlorine analysis to interferences by inorganic and organic N-chloramines (see Appendix D for a review). In the presence of certain N-chloramine compounds, these methods may erroneously provide positive readings for free chlorine residuals. Under such conditions, inadequate disinfection may be observed while maintaining measured free chlorine residues that should be more than adequate. This vulnerability of current methods for free chlorine analysis makes correct assessment of compliance with the new EPA "Surface Water Treatment Rule" difficult (U.S. EPA 1987).
Direct Chromatographic Methods Applied to the Analysis of N-Chloramines

To date, development of direct chromatographic methods for N-chloramine analysis has received little attention. This likely has resulted from concerns among researchers about the inherent instability of these compounds under even relatively mild conditions. To a large extent the distribution of chlorine among reactive nitrogenous substrates is kinetically controlled and strongly a function of pH (Morrison 1967; Margerum et al., 1978; and Gray et al., 1978). Thus, due to the high lability of the nitrogen-chlorine bond, the potential effects of elements common to most analytical protocols cannot be ignored. For example, procedures calling for pH adjustments and lengthy sample preconcentration steps must be viewed with skepticism since these operations can alter the distribution of active chlorine among components of the total residual pool as well as change the chlorine demand characteristics of the sample. An additional question relevant to chromatographically based methods is the extent to which N-chloramines interact with the separation media; e.g. are they reduced by chlorine demand? To accurately quantify these oxidant compounds, the separation media must be chemically inert.

Acknowledging the instability of these oxidant compounds, some investigators have chosen the mild conditions of thin layer chromatography (TLC) for their analyses. Patton and coworkers (1972) used cellulose coated TLC plates for the analysis of products formed during aqueous chlorinations of cytosine, 5-chlorocytosine and 5-methylcytosine. Similarly, Hoyano et al. (1973) used a cellulose based stationary phase and 70 percent aqueous methanol to separate and identify products of the chlorination of pyrimidine and purine nucleic acid bases. In both reports N-chloro compounds were detected by color development after spraying the plate with potassium iodide and starch solutions. More recently, Gould and colleagues employed silica gel TLC plates and a 4:1 solvent mixture of methylene chloride and methanol during the analysis of chlorination products of uracil (1984b) and cytosine and 5-methylcytosine (1984a). As above, oxidants were identified by color development following their reaction with solutions of potassium iodide and starch.

Gas and liquid chromatographic methods have been a mainstay of environmental research due to their resolving power and versatility and the availability of detectors offering high sensitivity and selectivity. Because of these attributes, it is natural to consider their applicability to analysis of N-chloramines. Kearney and Sansone (1985) employed gas chromatography in the analysis of N-halogenated organic methylamines produced during the chlorination of seawater. A 30 m - thick film (1.0 μm) DB5 column was used at ambient temperatures to separate the N-halogenated amines. Compounds were detected and quantified with a Hall electrolytic conductivity detector operated in either the halogen or nitrogen mode. Peak identities were confirmed by mass spectrometry. Peters et al. (1990) have recently reported the analysis of N-chloroimidates by GC/MS. These compounds were produced by derivatization of N-chloroamides with
diazomethane. Together, these reports constitute the only reported applications of gas chromatographic methods to the direct analysis of N-halogenated compounds.

Several researchers have applied HPLC methods to the analysis of chlorine produced oxidants. Hand and Margerum (1983) used HPLC to quantify products in studies of the mechanism of inorganic dichloramine decomposition. A C₁₈ column and an isocratic 0.1 M, pH 7.0 sodium phosphate mobile phase were used to resolve and quantify monochloramine and dichloramine. Compounds were detected by monitoring UV absorbance at 231 nm. More recently, Coburn et al. (1983) employed a C₈ column and an isocratic 5:95 acetonitrile/water mobile phase to resolve monochloramine, N-chloroglycine and N-chlorolysine. Detection employed post-column reaction of the sample with hydrogen peroxide and iodide. The oxygen produced from reaction with N-chloro compounds was measured by membrane-covered electrode amperometry. In this preliminary work no attempts were made to examine quantitative applicability of the technique.

Mazina et al. (1990) used HPLC to fractionate model N-chloropiperidine solutions and stomach fluids of rats treated with ³⁵Cl-N-chloropiperidine. Reversed-phase separations employed gradient elution by mixed water/acetonitrile mobile phases and collected fractions were assayed by liquid scintillation counting. Under these conditions, N-chloropiperidine was found to have a retention time of 20 to 30 minutes. In studies using a nearly identical analytical approach, Nweke and Scully (1989) identified N-chloroisoleucine and N-chloro-2-methyl butyraldime. Together these works constitute the only reported applications of liquid chromatography to the direct analysis of N-chloro compounds. Method development work in these reports was either subsidiary to the main research objective or of limited scope. Little emphasis was placed upon method evaluation and validation. Consequently, data presented was inadequate to critically assess the validity of the reported HPLC based methods.

Indirect Chromatographic Methods Applied to the Analysis of N-chloramines

Acknowledging the inadequacy of traditional analytical methods for the analysis of N-chloramines, Scully et al. (1983 and 1984b) sought to analyze N-chloramines by a derivatization based procedure. The chemistry underlying their derivatization technique is as follows. Solutions of chlorinated nitrogenous compounds are reacted with dansyl sulfinic acid at pH 9.5. The highly nucleophilic dansyl sulfinic acid abstracts the electrophilic chlorine of N-chloramines to form dansyl chloride and the parent amine. The dansyl chloride produced is then free to react with any amines in solution to produce highly fluorescent dansyl derivatives. The latter step in this derivatization, proposed as rate limiting, is exactly analogous to the widely accepted dansylation procedure used for analysis of amino acids (Gray 1972). The product composition of the two step derivatization reaction was stated to be representative of the N-chloramine pool since the fastest
amino compounds to chlorinate would also react most rapidly with the dansyl chloride intermediate.

This derivatization technique has been widely applied to the analysis of model compound solutions, stomach fluids and domestic wastewaters (Scully et al., 1983, 1984b, 1985, 1986 and 1990; Choshen et al., 1990). This derivatization technique, however, has shortcomings severe enough to preclude its use to identify and quantify N-chloramines in chlorinated waters (Jersey et al., 1990). Among the technique's potential limitations or disadvantages are matrix effects, low product yields at dilute N-chloramine concentrations and a marked dependence of yield upon reaction conditions. In addition, the relative slowness of the derivatization procedure, requiring hours for complete derivatization, biases the method towards detection of the more stable chloramines. As evidence supporting the potential importance of this problem, half-lives for many of the N-chloramino acids, an important component of the chloramine pool, are on the order of hours or less (Isaac and Morris 1983b). And finally, the rather significant pH adjustment to alkaline conditions required by the derivatization will alter the very composition of the solution being examined.

Lukasewycz et al. (1989) have recently reported a derivatization method based upon reactions of N-chloramines with 2-mercaptobenzothiazole. In this method, stable sulfenamide derivatives are analyzed by HPLC with UV or electrochemical detection. Like the dansylation technique, this derivatization proceeds indirectly through formation and reaction of an intermediate which in turn reacts with free amines to produce the detected product. Although the yields are somewhat higher and the kinetics faster than the dansylation method, this derivatization procedure appears to suffer problems similar to those of the dansylation method discussed above. Specifically, yields are a function of the Cl:N ratio and the chemistry of the precursor amine. In addition, yields at dilute concentrations of N-chloramines were only on the order of a few percent.

**ELECTROCHEMISTRY OF INORGANIC AND ORGANIC N-CHLORAMINES**

Because they are strong oxidants, free available chlorine and the inorganic and organic N-chloramines can be expected to readily undergo electrochemical reductions. Historically, the majority of research concerning the electrochemistry of chlorine and chlorine produced oxidants has been focused on determination of these compounds by amperometric titration (Marks and Glass 1942; Marks and Bannister 1947; Haller and Listek 1948; Marks et al., 1951; and Williams 1951). Estimated standard redox potentials for chlorine and inorganic and organic N-chloramines are summarized in Tables 1 and 2. Redox potential values listed in these tables were calculated by the cited authors using available thermodynamic data.
### Table 1. Standard reduction potentials for chlorine, iodine and inorganic N-chloramines

<table>
<thead>
<tr>
<th>Compound</th>
<th>Reaction</th>
<th>$E^\circ$ (V)</th>
<th>Ref.</th>
<th>Stated Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorine</td>
<td>$\text{Cl}_2 + 2e = 2\text{Cl}^-$</td>
<td>1.36</td>
<td>Jolley 1956, Drago 1957</td>
<td></td>
</tr>
<tr>
<td>Iodine</td>
<td>$\text{I}_2 + 2e = 2\text{I}^-$</td>
<td>0.54</td>
<td>CRC Press; Handbook Chem. Phys.; 62nd ed.</td>
<td></td>
</tr>
<tr>
<td><strong>Inorganic N-chloramines</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{NH}_2\text{Cl}$</td>
<td>$\text{NH}_2\text{Cl} + 2\text{H}^+ + 2e = \text{Cl}^- + \text{NH}_4^+$</td>
<td>1.48</td>
<td>Jolley 1956, Drago 1957, Sisler 1983</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 M $\text{H}^+$, $\text{NH}_4^+$</td>
<td>none stated</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.44</td>
<td>Gray et al. 1978</td>
</tr>
<tr>
<td>$\text{NHCl}_2$</td>
<td>$\text{NHCl}_2 + 3\text{H}^+ + 4e = 2\text{Cl}^- + \text{NH}_4^+$</td>
<td>1.39</td>
<td>Jolley 1956, Drago 1957</td>
<td></td>
</tr>
<tr>
<td>$\text{H}^+, \text{NH}_4^+$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.34</td>
<td>Gray et al. 1978</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\text{NHCl}_2 + \text{H}^+ + 2e = \text{Cl}^- + \text{NH}_2\text{Cl}$</td>
<td>1.24</td>
<td>Gray et al. 1978</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 M $\text{H}^+$, $\text{NH}_2\text{Cl}$</td>
</tr>
<tr>
<td>$\text{NCl}_3$</td>
<td>$\text{NCl}_3 + 4\text{H}^+ + 6e = 3\text{Cl}^- + \text{NH}_4^+$</td>
<td>1.37</td>
<td>Jolley 1956, Drago 1957</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 M $\text{H}^+$, $\text{NH}_4^+$</td>
</tr>
<tr>
<td>Compound</td>
<td>Reaction</td>
<td>$E^\circ$</td>
<td>$E_{1/2}^a$</td>
<td>Ref., Stated Conditions</td>
</tr>
<tr>
<td>------------------------</td>
<td>-----------------------------------------------</td>
<td>-----------</td>
<td>-------------</td>
<td>-----------------------------------------------------</td>
</tr>
<tr>
<td>N-chloroalkylamines</td>
<td>$\text{RNR'Cl} + \text{H}^+ + 2e = = \text{Cl}^- + \text{RR'NH}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-chlorodimethylamine</td>
<td>1.37</td>
<td>1.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-chlorodiethylamine</td>
<td>1.37</td>
<td>1.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-chlorodipropylamine</td>
<td>1.37</td>
<td>1.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-chlorodisopropylamine</td>
<td>1.43</td>
<td>1.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-chlorobutylamine</td>
<td>1.37</td>
<td>1.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-chloromethylbutylamine</td>
<td>1.37</td>
<td>1.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-chloromorpholine</td>
<td>1.32</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-chloropiperidine</td>
<td>1.34</td>
<td>1.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\text{RNR'Cl} + 2 \text{H}^+ + 2e = = \text{Cl}^- + \text{RR'NH}_2^+$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-chloromethylamine</td>
<td>1.39</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-chloroquinoxulidium</td>
<td>1.35</td>
<td>1.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-chlorosuccinimide</td>
<td>1.27</td>
<td>1.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dichloramine-T</td>
<td>1.31</td>
<td>1.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloramine-T</td>
<td>1.27</td>
<td>1.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-chloro-N-methylbenzene</td>
<td>1.26</td>
<td>1.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sulfphonamide</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{N}_2\text{N}$-dichloro alkylamines</td>
<td>$\text{RNCl}_2 + 3\text{H}^+ + 4e = = 2 \text{Cl}^- + \text{RNH}_3^+$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{N}_2\text{N}$-dichloromethylamine</td>
<td>1.30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{RNCl}_2 + \text{H}^+ + 2e = = \text{Cl}^- + \text{RNHCl}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{N}_2\text{N}$-dichloromethylamine</td>
<td>1.20</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$E_{1/2}^a$ is defined as the potential at which current is one half $i_L$, the diffusion limited value where:

$$E_{1/2}^a = E^\circ - 0.059/\nu \log \frac{D_{\text{O}^{1/2}}}{D_{\text{R}^{1/2}}}$$

$D_{\text{O}^{1/2}}$ and $D_{\text{R}^{1/2}}$ equal the square root of the diffusion coefficients of the oxidized and reduced species, respectively.

---

*Table 2. Standard reduction potentials of organic N-chloramines*
At the dropping mercury electrode, Heller and Jenkins (1946) found aqueous chlorine and N-chloro-p-toluene sulfonamide (chloramine-T) to be irreversibly reduced by a 2 electron mechanism ($E_{1/2}$ 0.08 and -0.13 V vs a saturated calomel electrode (SCE) at neutral pH, respectively). Currents were proportional to concentration. Monochloramine was found to be reduced irreversibly in the pH range 7 to 11 with $E_{1/2} = -0.65$ V vs. SCE. Chao (1968) used chronopotentiometry to determine the diffusion coefficients of species constituting free available chlorine. Harrison and Khan (1971) studied the reduction of hypochlorite at a rotating platinum electrode. Coulometric experiments indicated a 2 electron mechanism requiring large overpotentials for an irreversible reduction. A two step mechanism involving an initial slow reduction of hypochlorite to surface adsorbed elemental chlorine was proposed. Problems concerning reproducibility were noted when working with free chlorine and glassy carbon electrodes due to formation of surface oxides. Hine and Yasuda (1971) reported similar results for reduction of hypochlorite at the rotating platinum electrode. Limiting currents were proportional to concentration at -0.5 V vs SHE. Morrison et al. (1983) determined free chlorine in a flow injection apparatus using a silver iodide working electrode held at -0.06 V vs. SCE. Free chlorine and monochloramine could be detected simultaneously at an working electrode potential of -0.22 V vs. SCE. Currents were found to be proportional to oxidant concentrations. Morrow and Roop (1975) developed a continuous flow stream method to analyze free chlorine by selectively detecting bromine formed by reaction of free chlorine with bromide. Bromine was measured by a gold working electrode maintained at + 0.52 V vs. SCE. Evans (1982) used linear sweep voltammetry to quantify inorganic N-chloramines in kinetic studies of their decomposition. Shifts in $E_{p,c}$ observed in going to lower pH values were a result of changes in species compositions.

With respect to the electrochemistry of organic N-chloramines, there has been only one published study which examined their behavior directly. In this work, Scully et al. (1984a) studied the electrochemistry of free chlorine and inorganic and organic N-chloramines by cyclic voltammetry. Platinum and glassy carbon working electrodes were examined at solution pH values of 2 and 8. At pH 8, hypochlorite was found to reduce irreversibly on platinum at 0.45 V vs. SCE. For hypochlorite at glassy carbon, only large background currents were observed at this pH. N-chloropiperidine was not reduced at either working electrode at this pH. When the pH was lowered to 2, free chlorine was found to be reduced irreversibly at 0.75 and 0.85 V vs. SCE at glassy carbon and platinum working electrodes, respectively.

At pH 2, Scully et al. (1984a) found that inorganic monochloramine was reduced on glassy carbon at -0.5 V vs. SCE. In addition, the electrochemical behavior of monochloramine under these conditions was shown to be time dependent. At long times following a pH jump to 2, the wave originally appearing at -0.5 V vs. SCE decreased in magnitude while a new wave appeared at + 0.5 V vs. SCE. This behavior is consistent with a shift of chlorine from dichloramine to trichloramine. This suspicion of species
redistribution was later confirmed by examination of UV absorbance spectra of similar solutions. Scully and coworkers (1984a) also examined the aqueous reduction of a series of primary and secondary N-chloroalkylamines. N-chloroderivatives of secondary amines (piperidine, diethylamine, and pyrrolidine) were found to reduce irreversibly at +0.05 V vs. SCE on glassy carbon at pH 2. The N,N-dichloralkylamines were found to reduce at -0.4 V vs. SCE under the same conditions. The reduction potential for these compounds was found to shift to more anodic potentials with increasing alkyl chain length.
EXPERIMENTAL APPROACH

OVERVIEW OF DESIGN AND RATIONALE OF RESEARCH

The fundamental objective of analyses performed on environmental samples is the successful measurement of substances at concentrations believed to be less than toxic, carcinogenic or otherwise detrimental. Current methods of N-chloramine analysis fall far short of fulfilling this objective. By current methods it is not possible to measure individual organic N-chloramines since these methods were developed with an emphasis placed upon specificity for free chlorine analysis. Through reaction with iodide and pH control, certain of these methods are proclaimed to provide specificity for analysis of inorganic monochloramine (APHA et al., 1985). This approach to analysis, however, is especially prone to interferences since delineations are operationally defined. In addition, all species oxidizing iodide, even those reacting partially, are measured simultaneously.

The goal of this research was to develop an analytical method capable of separating and detecting organic N-chloramines and inorganic monochloramine in chlorinated waters. Once developed, the method was applied to analyses of laboratory chlorinated North Carolina surface water samples. Specifically, analyses of chlorinated Jordan Reservoir and High Rock Lake samples were performed.

EXPERIMENTAL APPROACH AND OBJECTIVES

Due to the inadequacies of reported derivatization-based methods discussed earlier in this document, it was decided that the best approach towards developing an analytical method for these relatively unstable compounds would be one that analyzed them directly in aqueous solution. Separations could employ the inherently mild conditions of HPLC and be combined with either a general iodometric or a more specific direct amperometric detection technique. Electrochemical detection of N-chloramines was attractive due to the potential advantages it offered, which in decreasing order of significance were: selectivity, sensitivity and the relatively low cost of the required instrumentation.

The overall research objectives and their interdependencies as envisioned by this approach are depicted schematically in Figure 3. Research objectives were delineated according to the rationale which follows. Due to the relative instabilities of N-chloramines, it was decided to examine and verify the underlying principles of the proposed analytical approach, chromatography and electrochemistry, individually before their combined application in the final method. Unfortunately, the suitability of a chroma-
Figure 3. Flow diagram of experimental objectives for N-chloramine analytical method development employing HPLC and amperometric detection.
tographically based method for their analysis could not be properly assessed from an examination of the literature. There were only a few scattered reports of such applications and none provided sufficient data or analytical detail to allow such an assessment. The situation regarding the electrochemistry of organic N-chloramines was much the same. In the only work reported in the literature (Scully et al., 1984a), the ranges of working electrode materials and solution pH examined were limited. By individually examining and verifying the applicability of these underlying principles in experimentally simple systems, they could be combined with confidence in the proposed direct analytical method.

Early on it was evident that post-column reagent addition would be required for either the direct or indirect detection scenarios envisioned. Results obtained from the research objectives described above were to be used to guide the selection and optimization of the post-column reaction detector. The next research objective involved applying the optimized method to analysis of model compound solutions. This work was intended to evaluate the characteristics of the post-column reaction detector (sensitivity, selectivity, linearity, etc.) and to verify its applicability by performing analyses of solutions having known composition and chemistry.

The remaining research objectives involved developing independent methods to confirm identities of compounds detected by the post-column reaction detector and to apply the optimized method to analysis of chlorinated waters. Identities arrived at by retention time matching in liquid chromatography are tentative due to the probability of compounds coeluting within peaks. Confirmations could be provided by mass spectrometry performed directly on collected fractions or indirectly on solutions prior to chlorination.

The final research objective involved application of the optimized method to the analysis of chlorinated wastewaters and surface waters. While not mentioned in the original proposal, wastewaters were added as an application since concentrations of amino compounds in these samples would be quite high. Surface waters were selected since they serve as the primary source of drinking water in many areas, including much of the State of North Carolina. The occurrence and distribution of N-chloramines in these systems are necessarily issues of concern. Concentrations of reduced organic nitrogen compounds in these samples would be expected to be considerably lower than those encountered in wastewaters.

**RATIONALE AND SELECTION OF MODEL COMPOUNDS**

Organic nitrogen compounds occur in great numbers and in widely varying concentrations within natural waters and wastewaters (Hunter 1971; Ram and Morris 1980; and Morris et al, 1980). The chemistry of their reaction with chlorine is similarly diverse, culminating in a variety of N-chloro products formed (Morris 1967). This diversity in
structure and reactivity thus makes it necessary to delineate a subset of amino compounds to serve as model compounds for method development work.

There were several criteria employed to guide the selection of compounds used as model N-chloramines in this research. First and most importantly, it was necessary that the model N-chloramines be as stable as possible to facilitate method development work. Second, the amine precursors of the targeted analytes should be abundant in wastewaters and natural waters in order to be representative of the systems to be studied. Also important was their compatibility with the limitations inherent to the analytical method proposed, in this case HPLC/EC. And finally, due to the potential complexity of chlorination reactions, it was important that the model N-chloramines be easily synthesized in relatively pure form.

By far the most important class of amino compounds occurring in water are the amino acids. In spite of their relative abundance, however, there are several significant disadvantages associated with their selection as model compounds. Unlike some N-chloramines, the N-chloro derivatives of alpha amino acids are particularly unstable (Stanbro and Smith 1979; Hand et al., 1983; Isaac and Morris 1983b; LeClloirec and Martin 1985; and Stanbro and Lenkovitch 1985). With the exception of N-chloroglycine, measured half-lives have been on the order of one hour or less for the majority of N-chloro amino acids examined. An additional disadvantage associated with the amino acids is that many exhibit complex chlorine demand kinetics attributable to the nature of their R groups or sidechains.

Many heterocyclic nitrogen compounds of biologic origin, including the purine and pyrimidine bases of nucleic acids, have been identified in surface waters and wastewaters (Ram and Morris 1980; Morris et al., 1980; Pitt et al., 1975; and Jolley et al., 1983). It is not unreasonable to anticipate that the N-chloro derivatives of these compounds would be amendable to analysis by reversed-phase HPLC/EC. These compounds, however, have been shown to exhibit complex chlorine demand kinetics (pyrimidine bases) or little reactivity at all (purine bases). In addition, upon chlorination, the presence of multiple reactive nitrogens can result in formation of complex N-chloro product mixtures. Thus, selection of the purine and pyrimidine bases as model compounds is inappropriate due to problems associated with product yields, purities and stabilities.

The N-chloro alkylamines and monochloramine have been selected as model compounds for this research because they best satisfy the criteria delineated above. In many polluted and natural waters, ammonia is the dominant form of dissolved nitrogen. In addition, in non-nitrified wastewaters ammonia concentrations can be quite high. While not the most abundant form of nitrogen, the presence of alkylamines in surface water and wastewater has nonetheless been reported (Koga et al., 1982; Scully et al., 1988; U.S. EPA, 1978; and Choshen et al., 1990).
The chlorine demand kinetics of primary and secondary alkylamine chlorinations are uncomplicated, the reactions terminating in the production of only singly and doubly substituted N-chloro products. By careful control of reaction pH and chlorine-to-nitrogen molar ratios, it is possible to produce single products in relatively pure and stable form. Concentrations of pure model compounds can thus be quantified by existing methods for chlorine residual analysis to serve as standards. A final advantage of selecting the alkyl amines to serve as model compounds is that their N-chloro derivatives are compatible with analyses conducted by reverse-phase HPLC, e.g. they are suitably nonpolar.
MATERIALS AND METHODS

REAGENTS

Reagent water was deionized by mixed bed ion-exchange, passed through an activated carbon bed and glass distilled. Chlorine-demand-free water (CDFW) used in some experiments was prepared according to Standard Methods (APHA et al., 1985). Reagent grade organic nitrogen compounds were obtained from Sigma Chemical Co. (St. Louis, MO) as free bases, acids or salts. Reagent grade ammonium chloride (Fischer Scientific Co., Pittsburgh, PA) served as the ammonia source. Sodium hypochlorite (5 percent OCl') and phenylarsine oxide stock solutions were obtained from Fischer Scientific Co. Chemicals of reagent grade purity were used without further purification. Glassware used in all experiments was tap water washed, soaked in 6 N HNO₃ and thoroughly rinsed with distilled and deionized water.

MEASUREMENT OF pH

Measurements of pH were performed with an Orion Research Model 601A pH Meter (Orion Research Inc., Boston, MA) equipped with a combination electrode (Model 13-639-252, Fischer Scientific Co.) The pH meter was calibrated before each use by measuring response of standard buffers (Fisher Scientific Co.) bracketing the pH range of interest and making the necessary adjustments.

MEASUREMENT OF OXIDANT CONCENTRATIONS

Concentrations of free and total residual chlorine employed to synthesize N-chloramino compounds were determined by the forward amperometric titration procedure (APHA et al., 1985). This technique was also used to measure all total residual chlorine concentrations in surface and wastewater samples. A hook-type rotating platinum electrode (rotator S-76485; Pt electrode S-30421; Sargent-Welch Scientific Co., Skokie, IL) and MP-1000 series voltage source and signal conditioning components (McKee-Pedersen Instruments, Danville, CA) were employed. A laboratory constructed saturated calomel electrode (SCE) served as the reference electrode. All measurements were made at +200 mV vs SCE. Phenylarsine oxide titrant solutions (Fischer Scientific Co.) were iodometrically standardized by titrating a known amount of iodine generated by the iodate-iodide reaction at low pH.

Concentrations of NH₂Cl were measured by the DPD-FAS titrimetric procedure of Standard Methods (APHA et al., 1985). This procedure was also routinely employed to
measure free chlorine stock concentrations as a cross check to validate the ongoing accuracy of the amperometric titration technique. The DPD-FAS procedure was also selected for measurement of free chlorine and NH₂Cl in surface water and wastewater samples due to its simplicity and speed. Difficulties in removing all traces of I⁻ made it difficult to quickly switch among measurements of free and combined chlorine by the amperometric titration procedure without interferences.

PREPARATION OF CHLORINATED MODEL COMPOUND SOLUTIONS

Due to their instability, standard reference materials are unavailable for most N-chloro compounds. Consequently, working standards were made from dilutions of fresh stock N-chloramine solutions prepared by combining standardized chlorine solutions with solutions containing an excess amine. The concentrations of the resulting N-chloramine stock solutions were themselves standardized by amperometric titration. Single component standard solutions were synthesized in all instances since it is not possible to prepare solutions with multiple N-chloro compounds at known individual concentrations.

Stock solutions of N-chloramines were prepared immediately before use by combining equal volumes of amine and chlorine solutions with rapid mixing. Each solution was buffered at pH 7.0 by a 0.02 M phosphate buffer and concentrations were typically on the order of 10⁻³ to 10⁻⁴ M. To minimize formation of the unwanted N,N-dichloramines of ammonia and primary amines, chlorinations were performed at molar reduced nitrogen-to-chlorine ratios of ≥ 2. Solutions were allowed to equilibrate for 15 to 30 minutes at ambient temperature and in darkness before further use. Two component N-chloramine solutions were prepared by combining, with rapid mixing, equal volume solutions of single N-chloramines prepared in the manner described above. Chlorinations of model compound solutions in breakpoint experiments were performed by small volume concentrated chlorine stock solution spikes in order to minimize dilution effects. These chlorinations were buffered at pH 7.0 (0.02 M phosphate buffer) and allowed to proceed for 1 hour in darkness before residual analysis. Inorganic N,N-dichloramine was formed by adjusting the pH of NH₂Cl solutions to pH ≤ 2.

PROCEDURES FOR SPECTROPHOTOMETRIC MEASUREMENTS

A Cary Model 219 spectrophotometer (Varian Instruments, Sunnyvale, CA, U.S.A.) was used to perform all UV spectrophotometric measurements. Molar extinction coefficients were estimated from absorption measurements obtained on a series of solutions made from freshly prepared N-chloramine stock solutions. The resulting dilution series data was analyzed by simple linear regression to estimate constants. Stock N-chloramine solu-
tions were standardized by amperometric titration and experiments estimating molar extinction coefficients were performed in duplicate.

Concentrations of N-chloramines in two component solutions were determined spectrophotometrically by a method analogous to that described by Isaac and Morris (1983a). Since each species possesses characteristic absorbances (molar extinction coefficients) at the two wavelengths selected for study, simultaneous measurement of absorbance at each wavelength leads to two equations which can be solved for the concentrations of the individual N-chloramines. The two simultaneous equations used in this study are listed below:

\[
A_{245} = C_1 E_1^{245}L + C_2 E_2^{245}L
\]

\[
A_{270} = C_1 E_1^{270}L + C_2 E_2^{270}L
\]

where \(A_{245}\) and \(A_{270}\) are the measured UV absorbances at 245 and 270 nm, respectively, \(L\) is the path length (cm), \(C_1\) and \(C_2\) are the concentrations (M) of the two N-chloramines and \(E_1\) and \(E_2\) (\(M^{-1}cm^{-1}\)) are the empirically derived molar absorptivities for N-chloramines 1 and 2 at the selected wavelengths.

CYCLIC VOLTAMMETRY

Collection of cyclic voltammographic data employed a BAS (Bioanalytical Systems Inc., West Lafayette, IN) CV-1B cyclic voltammograph equipped with a Houston Instruments (Houston Instrument, Austin, TX) Model 100 Omnigraphic X-Y recorder. A platinum wire auxiliary electrode and saturated calomel (laboratory constructed) (SCE) or silver/silver chloride (Ag/AgCl) reference electrodes were used to collect all data. Performance characteristics of platinum (Pt), glassy carbon (GC) carbon paste (CP) and in one instance gold (Au) working electrodes (BAS Inc., West Lafayette, IN) were investigated in this study.

Ionic strength was maintained at greater than or equal to 0.1 by additions of reagent grade buffers for pH control (see Table 3). Deoxygenation was achieved by sparging the voltammetry cell with high purity nitrogen gas. Surfaces for each of the working electrode materials studied were regenerated immediately before collection of data for a given N-chloramine or oxidant. Glassy carbon, platinum and gold surfaces were polished.
Table 3. Summary of buffer compositions for cyclic voltammetry experiments

<table>
<thead>
<tr>
<th>Solution pH</th>
<th>Buffer Species</th>
<th>Ionic Strength</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.2</td>
<td>HCl/KCl</td>
<td>~0.1</td>
</tr>
<tr>
<td>2.1</td>
<td>HCl/KCl</td>
<td>~0.1</td>
</tr>
<tr>
<td>3.1</td>
<td>H₃PO₄/H₂PO₄⁻</td>
<td>~0.1</td>
</tr>
<tr>
<td>4.1</td>
<td>HAc/Ac⁻</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>5.0</td>
<td>HAc/Ac⁻</td>
<td>0.08</td>
</tr>
<tr>
<td>6.0</td>
<td>H₃PO₄⁻/HPO₄⁻²</td>
<td>0.08</td>
</tr>
<tr>
<td>7.0</td>
<td>H₂PO₄⁻/HPO₄⁻²</td>
<td>0.18</td>
</tr>
<tr>
<td>8.2</td>
<td>H₂PO₄⁻/HPO₄⁻²</td>
<td>0.40</td>
</tr>
</tbody>
</table>

by abrasion with alumina as recommended by the manufacturer. Carbon paste electrode surfaces were regenerated by removing a small amount of material from within the electrode cavity and carefully repacking it with fresh carbon paste. A planar surface was achieved by sliding the perpendicularly held electrode across an index card.

A schematic depicting the sequence of steps employed in collecting cyclic voltammographic data for each compound is present in Figure 4. Solutions of N-chloramines were synthesized immediately before collection of data as described above. The composition of buffers used for pH control and as supporting electrolyte are summarized in Table 3.

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

Pumps and Columns

A Waters (Milford, MA) HPLC system, consisting of two model 6000A pumps and a model 660 solvent programmer, was used to perform all separations. Isocratic separations employed a single model 6000A pump and mobile phases containing mixtures of HPLC grade acetonitrile (CH₃CN) (EM Science, Gibbstown, NJ and Fischer Scientific, Pittsburgh, PA) and pH 6.2 buffered (0.02 M phosphate) reagent water. Mobile phase
Figure 4. Flow diagram of experimental procedures for collection of N-chloramine electrochemical data by cyclic voltammetry

Chlorination
(pH > 7, Molar N:Cl > 1)

Equilibration
(0.5 - 1.0 h)

Electrochemical Variables
(pH, working electrode, N-chloramine)

Cyclic Voltammetry

Deaeration and/or Oxidant Quench

Cyclic Voltammetry
composition was varied during isocratic runs to provide minimum analysis time while still retaining adequate separation of analytes. Gradient runs in all cases were linear and employed the same solvents used during isocratic runs. Unless otherwise noted flows were 1.0 ml/min. Prior to use, mobile phases were filtered through 0.2 μm PTFE filters (Millipore, Bedford, MA). Pulse dampening was provided by built-in high pressure filters in each pump and an additional diaphragm type pulse dampener immediately downstream of the high pressure mixing point.

Zorbax (Dupont, Wilmington, DE) or J & W C18 analytical columns (J & W Scientific, Folsom, CA; 5 μm particle size, 25 cm x 4.6 mm I.D.), fitted with laboratory packed guard columns [2 cm x 2 mm I.D.; 37-50 μm pellicular C18 μBondapack (Waters)], were used to perform all separations in these experiments.

Detection

A Waters (Milford, MA) model 440 absorbance detector was used in preliminary work evaluating post-column reactor design performance. A detection wavelength of 254 nm was used in all instances. The electrochemical detector was comprised entirely of BAS components (Bioanalytical Systems, Inc.). A thin-layer flow through cell (pre-1986 design) enclosed in a Faraday cage was used in conjunction with a LC4B amperometric controller. Reference and auxiliary electrodes were Ag/AgCl and stainless steel, respectively, in all cases. Dual platinum and glassy carbon electrodes were operated singly in the serial configuration. The gasket defining detector cell volume was 0.015 in. unless otherwise noted. Signals from both detectors were processed by a Shimadzu C-R3A integrator (Shimadzu Inc., Columbia, MD).

A schematic of the HPLC/post-column reaction detector employed in this work is shown in Figure 5. In this system post-column reagent and HPLC eluate were combined at right angles by a small dead volume tee (1/16 in, 0.25 mm bore; Valco Instruments Co., Inc., Houston, TX). HPLC and post-column flows were maintained at a ratio of 2:1 in all experiments. A crocheted PTFE reactor (10 ft x 0.5 mm i.d., Supelco, Inc., Supelco Park, PA) connected directly to the mixing tee provided the time delay required for post-column reaction. Post-column reagent was delivered by an Ismatec peristaltic metering pump (Cole-Palmer Instrument Co., Chicago, IL). Flow was controlled by tensioner adjustments and selection of tubing inner diameter (Cole-Palmer Instrument Co., Chicago, IL). Dampening of flow pulsations from the peristaltic pump was provided by a 50 foot length of loosely coiled teflon tubing (1.5 mm o.d. x 0.8 mm i.d.) placed between the pump and the mixing tee.

The constituents of the post-column reagent were potassium iodide (KI), sodium acetate (NaAc) and glacial acetic acid (HAc)(Fischer Scientific Co.). Each component was reagent grade and used as received. The concentration of KI in all experiments was 0.09
Figure 5. Schematic of instrumentation for post-column reaction/electrochemical detection of N-chloramines
M unless otherwise noted. Post-column reagent was buffered at pH 4.0 ± 0.1 by a 0.294 M (0.24 M HAc, 0.054 M NaAc) acetate buffer prepared by dilution from a tenfold concentrate. To minimize unwanted iodine formation from air oxidation of iodide, post-column reagent was made fresh daily and stored in sealed amber glass vessels. This treatment was observed to successfully minimize background currents.

Injection

Direct injections were made by a Rheodyne model 7125 injection valve (Rheodyne Inc., Cotati, CA) fitted with 10, 20 or 50 μl loops and operated in the complete filling mode. The system utilized for injections by the on-line enrichment technique is shown schematically in Figure 6 and was very similar to one described by other workers (Lankelma and Poppe 1978; Koch and Kissinger 1980). Valves A and B were Rheodyne (Cotati, CA) Model 7000 and 7125 injection valves, respectively. A 2 ml sample loop (Alltech Associates, Inc., Deerfield, IL) was fitted to valve B. The enrichment column (5 cm x 0.46 cm I.D.) was laboratory packed with 50 μm pellicular υBondapack C₁₈ (Waters, Milford, MA) and inserted as the sample loop of valve A. The enrichment column mobile phase, chlorine demand-free-water, was delivered at 1 ml/min by a Milton Roy Mini-Pump (Baxter Healthcare Corporation, Scientific Product Div., Charlotte, NC).

Analyses of samples by the on-line enrichment technique followed four procedural steps. Initially, the loop of valve B was loaded via the complete filling mode with the sample to be analyzed. Valve B was next rotated, redirecting enrichment mobile phase flow and displacing the contents of the sample loop onto the enrichment column fitted as the sample loop of valve A. Following enrichment, valve A was rotated and the contents of the enrichment column were back-flushed onto the analytical column by the redirected flow of HPLC mobile phase. Once sufficient time had passed for elution of components from the enrichment column by HPLC mobile phase, valve A was rotated in order to wash the sample loop and to recondition the enrichment column in preparation for the next injection. Thorough reconditioning of the enrichment column with flow of chlorine demand-free-water was found to be essential for reproducible detector response and peak retention times.

Injection volumes greater than two ml were performed as follows: flow of enrichment mobile phase was stopped following displacement of the first loop volume; valve B was thrown and the sample loop refilled; valve B was thrown and flow of enrichment mobile phase was restarted. At times, poorly retained compounds were displaced from the enrichment column prior to analysis by allowing flows of enrichment mobile phase in excess of that needed to completely displace the contents of the sample loop of valve A.
Figure 6. Schematic depiction of tandem rotary injection valve on-line enrichment system used for analysis of N-chloramines by HPLC with post-column reaction detection.

INJECTION LOOP LOADING

ENRICHMENT

INJECTION AND ANALYSIS

Detector
COLLECTION AND CHLORINATION OF WASTEWATER AND SURFACE WATER SAMPLES

Surface water samples were taken from B. Everett Jordan Reservoir and High Rock Lake, North Carolina lakes typical of surface water impoundments found in the Piedmont region of the state. Surface water grab samples were collected at bridge crossings at each lake. Wastewater samples were collected from the Mason Farm Treatment Plant located in Chapel Hill, North Carolina. This is a 10 million-gallon-per-day municipal plant providing advanced secondary treatment by activated sludge processes after trickling filter conventional treatment. Primary and final clarifier effluent were collected during each sampling.

Surface water and wastewater were collected in amber 4 liter glass bottles with PTFE lined caps. Prior to sampling, bottles were tapwater rinsed, soaked in chromic acid and thoroughly rinsed with distilled and deionized water. Upon return to the laboratory, samples were filtered through Whatman glass fiber filters (Fischer Scientific Co.) and stored at 4°C. Sample preservation by acidification to pH ≤ 2 as recommended by Standard Methods (APHA et al., 1985) was unacceptable due to the large volume of buffer required to increase solution pH to neutral values prior to chlorinations. Time elapsed between sample collection and final use was less than one week.

Chlorinations of surface water and wastewater samples in all breakpoint and HPLC/EC experiments were performed by additions of small volume concentrated chlorine stock solution spikes with rapid mixing in order to minimize dilution effects. Chlorinations were buffered at pH 7.0 (0.02 M phosphate buffer) and allowed to react at ambient temperature for 1 hour in darkness before analysis. Measurement of chlorine residuals were replicated in duplicate or more in all experiments.
RESULTS AND DISCUSSION

ANALYSIS OF N-CHLORAMINES BY HPLC WITH UV DETECTION

Preliminary Chromatographic Assessment

Before conducting initial experiments it was necessary to select a subset of possible N-chloramines to serve as model compounds. Criteria for selection of model compounds included: ease of forming pure, monochloro substituted stable products; absence of chlorine demand reactions other than N-chloramine formation; and their compatibility with separation and detection by reverse-phase HPLC with UV detection at 254 nm. Based on these criteria, NH$_3$ and primary and secondary alkylamines were selected. These compounds, possessing a single reactive nitrogen and unreactive alkyl substituents, react with chlorine under proper conditions to form single N-chloramine products. In addition, substitution of Cl$^-$ for H$^+$ in these compounds provides two beneficial effects. First, the N-chloramine product absorbs light in the region of 240 to 300 nm, whereas the unsubstituted amines are UV transparent. Secondly, substitution greatly reduces nitrogen basicity. Thus, N-chloroalkyl amines exhibit good reversed-phase chromatographic behavior and detection at 254 nm provides selectivity for the chlorinated product in solutions containing quantities of the unchlorinated precursor amine.

Initial HPLC analyses of NH$_2$Cl and a number of N-chloroalkylamines resulted in sharp, well formed peaks free of tailing. In addition, peak retention time and area reproducibility were excellent. For concentrations in the range 10$^{-3}$ to 10$^{-5}$ M, relative standard deviations (RSD) for multiple injections were typically less than 1 percent and 2-4 percent for retention time and peak area, respectively. Observations of color development following peak fraction collection in mildly acidic KI/starch solutions confirmed the oxidant character of the detected compounds. This work also demonstrated that column materials could withstand repeated injections of these compounds, strong oxidants with $E^\circ$ values $> 1.0$ V, with no deterioration in performance.

Detection limits for N-chloramines examined were approximately 10$^{-5}$ to 10$^{-6}$ M at the detection wavelength of 254 nm. Figure 7 shows a HPLC/UV chromatogram obtained from analysis of a 10$^{-6}$ M NH$_2$Cl solution (70 $\mu$g/l as Cl$^-$). Since the detection wavelength of 254 nm was not within the region of maximum absorbance for the N-chloramines examined, sensitivity could be increased significantly simply through the use of a variable wavelength UV detector.
Figure 7. HPLC/UV chromatogram for injection of $10^{-6}$ M NH$_2$Cl.

Mobile phase 5:95 CH$_3$CN:pH 6.2 phosphate buffered (0.02 M) water at 1.0 ml/min, injection loop volume 20 μl.

The next sequence of experiments evaluated the quantitative applicability of the method by constructing calibration curves for a number of N-chloramines. Due to their reactive nature, standard reference materials are unavailable for nearly all of these compounds. Consequently, standards were made from dilutions of freshly made stock N-chloramine solutions. These solutions were standardized by amperometric titration and used immediately. Calibrations here and in all experiments were performed on single component solutions since it is not possible to form solutions with multiple analytes at known concentrations.

Results of calibration curves generated for several N-chloramines at the ppm level (as Cl$_2$) using least squares linear regression, are shown in Figure 8. For each of the compounds examined, calibrations were very successful with high correlation coefficients and near zero intercepts.

Linearity plots derived from analyses of NH$_2$Cl and N-chloromethylamine (MACl) are presented in Figure 9. These plots were constructed using a strict linear model which assumes no detector response at zero analyte concentration as described by Dorschel et
Figure 8. HPLC/UV calibration curves for N-chloramines.

Mobile phase 30:70 CH₃CN:pH 6.2 phosphate buffered (0.02 M) water at 1.0 ml/min, injection loop volume 50 μl.

al. (1989). The general form of the model is shown below as well as the specific case for a UV photometric detector:

General Model:

\[ R = SC \]

UV Photometric Detector Model:

\[ R = A = EbC \]

\[ R/C = A/C = Eb = S = RF \]
The terms in these equations are as follows: \( R \) equals the detector response, \( C \) the analyte concentration, \( A \) the measured absorbance, \( E \) the extinction coefficient, \( b \) the optical pathlength and \( S \) the response factor (RF) for the analyte of interest. The response factor can be considered simply as detector response per unit concentration for a specific substance and set of analytical conditions. The model above in essence constitutes a single point calibration with the linear range determined by the concentrations over which RF values remain within a defined tolerance. In the case of Figure 9 this tolerance has been chosen as ± 5 percent of the mean RF value.

The data of Figure 9 demonstrate that mean RFs are within the linear range for both compounds over the range of concentrations examined. The RF for a single injection may, however, fall outside specified limits. Thus for this system of analytes, analytical conditions and concentrations, unknowns could be quantified to an accuracy of ± 5 percent by multiple injections of unknowns and a single calibration point. Results for N-chlorodimethylamine (DMACl) were similar to those discussed above. The linearity observed for each analyte at the lower concentrations supports the premise that chlorine demand originating within the separation media is negligible. If there had been appreciable demand, the effects would have been most prominent at the lowest N-chloramine concentrations where absolute losses would have been most apparent. If present, chlorine demand would have manifested itself as nonlinear absorbance versus concentration behavior at the lowest N-chloramine concentrations.

**Examination of N-Chloramine Mixtures**

The objective of these experiments was to validate quantitative applications of the HPLC/UV method to analyses of N-chloramine mixtures. More specifically, the primary concern was whether the levels and distribution of N-chloramines remain unaltered during passage through the chromatographic system prior to their detection. In the single component solution work reported above, the only possible chlorine transfer reaction terminated in formation of the corresponding N,N-dichloramine. In multiple component N-chloramine solutions, the number of possible chlorine transfer reactions increases very rapidly as the number of species in solution increases. Transfers can, for example, occur among one N-chloramine and free amines which may be present. Similarly, transfers can occur from one N-chloramine to another to form N,N-dichloramines. Transfers can also occur through interactions with compounds strongly retained by the column. Many of these reactions are thermodynamically favored and the kinetics are relatively rapid (Higuchi and Hasegawa 1965; Synder and Margerum 1982; and Isaac and Morris 1983a). Thus, under some conditions concerns regarding the extent to which these reactions occur within the time frame of an analysis may be warranted.

The approach taken in these experiments was to make binary mixtures of N-chloramines and to compare spectroscopically determined concentrations with those determined by
Figure 9. Linearity plot for calibration of N-chloramines by HPLC/UV.

A. 

B. 

(A). monochloramine and (B). N-chloromethylamine. R equals response in peak area and S the slope of the calibration regression constant. Symbols represent RF values from single injections.
Figure 10. Plot of absorption versus concentration for model N-chloramines at 270 nm

<table>
<thead>
<tr>
<th>Compound</th>
<th>Slope</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH$_2$Cl</td>
<td>118.7</td>
<td>0.9998</td>
</tr>
<tr>
<td>CH$_3$NH$_2$Cl</td>
<td>165.5</td>
<td>0.9998</td>
</tr>
<tr>
<td>(CH$_3$)$_2$NCl</td>
<td>320.4</td>
<td>0.9997</td>
</tr>
</tbody>
</table>

HPLC/UV analyses performed on identical solutions. Total residual chlorine content was confirmed by an independent third measurement technique, amperometric titration. Empirically derived molar extinction coefficients were used to determine N-chloramine concentrations by UV spectroscopy. An example of data collected at 270 nm for the model compounds examined in this work is shown in Figure 10.

Results obtained from experiments analyzing mixtures of NH$_2$Cl and N-chloromethylamine and NH$_2$Cl and N-chlorodimethylamine are listed in Table 4. Experimental conditions were selected so that concentrations of the N-chloramines were approximately equal in order to minimize rates of chlorine transfers. A schematic depiction of the sequence of steps undertaken in these experiments is presented in Figure 11.

The data in Table 4 demonstrate the good agreement provided by the two measurement techniques for individual species at comparable times following formation. In addition, totals derived by each method were in good agreement with those determined by a third and independent measurement technique, amperometric titration. Under the conditions of these experiments, namely short reaction times and high molar nitrogen-to-chlorine
ratios, formation of N,N-dichloramines was minimized. Despite these precautions, spectroscopic measurements performed at longer reaction times (hours) for mixtures of NH$_2$Cl and MACl were hindered due to interferences arising from their formation. Unfortunately, spectroscopic methods are especially prone to interferences since absorbance is measured for all species simultaneously. For the NH$_2$Cl and DMACl mixtures, spectroscopic interferences at longer reaction times were much less. This was largely attributable to the inability of dimethylamine to form a N,N-dichloro product.

Results obtained from these experiments support the application of a chromatographically based method for analysis of N-chloramines mixtures. The data show that within experimental error and the time frame of an analysis, concentrations of N-chloramines in these mixtures remained unaltered by the chromatographic process.
Table 4. Species concentrations in (A) monochloramine and N-chloromethylamine (MACI) and (B) monochloramine and N-chlorodimethylamine (DMACI) mixtures as measured by UV spectroscopy and HPLC/UV.

<table>
<thead>
<tr>
<th>Measurement Technique</th>
<th>Time (min)</th>
<th>NH₂Cl</th>
<th>MACI</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC*</td>
<td>32ᵇ</td>
<td>0.64 (41)c</td>
<td>0.93 (59)</td>
<td>1.57</td>
</tr>
<tr>
<td>Spec</td>
<td></td>
<td>0.66 (41)</td>
<td>0.97 (59)</td>
<td>1.64</td>
</tr>
<tr>
<td>Amperometric</td>
<td></td>
<td></td>
<td></td>
<td>1.46</td>
</tr>
</tbody>
</table>

Experiment A

Trial 1

| HPLC | 32ᵇ | 0.58 (46) | 0.70 (54) | 1.27 |
| Spec |     | 0.63 (46) | 0.73 (54) | 1.36 |
| Amperometric |     |           |         | 1.46 |

Experiment B

Trial 1

| HPLC | 27  | 0.40 (53) | 0.35 (47) | 0.75 |
| Spec |     | 0.42 (56) | 0.33 (44) | 0.75 |
| Amperometric |     |           |         | 0.70 |

Trial 2

| HPLC | 27  | 0.67 (53) | 0.60 (47) | 1.27 |
| Spec |     | 0.71 (54) | 0.60 (46) | 1.31 |
| Amperometric |     |           |         | 1.15 |

a Concentration mean value of duplicate injections.
b Time elapsed from combining single N-chloramine solutions to form mixture.
c Figures in brackets refer to the percentage of the total as measured by each measurement technique.
Application to Breakpoint Chlorination of a Tertiary Mixture

The HPLC/UV method was next applied to analysis of solutions containing NH₃, methylamine and dimethylamine, each present at 20 mg/l, that were chlorinated at points across the breakpoint curve. The goal of this experiment was to discern whether the method could resolve components of the total residual chlorine pool at points along the breakpoint curve. Solution composition and chlorine dosages were selected so that typical breakpoint chemistry could be examined. Analyses by HPLC were limited to singly substituted N-chloramines. Total residual chlorine concentrations were measured by the forward amperometric titration procedure.

Concentrations of model compound N-chloramines measured as a function of Cl:N molar ratio are shown in Figure 12. An example chromatogram, obtained from analysis of the chlorinated amine solution at the lowest dose examined, is shown in Figure 13. Concentrations of NH₂Cl and MACl increased with chlorine dose to the chloramine maximum ([Cl]:[N] molar ratio = 1), whereafter they decreased, eventually reaching zero at the breakpoint. Levels of DMACl increased more slowly at low chlorine dosages, eventually reaching a maximum near the breakpoint where concentrations remained stable with further chlorine additions. Figure 14 presents total N-chloramines measured by HPLC/UV and amperometric titration. Measurements performed by HPLC were limited to singly substituted N-chloramines while amperometric titration measured all forms of chlorine, including N,N-dichloramines and any free chlorine which may have been present.

The concentration profiles observed for NH₂Cl were in accordance with the known breakpoint chemistry of NH₃, with the N-chloramine maximum and breakpoint occurring near solution molar chlorine-to-nitrogen ratios of 1 and 1.75, respectively (Palin 1975 and Isaac and Morris 1983b). Because analytical methods have been unavailable for analysis of organic N-chloramines, detailed studies of their behavior (concentration profiles) across the breakpoint curve have not been reported. Existing knowledge regarding the chlorination chemistry of primary organic amines suggests, however, that concentration profiles should mimic that of NH₂Cl. The data of Figure 12 show this to be the case. In addition, the earlier (lower chlorine dosage) maximum concentration observed for MACl relative to NH₂Cl is consistent with the relative rates of reaction of their precursor amines with aqueous chlorine (Morris 1967).

Unlike NH₂Cl and MACl, levels of DMACl increased steadily until reaching a maximum which remained constant with further chlorine additions. Concentrations peaked near the breakpoint as measured by amperometric titration (Figure 14). This behavior is consistent with that expected of secondary organic alkylamines, since in their reactions with aqueous chlorine only a singly substituted N-chloro product can be produced. The N-chloro derivatives of dialkylamines have been reported to be stable in the presence of free chlorine (Scully and Bempong 1980).
Totals for N-chloramines as measured by HPLC and amperometric titration (Figure 14) followed expected trends. Concentrations measured by amperometric titration were consistently higher, especially at points past a chlorine-to-nitrogen ratio of one, because this method measures all components of the total chlorine residual pool, including N,N-dichloro products and any free chlorine which may be present. In contrast, the HPLC method in this experiment measured only NH$_2$Cl, MACl and DMACl.

Conclusions of N-Chloramine Analysis by HPLC-UV

Results from these preliminary experiments demonstrated the applicability of a HPLC based method to analysis of N-chloramine compounds. Solutions of relatively unstable N-chloramines were analyzed directly. The mild conditions inherent to HPLC allowed
Figure 13. HPLC/UV chromatogram of tertiary solution at the lowest chlorine dose ([Cl]:[N] = 0.25).

Chromatographic conditions as described in Figure 12. Concentrations are 7.6, 5.7 and 9.0 mg/l as Cl₂ for the first eluting NH₂Cl, second eluting MACI and last eluting DMACI, respectively.

Potentially sample altering pH changes and handling operations to be avoided. Accurate and precise analyses were performed for specific compounds free of interferences with detection limits on the order of 10⁻⁶ M. Linearity of response observed for model N-chloramines at dilute concentrations supported the premise that chlorine demand originating within the separation media was negligible. Data from mixture experiments demonstrated that the distribution and concentration of the individual N-chloramines tested remain unaltered by the chromatographic process prior to their detection. The ability of the HPLC/UV method to determine individual concentrations of species measured as combined residual chlorine by current methods was demonstrated by analyses of model solutions chlorinated at points across the breakpoint curve.
Figure 14. Concentrations of total chlorine measured by HPLC and amperometric titration.

Chromatographic conditions as described in Figure 12.

ELECTROCHEMICAL BEHAVIOR OF CHLORINE, IODINE AND INORGANIC AND ORGANIC N-CHLORAMINES IN AQUEOUS SOLUTION

Cyclic voltammetry (CV) is a powerful technique capable of providing a wealth of information from an experimentally simple system. The true value of cyclic voltammetry lies in its ability to provide qualitative information about redox systems, including in-situ generated species. For the compounds and solution conditions under investigation, some of the more important information provided by CV experiments includes the positions of $E_{p,e}$ and or $E_{p,a}$ and the extent of electrochemical reversibility.
Results discussed in this section were obtained from experiments conducted during the initial stages of method development work. The primary goal of these experiments was to assess the feasibility of using electrochemical detection (EC) to selectively detect N-chloramines following their separation by HPLC. In addition, it was hoped that knowledge regarding N-chloramine aqueous redox chemistry could be sufficiently developed to provide a rational basis for selection of initial HPLC/EC detection parameters. No attempts were undertaken during these experiments to collect quantitative data.

**Electrode Materials and Background Currents**

Important attributes of electrochemical systems include the potential window afforded as well as the level of noise or background current observed. Potential windows are system specific and particularly a function of the working electrode materials employed. Oxidative and cathodic limits result from electrochemical reactions involving components of the supporting electrolyte and/or the solvent system itself. Background currents are observed in all systems and become limiting at the potential window extremes. Because thin-layer flow through electrochemical cells are mass sensitive detectors, pulsed flow generates unwanted noise. This noise in turn negatively effects detection limits; as noise increases detection limits proportionally decrease. An additional concern regarding N-chloramine detection by reductive amperometry were currents which result from reduction of dissolved oxygen. These currents further contribute to background noise and thus negatively affect detection limits. The following section describes results of experiments conducted to assess background currents and the reduction of dissolved oxygen at a number of working electrode materials and supporting electrolyte combinations.

Cyclic voltammograms were collected for each working electrode material and pH buffer over a wide potential range. The performance of glassy carbon and carbon paste were very similar under the conditions examined. Background currents at positive applied potentials were quite small and increased significantly as applied potentials exceeded -1.0 V vs Ag/AgCl. Similar observations regarding these electrode materials have been reported elsewhere (Zittel and Miller 1965; Bratin and Kissinger 1981 and 1982; Johnson et al., 1986). Background currents observed at platinum electrodes increased to significant levels at potentials more negative than +0.2 V vs. Ag/AgCl and scans produced complex waveforms. The inferior cathodic limits observed for platinum are consistent with the poor performance noted by others for reductive electrochemical applications employing this material (Adams 1969, Bratin and Kissinger 1981, Stulik and Pacakova 1985). For all the working electrodes and pH buffers examined, impurities in buffer salts did not cause unacceptably high background currents within the afforded potential windows of each material.
A distinct dissolved oxygen reduction wave was observed only at the glassy carbon electrode (see Figure 15). For the other working electrode materials examined, the oxygen reduction wave occurred in regions where high background currents obscured its presence. Dissolved oxygen was found to be effectively removed from solution by nitrogen sparging for several minutes, a technique shown previously by others to be effective (Bakaylar et al., 1978; Bratin and Kissinger 1981; and Brown et al., 1981). Reproducibility of background currents observed at carbon paste and platinum electrodes was quite good while for glassy carbon this was not the case. It was found that the glassy carbon electrode surface had to be carefully polished to obtain reproducible background currents. This observation has been reported by others and has been attributed to the formation of surface oxides (Lund et al., 1979; and Horvai and Pungor 1987) and continuous solvent wetting of pores within the solid electrode (Lankelma and Poppe 1978).

Aqueous Free Chlorine

In initial investigations of free and combined chlorine electrochemistry, cyclic voltammograms for individual species were obtained at pH 2 and 7. In addition, the oxidant character of each compound's waveform was confirmed by observing loss of reduction waves following solution quenching with arsenite additions. Similarly, cyclic voltammograms were also obtained for each compound following solution deoxygenation to examine what role, if any, dissolved oxygen had in the reductions of these compounds. Concentrations of chlorine and N-chloramines examined in these experiments were millimolar or greater. Periodically, potential at the working electrode was monitored to ensure that proper control of applied potential was maintained.

Cyclic voltammographic data obtained from electrochemical reductions of aqueous free chlorine are summarized in Table 5. Values of $E_{pc}$ are approximate and have been estimated graphically. Reductions of aqueous chlorine were found to generate distinct reduction waves for each working electrode material and pH examined. In all cases, the reduction was highly electrochemically irreversible. Reduction of aqueous chlorine at pH 2 and a carbon paste electrode is shown in Figure 16. For carbon paste and glassy carbon working electrodes, decreasing solution pH from 7 to 2 caused a significant anodic shift in the observed position of $E_{pc}$.

Aqueous Iodine

The electrochemistry of aqueous iodine was examined to guide the selection of conditions for its detection in the generalized N-chloramine post-column reaction detection scheme. The iodine/iodide couple was observed to undergo redox reactions very readily
Figure 15. Background currents at a glassy carbon working electrode in a phosphate buffered solution at pH 2.0
Table 5.  \( E_{pc} \) values for reduction of aqueous chlorine

<table>
<thead>
<tr>
<th>Working electrode</th>
<th>( E_{pc} ) (V vs Ag/AgCl)</th>
<th>pH 7</th>
<th>pH 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glassy carbon</td>
<td>-0.400</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>Carbon paste</td>
<td>-0.650</td>
<td>-0.050</td>
<td></td>
</tr>
<tr>
<td>Platinum</td>
<td>0.550</td>
<td>0.550</td>
<td></td>
</tr>
</tbody>
</table>

Table 6.  \( E_{pc} \) values for reduction of aqueous iodine

<table>
<thead>
<tr>
<th>Working electrode</th>
<th>( E_{pc} ) (V vs Ag/AgCl)</th>
<th>pH 7</th>
<th>pH 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glassy carbon</td>
<td>-0.050</td>
<td>0.400, 0.700</td>
<td></td>
</tr>
<tr>
<td>Carbon paste</td>
<td>0.050</td>
<td>0.350, 0.700</td>
<td></td>
</tr>
<tr>
<td>Platinum</td>
<td>0.150</td>
<td>0.450, 0.750</td>
<td></td>
</tr>
<tr>
<td>Gold</td>
<td>0.150</td>
<td>0.400, 0.750</td>
<td></td>
</tr>
</tbody>
</table>

and with a degree of electrochemical reversibility. Redox chemistry of iodine was examined following its in-situ generation from a solution of iodide. Results observed for aqueous iodine reductions at a variety of working electrode materials are summarized in Table 6.

For the pH (supporting electrolyte) and working electrode material combinations examined in these experiments, iodine was found to undergo reductions at moderately high positive applied potentials. In addition, at pH 2 reductions gave a two peak wave-
Figure 16. Cyclic voltammogram of aqueous chlorine at pH 2 and a carbon paste electrode
form at each of the working electrode materials evaluated. Factors responsible for the two peak waveforms observed at pH 2 are not understood at this time.

The positive potentials required for reduction of aqueous iodine offer an advantage for the general amperometric detection method since the applied potential \( E_{\text{applied}} \) can be in a region of low background current and well away from the dissolved oxygen reduction wave. These low background currents are conducive to good detection limits. Similarly, the relatively positive applied potential required to reduce iodine constitutes a very significant advantage in terms of detection selectivity.

**Organic and Inorganic N-Chloramines**

A summary of cyclic voltammographic data obtained from reductions of N-chloro derivatives of ammonia and primary organic amines is provided in Table 7. An examination of the data in this table quickly reveals the superiority of the glassy carbon electrode for these compounds and matrices. Reduction waves for all compounds and working electrodes were highly irreversible and required applications of large overpotentials.

The high and irregular background currents observed for platinum severely limited this material's usefulness for detecting these compounds directly by reductive amperometry. Similar limitations were applicable to the use of carbon paste electrodes. At pH 7 clearly resolved reduction waves were not observed. Reducing solution pH to 2 caused the appearance of reduction waves but unfortunately they occurred very near regions where background currents rapidly increased. This limitation, in addition to their mechanical fragility in solutions of significant organic solvent content, severely restricted the potential usefulness of carbon paste electrodes to detect N-chloramines by reversed phase HPLC with reductive amperometric detection.

Reductions at glassy carbon electrodes produced distinct and well resolved waves for monochloramine and the organic N-chloramines examined. A two peak waveform was observed for solutions of N-chlorohexylamine at pH 7 and 2. The larger peak occurred at more cathodic potentials while the second peak was much smaller and appeared at more positive applied potentials. The reasons for this two peak waveform remain unknown at this time, though initially it was suspected to have arisen from partial formation of N,N-dichlorohexylamine via the disproportionation reaction. This suspicion appears unfounded, however, based upon the findings observed for the other primary N-chloramines and N,N-dichloramines as discussed later in this document.

Cyclic voltammographic data collected during examinations of aqueous N-chloro secondary amines are summarized in Table 8. In general, these compounds were reduced at slightly more positive potentials than the N-chloro primary amines. Like the
Table 7. $E_{pc}$ values for monochloramine and organic N-chloramines in aqueous solution.

<table>
<thead>
<tr>
<th>Compound</th>
<th>pH</th>
<th>glassy carbon</th>
<th>carbon paste</th>
<th>platinum</th>
</tr>
</thead>
<tbody>
<tr>
<td>monochloramine</td>
<td>7</td>
<td>-0.700</td>
<td>nd*</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-0.700</td>
<td>at bckgrd**</td>
<td>0.400</td>
</tr>
<tr>
<td>N-chloromethylamine</td>
<td>7</td>
<td>-0.600</td>
<td>at bckgrd</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-0.600</td>
<td>-0.900</td>
<td>0.500</td>
</tr>
<tr>
<td>N-chloroethylamine</td>
<td>7</td>
<td>-0.600</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-0.600</td>
<td>-0.900</td>
<td>0.450</td>
</tr>
<tr>
<td>N-chlorohexylamine</td>
<td>7</td>
<td>-0.400, -0.650</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-0.350, -0.600</td>
<td>at bckgrd</td>
<td>nd</td>
</tr>
</tbody>
</table>

* Reduction currents not seen clearly resolved from background currents.

** Reduction currents seen clearly but at potentials very close to the regions where background currents rapidly increase.

N-chloro primary amines, however, all reductions were highly irreversible and required application of large overpotentials.

For the N-chloro secondary alkylamines the performance of glassy carbon was again superior to that of the other electrode materials examined. Reduction waves for these compounds were readily discernable at a platinum electrode, but proximity to regions of high background current severely limited this electrode material’s potential usefulness. The performance of carbon paste was intermediate between that of glassy carbon and platinum. Well resolved reduction waves were observed at the carbon paste electrode at pH 2. At pH 7, reduction waves were observed for each secondary N-chloramine but they occurred in a region close to high background currents. For nearly all the model
Table 8. $E_{p,e}$ values for secondary N-chloramines in aqueous solution.

<table>
<thead>
<tr>
<th>Compound</th>
<th>pH</th>
<th>glassy carbon</th>
<th>carbon paste</th>
<th>platinum</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-chlorodimethylamine</td>
<td>7</td>
<td>-0.700</td>
<td>near bckgrd.</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-0.400</td>
<td>-0.400</td>
<td>0.600</td>
</tr>
<tr>
<td>N-chlorodiethylamine</td>
<td>7</td>
<td>-0.800</td>
<td>near bckgrd.</td>
<td>0.050</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-0.250</td>
<td>-0.400</td>
<td>0.500</td>
</tr>
<tr>
<td>N-chloropiperidine</td>
<td>7</td>
<td>-0.700</td>
<td>near bckgrd.</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-0.200</td>
<td>-0.500</td>
<td>0.550</td>
</tr>
<tr>
<td>N-chloromorpholine</td>
<td>7</td>
<td>near bckgrd.</td>
<td>near bckgrd.</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-0.800</td>
<td>-0.900</td>
<td>0.550</td>
</tr>
<tr>
<td>N-chloropyrrolidine</td>
<td>7</td>
<td>near bckgrd.</td>
<td>near bckgrd.</td>
<td>0.100</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-0.300</td>
<td>-0.500</td>
<td>0.600</td>
</tr>
</tbody>
</table>

* Reduction currents not seen clearly resolved from background currents.

In an attempt to confirm the cyclic voltammographic data obtained for the N-chloramines of primary amines, cyclic voltammograms were collected for the N,N-dichloro derivatives of these same compounds. The dichloramines were formed at a Cl:N molar ratio of approximately 2. Results of these measurements are summarized in Table 9.
Table 9.  $E_{pc}$ values for N,N-dichloramines in aqueous solution.

<table>
<thead>
<tr>
<th>Compound</th>
<th>pH</th>
<th>$E_{pc}$ (V vs. Ag/AgCl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>glassy carbon</td>
</tr>
<tr>
<td>N,N-dichloromethylamine</td>
<td>7</td>
<td>-0.600</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-0.600</td>
</tr>
<tr>
<td>N,N-dichloroethylamine</td>
<td>7</td>
<td>-0.600</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-0.550</td>
</tr>
<tr>
<td>N,N-dichlorohexylamine</td>
<td>7</td>
<td>-0.450</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-0.400</td>
</tr>
</tbody>
</table>

* Reduction currents not seen clearly resolved from background currents.

An example cyclic voltammogram collected for N,N-dichloromethylamine is provided in Figure 17.

As seen earlier with singly substituted N-chloro compounds, reductions of all N,N-dichloramines were highly electrochemically irreversible and required large over-potentials. The data in this table are surprisingly in agreement with that of the singly substituted N-chloramines shown in Table 8. This agreement suggests that either the reduction waves seen earlier for N-chloramines at pH 7 result from the presence of high level N,N-dichloro impurities or that both the N-chloro and N,N-dichloro derivatives of these amines are reduced at the same applied potential. Several observations are consistent with the latter scenario.

First, no difference in $E_{pc}$ was observed for solutions of N-chloro primary amines when going from pH 7 to 2. N,N-dichloramines are rapidly formed under these conditions from inorganic monochloramine and primary organic N-chloramines by the disproportion-
Figure 17. Cyclic voltammogram for N,N-dichloromethylamine at pH 2 and a glassy carbon electrode
tionation reaction. Thus, if the N-chloro and N,N-dichloro derivatives were reduced at different applied potentials one would expect to see differences in \( E_{pc} \) in going to the lower pH for the N-chloro primary amines.

Secondly, for solutions of N,N-dichloramines there was not a dramatic shift in \( E_{pc} \) towards more anodic potentials with a decrease in pH. Anodic shifts of \( E_{pc} \) with decreasing pH are common to reductions of most organic compounds since proton transfers are involved (Bratin and Kissinger 1981). Substitution of chlorine at nitrogen dramatically reduces the basicity of amino compounds. Weil and Morris (1949b), for example, estimated N-chlorodimethylamine and N-chlorodiethylamine to have \( pK_a \)s of approximately 0.5 and 1.0, respectively. Thus, the large shifts in \( E_{pc} \) observed for the secondary N-chloramines in going from pH 7 to 2 are consistent with the involvement of the protonated N-chloramine species. Substitution by two chlorine atoms at nitrogen as in N,N-dichloramines would further decrease nitrogen basicity. Consequently, even at pH 2 the degree of protonation of these compounds would be expected to be immeasurably small. One would, therefore, not expect to observe an anodic shift in \( E_{pc} \) in going to lower pH with the N,N-dichloramines.

And finally, solutions of monochloramine and N-chloro primary amines were made under conditions where formation of N,N-dichloramines was minimized. It was highly unlikely that significant N,N-dichloro impurities were present in the N-chloro primary amine solutions. Solution pH was neutral or higher and molar nitrogen to chlorine ratios were well in excess of 1.

For the glassy carbon electrode and N-chloropiperidine, there were clear trends of more positive \( E_{pc} \) with decreasing solution pH. The trend was gradual at the higher pH values and increased abruptly in going from pH 3 to 2. As discussed earlier, this trend is consistent with known chemistry concerning N-chloro secondary amines. In general, shifts in \( E_{pc} \) to more anodic potentials are common among reductions of organic compounds since they invariably involve proton transfer reactions. The abrupt shift observed in \( E_{pc} \) in going to low pH is consistent with a change in electroactive species from a neutral to protonated form.

**Conclusions of Electrochemical Reduction Studies**

In general, our findings concerning the electrochemical behavior of aqueous inorganic and organic N-chloramines are in agreement with those reported by Scully et al. (1984a), though for the majority of compounds we observed reductions at slightly more negative potentials. Reductions for all compounds examined were highly irreversible, required large overpotentials and proceeded more favorably under acidic conditions. The performance of glassy carbon was superior to platinum due to proximity of reduction waves at the latter near regions of high background current. Reductions of N-chloro-
primary alkylamines were observed with \( E_{pc} \) values of approximately 0.400 and -0.600 to -0.700 V vs Ag/AgCl at pH 2 for Pt and GC working electrodes, respectively. Under similar conditions, N-chloro secondary amines were reduced at slightly more positive potentials.

Based upon results of the electrochemical studies above, detection of N-chloramines by direct reductive amperometry constitutes an analytical approach potentially fraught with problems since these substances fail to undergo rapid heterogeneous electron transfer at, or even near, their thermodynamic \( E^0 \) values. This behavior in turn necessitates application of large overpotentials (\( E_{applied} \geq -0.80 \) V vs Ag/AgCl) which compromise detection selectivity. Similarly, sensitivity is degraded by the resulting high background currents. And finally, while noise associated with the reduction of dissolved oxygen at these negative potentials can be reduced, this requires experimentally cumbersome deoxygenation procedures.

Due to the problems described above, we investigated conditions that might be employed in a general N-chloramine detection scheme based upon post-column reaction with iodide. The H\(^+\) catalyzed oxidation of I\(^-\) to I\(_2\) by inorganic and organic N-chloramines is employed in nearly all standard methods for combined chlorine residual analysis (APHA et al., 1985). In the studies discussed above, the iodide/iodine couple was found to readily undergo reduction and oxidation reactions at both glassy carbon and platinum electrodes at pH 2 and 7. Unlike chlorine, however, reduction of aqueous iodine exhibited a degree of electrochemical reversibility. Reductions at pH 7 and GC and Pt electrodes were observed with \( E_{pc} \) of 0.05 and 0.150 V vs Ag/AgCl, respectively. Based upon these experimental results, we decided it would be most fruitful to pursue detection of N-chloramines by employing post-column reaction with iodide followed by detection of the iodine product by reductive amperometry. The faster kinetics of homogeneous electron transfer reactions between N-chloramines and iodide, relative to the heterogeneous electron transfer reactions between N-chloramines and the electrode surface, can be used to advantage in post-column reaction detection by lowering the potential necessary for detecting and measuring the N-chloramines. Detection in this manner affords potential sensitivity enhancements (lower noise levels), maintains selectivity (oxidant analysis) and eliminates the need for sample and mobile phase deoxygenation.

ANALYSIS OF N-CHLORAMINES BY HPLC WITH POST-COLUMN REACTION ELECTROCHEMICAL DETECTION

Sensitive and selective determination of organic and inorganic N-chloramines by HPLC with spectrophotometric detection is hampered by the absence of natural fluorophoric functionalities and the weakness of chromophoric properties imparted to these compounds by chlorine substitution at nitrogen. While this weak absorbance in the UV
region allows for selective detection of some N-chloramines, detection by this method is neither selective nor sensitive enough for analyses at trace levels in complex matrices such as chlorinated wastewaters and natural waters. The fact that the N-chloramines are strong oxidants does, however, provide for the possibility of highly selective and sensitive detection via electrochemical methods. Specifically, reductive amperometry can be used to detect N-chloramines in HPLC eluate directly or indirectly as iodine following post-column reaction with iodide.

The following text describes results of experiments whose objectives included the design and optimization of a post-column reaction electrochemical detector for analysis of N-chloramines in HPLC eluate. N-chloroalkylamines were once again employed as model compounds in this work.

Optimization of Post-Column Reaction Detector Design

**Chromatography.** Because we wished to develop a method for analyses of dilute N-chloramines in complex solutions, it was essential in preliminary work to design the post-column reaction detector so that loss of chromatographic resolution was minimized. From the many designs reported in the literature, use of a knitted or crocheted open tubular reactor (KNOT) seemed most appropriate for our application. A knitted open tubular reactor is comprised of a length of tubing which is tightly knitted or crocheted to provide a constantly changing direction of flow. From a resolution standpoint, performance improves as tubing inner diameter decreases. Knitted open tubular reactors are experimentally simple, provide the needed delay time and furnish enhanced mixing while minimizing peak broadening by axial dispersion (Selavka et al., 1987; Engelhardt and Neue 1982; Lillig and Engelhardt 1986; Poulsen et al., 1986; and Clark et al., 1989).

Results are presented in Figure 18 for an experiment which examined effects of the selected post-column reactor's (10 foot x 0.5 mm i.d.; crocheted PTFE) components upon the chromatographic performance of a column test mixture separation. These results demonstrated that inclusion of the post-column reactor upstream of the detector did not significantly degrade the chromatographic resolution achieved by the analytical column. From these experiments, the volume of the post-column reactor was estimated to be 0.58 ml, in agreement with the dimensionally calculated value of 0.6 ml. The volume was estimated by measuring test mixture retention time differences with and without the post-column reactor. In this and all post-column reactor evaluation experiments the ratio of HPLC to post-column reagent flow was maintained at two. At a combined flow of 1.5 ml/min, the residence time within the post-column reactor was approximately 24 seconds.

**Background Currents.** Because short-term noise levels negatively influence detection limits, variables affecting background currents in the electrochemical cell were examined.
Figure 18. Effects of post-column reactor components upon the chromatographic performance of a column test mixture analysis.

Chromatographic conditions as follows: (a) inj. vol. 50 μl; (b) analytical column 25 cm x 0.46 cm I.D. J&W C₁₈; (c) HPLC mobile phase 70:30 methanol/pH 6.2 (0.02 M phosphate) water at 1.0 ml/min; (d) post-column flow pH 6.2 (0.02 M phosphate) water at 0.5 ml/min; (f) UV detection at 254 nm. Chromatograms A and B separations performed by guard and analytical column with and without post-column reactor, respectively.
Background currents observed for the thin-layer cell at GC and Pt electrodes are shown in Figure 19 as a function of the applied potential. Under the conditions of these measurements (mobile phase a mixture of acetate buffered reagent water and acetonitrile), GC provides lower background currents and wider potential limits, particularly in the cathodic region. Due to platinum's elevated background currents and high surface reactivities, in addition to its commonly assumed predisposition towards memory effects (Adams 1969 and White 1986), remaining investigations focused primarily on use of GC as the working electrode material.

The influence of post-column reagent iodide concentration upon background currents at a GC electrode was also examined. Background currents were observed to increase.
sharply for I− concentrations in excess of 0.10 M. For concentrations less than 0.10 M, cathodic currents were normally less than 10 namps. Initial experience with this detection system demonstrated a clear trend of increasing background current with age of post-column reagent, an observation consistent with air oxidation of I− to I2. Subsequent to these early observations, post-column reagent was prepared fresh daily and pumped from sealed amber glass bottles to minimize background currents attributable to unwanted I2 formation.

Under isocratic conditions with standard post-column reagent composition and flow, peak to peak noise observed at a GC electrode in the frequency domain of a chromatographic peak was approximately 50 picoamps. Peak heights for many of the model compounds examined under these conditions were on the order of 3-5 namp/ng Cl+ equivalent injected. Since all N-chloramines are detected as their common I2 product, detection limits under these conditions can be theoretically estimated. Based on this approach, N-chloramine detection limits (S:N=2.5) should approach approximately 50 pg Cl+ equivalent injected.

Levels of background current were found to drift significantly during gradient separations, the magnitude being reproducible and a function of change in mobile phase composition across the gradient. For a gradient from 0 to 50 percent CH3CN in 15 min, drift was on the order of several namp, with currents increasing as CH3CN content decreased. Peak to peak noise increased slightly under these conditions.

**Applied Potential.** A hydrodynamic voltammmogram of iodine produced during the post-column reaction of NH2Cl with iodide is shown in Figure 20. The curve for detection of iodine shows the expected rise in current and thus peak area with increasingly negative applied potentials. The presence of the expected clear plateau is, however, somewhat obscured by peak area variability measured at potentials more negative than -0.2 V vs Ag/AgCl. Causes for the unanticipated behavior regarding measurements performed in this potential region remain unknown at this time.

**Kinetic Considerations.** Mechanisms by which iodide concentration can affect post-column reaction detector performance are twofold. First, since background currents appear to be chiefly attributable to the iodine content of the post-column reagent, low detection limits are favored by dilute iodide concentrations. On the other hand, the kinetics of reaction between iodide and N-chloramines are favored by high iodide concentrations since this reaction is first order with respect to iodide (Gray and Workman 1983). Data shown in Figure 21 demonstrate the importance of iodide concentration upon the kinetics of post-column reaction for N-chlorodimethylamine. At the high N-chloramine concentration, peak areas noticeably decreased at iodide concentrations less than 5.0 x 10−4 M. In contrast, peak areas for the lower N-chlorodimethylamine concentration decreased much earlier, at iodide concentrations less than 2.0 x 10−2 M. At low iodide and N-chloramine concentrations, therefore, the linearity of post-column reaction detection clearly may become questionable due to kinetic considerations.
Figure 20. Hydrodynamic voltammogram at a GC electrode of iodine produced by post-column reaction of \( \text{NH}_2\text{Cl} \) (17 \( \mu \text{M} \) inj.) and iodide.

![Graph showing hydrodynamic voltammogram](image)

Conditions as follows: (a) HPLC mobile phase 10:90 \( \text{CH}_3\text{CN} / \text{pH 6.2 water at 1.0 ml/min} \); (b) post-column reagent 0.09 M KI, pH 4.0 at 0.5 ml/min; (c) error bars represent standard deviations for multiple measurements performed at each applied potential.

The selection of post-column iodide concentration must consequently strike a balance between minimizing concentration to limit noise and maintaining concentration sufficiently high to give complete post-column reaction independent of N-chloramine concentration. For low concentrations of both iodide and N-chloramine, detection limits may be independent of iodide concentration since the kinetics of post-column reaction are limiting. At the other extreme, that of complete post-column reaction, detection limits may be a function of reagent iodide concentration since this determines the level of noise above which a chromatographic peak must be detected.
Figure 21. Effect of post-column iodide concentration upon peak area measurements of N-chlorodimethylamine.

![Graph showing the effect of iodide concentration on peak area measurements.]

Conditions as follows: (a) HPLC mobile phase 30:70 CH₃CN/pH 6.2 water at 1.0 ml/min; (b) post-column reagent pH 4.0 with varying I⁻ concentration at 0.5 ml/min; (c) GC working electrode at -0.100 V vs Ag/AgCl; (d) error bars represent standard deviations for multiple measurements performed at each I⁻ concentration.

Detector Volume. Detector volume can be adjusted in thin-layer flow through cells by selection of cell gaskets of varying thickness. Thus, at fixed flow a decrease in cell gasket thickness is equivalent to an increase in fluid velocity across the electrode surface, which in turn leads directly to increases in measured currents. Our observations regarding N-chlorodimethylamine peak area as a function of cell gasket thickness, as shown in Figure 22, were in agreement with the expected trends. Sensitivity, as measured by the slope of the fitted regression lines, increased approximately two- and five-fold when going from a 381 µm cell gasket to ones 127 and 51 µm thick, respectively. Unfortunate-
Figure 22. Effect of cell gasket thickness upon analysis of N-chlorodimethylamine standards.

![Gasket Thickness Graph]

Gasket Thickness

<table>
<thead>
<tr>
<th>Thickness (um)</th>
<th>Log S</th>
<th>n</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>381</td>
<td>11.55</td>
<td>1.06</td>
<td>0.9996</td>
</tr>
<tr>
<td>127</td>
<td>11.93</td>
<td>1.09</td>
<td>0.9973</td>
</tr>
<tr>
<td>51</td>
<td>12.27</td>
<td>1.16</td>
<td>0.9897</td>
</tr>
</tbody>
</table>

Log Peak Area

Log Conc. (CH₃)₂NCl (M)

Regression Model

\[ R = S \cdot C^n \]

Conditions as follows: (a) HPLC mobile phase 30:70 CH₃CN/pH 6.2 water at 1.0 ml/min; (b) post-column reagent at 0.5 ml/min; (c) GC working electrode at -0.100 V vs. Ag/AgCl.

Consequently, concurrent with the increase in analytical signal was an increase in noise so that overall no net S:N improvement was achieved by going to smaller detector cell volumes. These findings are in agreement with those reported elsewhere (Brunt and Bruins 1979 and Stulik and Pacakova 1985).

**Standard Post-Column Reaction Detector Conditions.** Based upon the results presented in the preceding sections, we adopted a set of standard operating conditions for the remaining experimental work reported here. These conditions, shown in Table 10, were selected to strike a balance among several important detector performance variables, including selectivity and detectability, in terms of both the absolute mass injected as well as dynamic range.
Table 10. Standard operating conditions for analysis of N-chloramines by post-column reaction detection

1. HPLC: Mobile phase CH₃CN/H₂O as required, 1.0 ml/min.
2. Post-Column Reagent: 0.09 M KI, pH 4.0 ± 0.1 (0.294 M total Ac⁻/HAc), 0.5 ml/min.
3. Electrochemical Detector: Glassy carbon electrode operated at -0.100 V vs Ag/AgCl.
4. Post-Column Reactor: 10 m x 0.5 mm I.D. knitted open tubular PTFE reactor, 24 s residence time.

Post-Column Reaction Detector Evaluation Employing Model Compounds

**Precision and Sensitivity.** Data presented in Table 11 show the level of precision obtained from multiple measurements of several N-chloramino compounds by post-column reaction detection. At ng injection levels, percent relative standard deviations of absolute peak area measurements were on the order of a few percent. The precision of these measurements are comparable to those provided by other detection methods commonly employed with HPLC.

Chromatograms presented in Figure 23 show detections of less than 0.6 ng quantities of NH₃Cl, N-chloromethylamine and N-chlorodimethylamine at S:N values greater than 10. Reductions in post-column iodide concentration, and thus short-term detector noise, did not significantly improve detection limits. It appears that for these model compounds and this system, detection limits attainable while employing high post-column iodide concentration, a condition where noise originating from iodide is limiting, roughly coincide with those attainable when utilizing low iodide concentrations, where the kinetics of post-column reaction become detection limiting. Assuming an injection volume of 50 µl and detection limits on the order of 200 pg of injected material, the post-column reaction detector described here allows determinations of aqueous N-chloramines at 10⁻⁸ M without any sample pretreatment procedures.
Table 11. Percent relative standard deviation of absolute peak area measurements for several model N-chloramines

<table>
<thead>
<tr>
<th>Compound</th>
<th>NH$_2$Cl</th>
<th>(CH$_3$)NHCl</th>
<th>(CH$_3$)$_2$NCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>ng injected</td>
<td>5.9</td>
<td>1.9</td>
<td>9.2</td>
</tr>
<tr>
<td>% RSD</td>
<td>3.1</td>
<td>2.3</td>
<td>3.2</td>
</tr>
</tbody>
</table>

**Calibration.** Linearity of post-column reaction detector response was examined by constructing calibration curves for a number of model N-chloramines. Results of calibrations for several N-chloramines over concentrations ranges greater than 3 orders of magnitude and using a simple nonlinear model are shown in Figure 24. Constants were estimated from log-transformed data by simple linear regression techniques. For each of the compounds examined, calibrations employing this model were successful with high correlation coefficients and small residual errors over the full concentration range evaluated.

In Figure 25 linearity plots derived from data generated during analyses of N-chlorodimethylamine, using the data shown in Figure 24, are presented. Response data were fitted to both simple linear and nonlinear models in a manner analogous to that described by Dorschel et al. (1989). The general forms of the models are shown below:

**Linear Model:** $R = SC$

**Nonlinear Model:** $R = SC^n$

The terms in these equations are as follows: $R$ equals the detector response; $S$ a constant describing detector sensitivity for the analyte of interest; $C$ the analyte concentration and $n$ a constant related to the curvature of detector response as function of concentration.

The simple linear model in essence constitutes a single point calibration wherein the linear range is determined by the concentrations where values of $S$ remain within a defined tolerance, chosen in the case of Figure 25 as $\pm$ 5 percent of the mean value.
Figure 23. HPLC/EC chromatograms for detection of model N-chloramines at low levels: (A) 0.59 ng NH₂Cl; (B) 0.38 ng N-chloromethylamine; (C) 0.37 ng N-chlorodimethylamine.

Conditions as follows: (a) HPLC mobile phase CH₃CN/pH 6.2 water at 1.0 ml/min; (b) Post-column reagent 0.09 M KI, pH 4 at 0.5 ml/min; (c) GC working electrode at -0.100 V vs Ag/AgCl.
Figure 24. Calibration curves for analyses of model N-chloramines by HPLC/post-column reaction detection.

Conditions as follows: (a) HPLC mobile phase CH$_3$CN:pH 6.2 water at 1.0 ml/min; (b) Post-column reagent 0.09 M KI, pH 4 at 0.5 ml/min; (c) GC working electrode at -0.100 V vs Ag/AgCl. Observed peak areas shown by data markers; predicted values by regression lines.

The nonlinear model, in contrast, describes a calibration in which sensitivity is not constant but rather a function of concentration. The value of $n$, the constant describing curvature of detector response versus concentration, is empirically derived from the regressed log-transformed data.

The data in Figure 25 clearly demonstrate the superiority of the nonlinear calibration model. Under conditions slightly less stringent than those shown in the figure (e.g. an allowable deviation of ±10 percent), detector response can be accurately described by the nonlinear model over the full concentration range examined, in this case over 3 orders of magnitude. In contrast, the simple linear model fails to describe detector response over a concentration range much greater than an order of magnitude. Under
Figure 25. Linearity plots for analysis of N-chlorodimethylamine by post-column reaction detection employing simple linear (A) and nonlinear (B) calibration models. Conditions as described in Figure 10.
most circumstances, results derived from calibrations for other N-chloramines were very similar to that shown for N-chlorodimethylamine in Figure 25. There were instances, however, when use of the nonlinear model failed to significantly improve description of detector response.

**Detector Durability.** The often expressed concern of electrochemical detectors regarding changes in their absolute detector response (Kissinger 1984), induced by alterations occurring at the electrode surface, was found to be unwarranted in this work. Under the conditions employed in these experiments (dilute N-chloramine concentrations, aqueous/organic mobile phases, etc.), absolute detector response at the glassy carbon electrode was found to be highly reproducible over periods of months. Only a very slight decrease in detector response was observed to occur with the passage of time. The need for electrode repolishing was found to be very infrequent.

**Application to Analysis of an N-chloramine Mixture Subjected to Breakpoint Chlorination**

The HPLC/post-column reaction detection system was next employed to analyze chlorinated solutions containing NH₃, methylamine, ethylamine and dimethylamine. In the solution, each amino compound was present at 100 µg/l prior to chlorination. The objective of this experiment was to determine whether the method could resolve components comprising the total residual chlorine pool solution at points along the breakpoint curve. Solution composition and chlorine dosages were selected so that typical breakpoint chemistry could be probed. Amine concentrations were selected so that concentrations of N-chloramines formed by chlorination would be at the ppb level. In this experiment analyses by HPLC were limited to singly substituted N-chloramines. No efforts were made to quantify N,N-dichloramine formation and concentration. For purposes of comparison and to serve as an independent reference technique, total residual chlorine concentrations were measured by the forward amperometric titration procedure.

Measured concentrations of N-chloramines and total chlorine residuals as a function of Cl:N molar ratio are shown in Figure 26. Concentrations in this figure are expressed on a Cl₂ equivalent weight basis, a convention common to the water and wastewater treatment industry. Levels of N-chloromethylamine and N-chloroethylamine were found to increase with chlorine dose to the chloramine maximum (molar chlorine-to-nitrogen ratio = 1), whereafter they decreased, eventually reaching zero near the breakpoint (molar chlorine-to-nitrogen ratio = 2). The concentration profile of NH₂Cl was similar except that the maximum concentration occurred at a slightly higher dosage and levels failed to reach zero at the breakpoint. Levels of N-chlorodimethylamine increased with chlorine dosage, eventually maximizing at a molar chlorine-to-nitrogen ratio slightly
Figure 26. Concentrations of individual N-chloramines (A) and total chlorine residuals (B) in a model solution chlorinated at points across the breakpoint curve.

A.

Concentrations of individual N-chloramines (A) and total chlorine residuals (B) in a model solution chlorinated at points across the breakpoint curve.

B.

 Conditions as follows: (a) HPLC mobile phase 30:70 CH₃CN/water at 1.0 ml/min; (b) post-column reagent 0.09 M KI, pH 4 at 0.5 ml/min; (c) injection volume 50 µl; (d) GC working electrode at -0.100 V vs Ag/AgCl; (e) results are mean value of duplicate determinations; (f) HPLC total considers only singly substituted N-chloro compounds.
greater than one. Concentrations of N-chlorodimethylamine remained stable with further chlorine additions. Also presented in Figure 26 are total chlorine residuals, as µg/L as Cl₂, determined by HPLC and amperometric titration. Totals measured by HPLC in this instance are limited to singly substituted N-chloramines which were summed following individual measurement. Results for amperometric titration, in contrast, measure all forms of oxidant, including N,N-dichloramines and any free chlorine which may be present.

The concentration profiles for model N-chloramines depicted in Figure 26 are in accordance with known breakpoint chemistry involving reactions of aqueous chlorine with nitrogen containing compounds (Morris 1967; Palin 1975 and White 1986). The concentration profile for NH₂Cl displays the classic rise and fall with increasing chlorine dosage. An exception in this case, however, is the fact that concentrations of NH₂Cl fail to reach zero at the breakpoint dosage and beyond. The reason for this discrepancy is unclear at this time.

Due to the unavailability of analytical methods for analysis of organic N-chloramines, detailed studies describing their behavior across the breakpoint curve have not been reported. Known chlorination chemistry for primary organic amines suggests, however, that concentration profiles should mimic that of NH₂Cl. The data of Figure 26 show this to be the case. The earlier maximum concentration observed for N-chloromethylamine and N-chloroethylamine relative to NH₂Cl is consistent with the relative rates of reaction of the respective precursor amines with aqueous chlorine (Morris 1967).

In contrast to the other N-chloramines, concentrations of N-chlorodimethylamine increased steadily until reaching a maximum which remained constant with further chlorine additions. This behavior is consistent with that expected of secondary organic alkylamines since their reactions with aqueous chlorine terminate in production of only singly substituted N-chloro products. The N-chloro derivatives of dialkylamines have been reported to be stable in the presence of free chlorine (Scully and Bempong 1980; Scully et al., 1984b).

Total chlorine residuals derived by summing HPLC measured concentrations and totals measured by amperometric titration followed expected trends. At low dosages, totals measured by HPLC and amperometric titration were in agreement and roughly equal to the chlorine dosage. At dosages past a molar chlorine-to-nitrogen ratio of 1, totals measured by amperometric titration were consistently higher since this method does not discriminate among components of the total chlorine residual pool as the HPLC technique does (e.g. in this case measuring only the singly substituted N-chloramines) but rather measures all forms simultaneously. Total chlorine residuals measured by amperometric titration at these dosages deviated from the applied dosage due to the occurrence of chlorine demanding breakpoint reactions for these compounds.
Conclusions of HPLC with Electrochemical Detection Method
Development

The method reported here constitutes the first detailed description of an analytical method for the direct determination of aqueous N-chloramines at trace levels. The mild conditions of HPLC were combined with post-column reaction detection to provide a highly selective and sensitive detection system. Aqueous N-chloramines can be detected directly at concentrations down to $10^{-8}$ M without the need for potentially sample altering concentration or pretreatment procedures. In addition, the kinetics of post-column reaction are sufficiently fast to allow calibrations for model compounds over concentration ranges in excess of 3 orders of magnitude with minimum error. Reproducibility of absolute detector response over time was excellent with electrode polishings required very infrequently. This method provides an avenue to explore the chemistry and possible environmental health consequences associated with N-chloramines that is otherwise inaccessible through existing analytical methods.

ANALYSIS OF N-CHLORAMINES IN CHLORINATED WASTEWATER AND SURFACE WATER BY ON-LINE ENRICHMENT HPLC AND POST-COLUMN REACTION ELECTROCHEMICAL DETECTION

Analysis of N-Chloramines by On-Line Enrichment

Model aliphatic N-chloramines were used to evaluate the effectiveness of the on-line enrichment technique. For 9.2 ng injections of N-chlorodimethylamine, a compound moderately retained by the enrichment column, peak areas and heights were observed to be independent of injection volume over the range of 50 to 2000 ul. Figure 27 shows a chromatogram obtained from analysis of a dilute aliphatic N-chloramine mixture by the on-line enrichment technique. Even for relatively poorly retained compounds, such as NH$_2$Cl and N-chloromethylamine for example, it can be seen that peak shapes remained relatively unaffected for 2 ml injection volumes.

The combination of HPLC with on-line enrichment and post-column reaction electrochemical detection provides a method for N-chloramine analysis that is extremely sensitive and highly specific. Detection limits (S:N > 2.5) for the N-chloramines shown in Figure 27 are less than 1 ppb (approximately $10^{-9}$ M concentrations) for 2 ml injection volumes. For very hydrophobic N-chloropiperididine, a compound strongly retained by the enrichment column, injection volumes of 10 ml provide detection limits of approximately 0.1 ppb ($8 \times 10^{-10}$ M). These very low detection limits are attainable without the need
Figure 27. Analysis of an aliphatic N-chloramine mixture by on-line enrichment HPLC post-column reaction detection.

Conditions as follows: (a) total residual chlorine $43 \pm 2 \mu M$ as $\text{Cl}_2$; (b) HPLC mobile phases A and B 10:90 and 70:30 CH$_3$CN/pH 6.2 water, respectively; linear gradient of 20 to 70% B in 20 minutes; flow 1.0 ml/min; (c) Econosil 25 x 0.46 cm I.D. C$_{18}$ column; (d) post-column reagent pH 4.0, 0.09 M KI at 0.5 ml/min; (e) enrichment mobile phase CDFW at 1.0 ml/min; (f) inj. volume 2 ml; (g) glassy carbon working electrode at -0.100 V vs Ag/AgCl; (h) N-chloro derivatives of the following amines: (1) NH$_3$, (2) methylamine, (3) ethylamine, (4) NH$_2$Cl, (5) dimethylamine, (6) isopropylamine, (7) propylamine, (8) tert-butylamine, (9) butylamine, (10) diethylamine (11) piperidine.
Table 12. Percent relative standard deviations of absolute peak area and height measurements for analysis of several model N-chloramines by the on-line enrichment technique

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (ppb)</td>
<td>(M)</td>
</tr>
<tr>
<td>N-chlorodimethylamine</td>
<td>9 5.8</td>
<td>7 x 10^{-8}</td>
</tr>
<tr>
<td></td>
<td>6 33</td>
<td>4 x 10^{-7}</td>
</tr>
<tr>
<td>N-chloropiperidine</td>
<td>8 16</td>
<td>1 x 10^{-7}</td>
</tr>
</tbody>
</table>

for potentially disruptive sample handling operations (sample extractions, concentrations, etc.) by direct analyses under the inherently mild conditions of HPLC.

Levels of precision measured for analysis of several model compounds are shown in Table 12. While not as reproducible as direct injections, relative standard deviations for measurements by this technique are nonetheless very good for these extremely dilute concentrations. Detector response was calibrated for several N-chloramines employing the on-line enrichment technique. For the compounds examined, responses were successfully calibrated for concentrations covering several orders of magnitude using the simple nonlinear model (R = SC^n, r^2 > 0.99) discussed in the previous section. Agreement between predicted and observed detector responses for very dilute N-chloramine concentrations demonstrated once again that the separation media did not exert a significant oxidant demand in this system.

The adsorptive capacity of the on-line enrichment column as it is related to organic substituent patterns in N-chloramines was next examined. A solution very similar to that shown in Figure 27 was analyzed as a function of injection volume. The solution of N-chloro aliphatic amines was, however, allowed to equilibrate for several days so that chlorine transfer reactions would be minimized during the course of this experiment. Results of these measurements are shown in Figure 28. Monochloramine (data not shown) and the N-chloro derivatives of methylamine (data not shown) and ethylamine were the least retained compounds. Injection volumes greater than two ml exceeded the adsorptive capacity of the enrichment column for these relatively polar compounds in this solution. In contrast, injection volumes for the highly hydrophobic N-chloro...
Figure 28. Relative peak areas of N-chloro aliphatic amines as a function of injection volume in the on-line enrichment technique.

![Graph showing the relative peak areas of N-chloro aliphatic amines as a function of injection volume.](image)

Analytical conditions as described in Figure 27. Total residual chlorine of mixture $2.0 \pm 0.3 \mu M$ as $Cl_2$, formed at pH 8 and a molar $[N]:[Cl]$ ratio of 13. Results are mean values of duplicate determinations.

derivatives of tert-butylamine, n-butylamine, diethylamine and piperidine could be as high as 10 ml without breakthrough from the enrichment column. These data reflect the marked degree to which chlorine substitution at nitrogen affects the polarity of these otherwise very hydrophilic amino compounds.

To assist the reader in assessing the adsorptive capacity of the enrichment column, please consider the following information regarding the composition of the N-chloramine solution employed in these measurements. The total residual chlorine concentration of
this equilibrated solution was determined to be $2.0 \pm 0.3 \mu M$. This residual was in turn distributed among, at a minimum, 11 individual N-chloramino species (see Figure 27). Concentrations for each N-chloramine were thus roughly on the order of $10^{-7} M$. Assuming an N-chloramino compound molecular weight of 100 g/mole, this concentration translates to approximately 10 ppb or 7 ppb as Cl₂. For a two ml injection volume, these concentrations in turn correspond to approximately 0.2 μg mass loadings of the enrichment column per individual N-chloramino species.

Applications to Model Compound Solutions

It is has been well established that NH₂Cl can behave as a potent chlorinating agent via chlorine transfer reactions with organic nitrogenous compounds (Higuchi and Hasegawa 1965; Synder and Margerum 1982; Isaac and Morris 1983a and 1985). In the practice of water disinfection by chloramination or preammoniation with chlorination, chlorine transfer reactions are of particular concern since organic N-chloramines are weak disinfectants (Feng 1966). In addition, the situation is further worsened by the fact that the organic N-chloramines are known to positively interfere in determinations of germicidal NH₂Cl by standard analytical methods (Wolfe et al 1985; Scully 1985). To date, research in this area has been limited to concentrated solutions (on the order of $10^{-5} M$) of an amine and N-chloramine since measurements of concentration have been performed solely by UV spectrophotometry.

Data presented in Figure 29 demonstrates the potential usefulness of the on-line enrichment HPLC/EC technique as a tool to study aspects of combined residual chlorine chemistry heretofore inaccessible. In this figure distribution of active chlorine among a mixture of N-chloramines, the precursor amines of which were each initially present at 10 ppm, is followed as a function of time following chlorination. Shortly after chlorination, active chlorine was found in kinetically determined product distributions, with residual chlorine composed primarily by N-chloro primary organic amines and NH₂Cl. With the passage time, however, distributions were observed to shift towards equilibrium controlled conditions, where distribution of active chlorine became increasingly concentrated in organic secondary N-chloramines. In fact, at eleven days following chlorination, N-chlorodimethylamine and N-chloropiperidine accounted for 70 percent of the solution's active chlorine content. Loss of total residual chlorine from that originally measured in this solution was 30 percent and 35 percent as measured by amperometric titration and the HPLC/EC methods, respectively.

Figure 30 presents chromatograms obtained from analyses of a chlorinated mixture of 22 amino acids as a function of time following chlorination. Conditions employed were such that formation of singly substituted N-chloro derivatives was strongly favored. Unlike the N-chloro aliphatic amines above, chromatograms for the N-chloro amino acids demonstrated their instability and rapid degradation under these conditions. Within 5 hours,
Figure 29. Kinetic versus thermodynamic product distributions from chlorination of an aliphatic amine mixture.

Analytical conditions as described in Figure 27. Total residual chlorine of mixture $43.0 \pm 2 \mu M$ as Cl$_2$; formed at pH 8 and a molar [N]:[Cl] ratio of 13.

degradation appeared to be nearly complete. Based upon HPLC/EC peak area measurements, the half-life of the combined residual chlorine in this solution was determined to be 63 minutes. These results are consistent with the known instability of these compounds (Stanbro and Smith 1979; Saunier and Selleck 1979; Hand et al., 1983; Hand and Margerum 1983; Isaac and Morris 1983b; LeCloirec and Martin 1985; and LeCloirec et al., 1985). Because of their transiency, N-chloro free amino acids are likely of minor consequence in determining the health effects of combined chlorine residuals.
Figure 30. HPLC/EC chromatogram of N-chloramino acid mixture at (A) 5, (B) 50, (C) 180 and (D) 360 min. following chlorination.

Analytical conditions as follows: (a) HPLC mobile phases A and B 0:100 and 50:50 CH₃CN/pH 6.2 water, respectively; linear gradient of 0% to 100% B in 60 minutes; flow 0.5 ml/min; (b) Econosil 25 x 0.46 cm I.D. C₁₈ column; (c) post-column reagent pH 4.0, 0.09 M KI at 0.25 ml/min; (d) inj. volume 50 ul; (e) glassy carbon working electrode at -0.100 V vs Ag/AgCl. Chlorination conditions as follows: pH 7.0; molar [N]:[Cl] ratio > 10; total residual chlorine approximately 3 x 10⁻⁴ M as Cl₂.

These compounds may, however, interfere in measurements of NH₂Cl at short time intervals following chlorination.

Application to Chlorinated Primary Wastewater

We next applied the on-line enrichment/HPLC post-column reaction detection technique to the analysis of chlorinated primary effluent wastewater, a matrix in which concentra-
tions of organic nitrogen compounds were likely to be relatively high. For the plant sampled, concentrations of NH$_4$-N and Total Kjeldahl-N (TKN) measured during routine monitoring were on the order of 15-20 and 30 mg/l as N, respectively. The breakpoint curve resulting from laboratory chlorinations of this effluent is presented in Figure 31. For the effluent described here, the N-chloramine maximum and breakpoint were observed at dosages of approximately 125 and 175 mg/l, respectively.

Samples of the chlorinated primary effluent were examined by HPLC/EC at different dosages to determine if the method could successfully resolve concentration profiles of compounds comprising the conventionally determined composite breakpoint curve. These results are presented in Figure 31. From an examination of these chromatograms, two observations are most apparent. First, as evidenced by a comparison of chromatogram A with chromatograms B through E, this method shows excellent selectivity for detection of chlorine produced oxidants in this complex matrix. With the exception of a minor peak occurring at the void volume, there were no significant peaks observed from analysis of a 2 ml injection of this very organically enriched and complex unchlorinated sample matrix. This excellent detection selectivity originates exclusively from the post-column reaction electrochemical detector employed.

The second most striking observation concerning these analyses is that, at dosages below the breakpoint, the total chlorine residual of the composite breakpoint curve is comprised nearly entirely of 2 components. Based upon retention time matches with analyses of synthesized standards, these compounds are tentatively identified as NH$_2$Cl and NHCl$_2$. A third minor peak eluting at approximately 26 minutes most likely is NCl$_3$. These tentative identifications are strongly supported by the known predominance of NH$_4$-N in the reduced-N content of domestic wastewaters (Metcalf and Eddy 1979; White 1986), the measurement of NH$_2$Cl residues by the DPD/FAS procedure and the rise and fall of their concentrations at points across the breakpoint curve.

Because all N-chloramines are detected as their iodine product following post-column reaction with iodide, detector response for individual compounds will be similar and solely a function of their active chlorine content. This assumes, however, uniformity concerning the extent of post-column reaction for detected compounds. In this sense then, total HPLC/EC peak area will measure the total chlorine content of a sample in a manner analogous to that measured by current standard methods. The one possible exception, however, is that not all combined chlorine residual is necessarily chromatographable under the conditions used in these experiments.

The parallelism exhibited by the amperometric titration and HPLC/EC measured concentration profiles demonstrated in Figure 32 is quite different from that seen previously in Figure 26B of this report. In the latter case, concentrations measured by the HPLC/EC technique were limited to only singly substituted N-chloro model compounds. Separations were isocratic and no attempt was made to measure N,N-dichloro species as they were formed. Consequently, as chlorine dosages exceeded
Figure 31. Primary wastewater effluent breakpoint curve and HPLC/EC chromatograms obtained at chlorine dosages: (A) 0, (B) 33, (C) 65, (D) 125 and (E) 167 mg/l (pH 7, 1 hour contact time at ambient temperature in darkness).

Analytical conditions as follows: (a) HPLC mobile phases A and B 5:95 and 70:30 CH₃CN/pH 6.2 water, respectively; linear gradient of 30% to 73% B in 23 minutes; flow 1.0 ml/min; (c) Econosil 25 x 0.46 cm I.D. C₁₈ column; (d) post-column reagent pH 4.0, 0.09 M KI at 0.50 ml/min; (e) enrichment mobile phase CDFW at 1.0 ml/min; (f) inj. volume 2 ml; (g) glassy carbon working electrode at -0.100 V vs Ag/AgCl. Breakpoint curve residuals are mean values of duplicate determinations.
that required to reach the chloramine maximum, the concentration profiles measured by the two techniques became divergent. In the case of Figure 32, chromatographic conditions were such (gradient elution) that all species, including any N,N-dichloro species that may have been formed, were measured. Thus, it is not unexpected that in this instance the two concentration profiles should closely mimic one another.

A comparison of total HPLC/EC peak area versus total chlorine residual as measured by amperometric titration is shown in Figure 32. The two curves in this figure are nearly identical. This observation supports the application of the method we report here to analysis of combined chlorine residuals in this sample. The excellent agreement
Figure 33. Concentration profiles of NH$_2$Cl, NHCl$_2$, and total peak area in chlorinated primary wastewater as determined by HPLC/EC.

Analytical conditions as described in Figure 31.

observed between the two curves indicates that the method is either analyzing most of combined chlorine residuals in this sample or that a nonchromatographable portion, if it does exist, remains relatively constant and small in magnitude across the breakpoint curve. Given our present knowledge of nitrogen chlorine chemistry, this latter situation appears unlikely.

Concentration profiles for tentatively identified inorganic N-chloramines as measured by HPLC/EC are presented in Figure 33. It was somewhat surprising, for this sample chlorinated at pH 7, to have observed the levels of NHCl$_2$ formed at dosages well before the N-chloramine maximum. Similarly, the nearness of the chloramine maximum and breakpoint (molar chlorine-to-nitrogen ratio of 1.36) provided further evidence supporting the somewhat unusual inorganic N-chloramine concentration profiles presented in
Figure 33. One must remember, however, that for this sample the rate of NHCl₂ formation becomes accelerated due to the high concentrations of NH₂Cl formed shortly following chlorination. Under these conditions, formation of NH₂Cl by the disproportionation reaction, which can be viewed as a chlorine transfer reaction, is competitive with its formation by chlorination of NH₂Cl by HOCl (Morris 1967; Margerum et al., 1978; Synder and Margerum 1982; Isaac and Morris 1985). That the NH₂Cl measured by HPLC/EC is not an artifact resulting from reactions occurring within the enrichment and separation media (e.g. disproportionation) is supported by two observations. First, the results of our earlier experiments concerning on-column changes in species composition and secondly our observation of only single chromatographic peaks for injections of large concentrations of NH₂Cl under similar analytical conditions.

The predominance of NH₃-N within the pool of reduced nitrogen compounds presents problems in detecting the more dilute organic N-chloramines by this method. The injection volumes required to maximize detection of the dilute organic N-chloramines necessarily result in injections of large quantities of inorganic N-chloramines. This in turn causes column overloading which changes retention times, degrades chromatographic resolution and distorts the resulting detector response scale. To some extent, this problem can be circumvented by displacing poorly retained NH₃Cl from the enrichment column prior to injection. The disadvantage inherent to this approach is that organic N-chloramines of similar polarity are similarly displaced.

Chromatograms obtained from analyses of identical wastewater samples chlorinated to the chloramine maximum but differing with respect to injection technique by the on-line enrichment procedure are shown in Figure 34. Pretreating the enrichment column by washing with several ml of chlorine-demand-free water prior to injection reduces column overloading. Treatment in this manner provides greater chromatographic resolution for the minor components, especially those strongly retained by the enrichment column. Based on relative retention times (RRT) matches (relative to NH₃Cl) with a standard mixture run before and after the analysis of this sample, the N-chloramines of methylamine, ethylamine, isopropylamine, tert-butylamine and n-butylamine were tentatively identified in this sample. Differences in RRT between identified peaks and standards were less than 1 percent.

Application to Chlorinated Wastewater Final Effluent

We next examined samples of a secondary wastewater effluent (advanced secondary treatment) by the on-line enrichment HPLC/EC analytical method. Concentrations of NH₄-N and Total Kjeldahl-N (TKN) typical of this effluent are 2-3 and 3-5 mg/l as N, respectively (routine plant monitoring data). A breakpoint curve and chromatograms resulting from analyses of this effluent sample are shown in Figure 35. An examination of the chromatograms quickly reveals the efficiency by which the treatment process
Figure 34. HPLC/EC chromatograms for primary wastewater chlorinated to the N-chloramine maximum (167 mg/l chlorine dosage).

Conditions in (B) as described in Figure 31. Conditions in (A) identical except that the enrichment column was washed with 4 ml of CDFW prior to injection.

removes reduced-N, the N-chloramine precursor material. The early and later eluting large peaks in these chromatograms correspond to NH$_2$Cl and NHCl$_2$, respectively. The retention time of the small peak preceding NH$_2$Cl in each chromatogram is similar to that of N-chloramino acids, many of which coelute near the void volume under these chromatographic conditions.

As observed during analyses of the chlorinated primary effluent, the inorganic N-chloramines overwhelmingly dominated the composition of combined chlorine residual in this matrix. Concentration profiles for inorganic N-chloramines measured in this effluent by HPLC/EC are shown in Figure 36. Relative to the chlorinated primary effluent examined previously, formation of NHCl$_2$ in this final effluent occurs at higher molar chlorine-to-nitrogen ratios and comprises significantly less of the pre-breakpoint

94
Figure 35. Secondary wastewater effluent breakpoint curve and HPLC/EC chromatograms obtained at chlorine dosages: (A) 0, (B) 1.18, (C) 2.92 and (D) 4.10 mg/l (pH 7, 1 hour contact time at ambient temperature in darkness).

Analytical conditions as described in Figure 31. Breakpoint curve residuals are mean values of duplicate determinations.

measured combined chlorine residual. This behavior is consistent with the lower concentrations of NH₂Cl initially formed in this water shortly following chlorination. The relatively constant levels of NH₂Cl measured by the DPD-FAS procedure at dosages beyond the breakpoint demonstrates the vulnerability of this method of NH₂Cl analysis to positive interferences. Similar results have been observed in chlorinated wastewater effluents by Saunier and Selleck (1979).

Application to Chlorinated Surface Waters

Surface waters from lakes which at present (High Rock Lake) or potentially may (Jordan Lake) serve as drinking water sources were next analyzed to examine the production of N-chloramines in these systems. Results from analyses of chlorinated High Rock Lake raw water samples are presented in Figure 37. Chlorinations of this water at varying dosages did not result in a breakpoint curve morphology typical of systems dominated by
Figure 36. Concentration profiles of NH$_2$Cl, NHCl$_2$ and total peak area in chlorinated advanced secondary treated wastewater as determined by HPLC/EC.

Analytical conditions as described in Figure 31.

NH$_4^+$-N. The high dissolved organic carbon content of this water (5.7 mg/l), collected during late spring (June 14, 1990), in conjunction with the visual abundance of algal biomass were indicative of the high level of biological activity in this matrix at the time of sampling. Under such conditions, it was not unexpected that levels of NH$_3$-N were possibly low. At this high level of activity, much of the aqueous nitrogen is tied up in biomass and levels of free dissolved NH$_3$-N are consequently very low.

Results from analyses of this surface water are very similar to that observed for the secondary effluent described above. The response arising from the inorganic N-chloramines dominated these HPLC/EC chromatograms. The peak preceding NH$_2$Cl, also observed in chlorinated secondary effluents, had a retention time consistent with a composition by N-chloramino acids. In chromatograms C and D, a few additional minor products can be seen. The identities of compound(s) forming these minor peaks unfortunately remain unknown. Concentration profiles for inorganic N-chloramines as
Figure 37. High Rock Lake surface water breakpoint curve and HPLC/EC chromatograms obtained at chlorine dosages: (A) 0, (B) 0.94, (C) 1.67 and (D) 2.21 mg/l (pH 7, 1 hour contact time at ambient temperature in darkness).

Analytical conditions as described in Figure 31. Breakpoint curve residuals are mean values of duplicate determinations.

measured by HPLC/EC, shown in Figure 38, were very similar to those observed in the chlorinated secondary effluent.

To verify that we could detect N-chloramines at the ppb level, aliquots of this surface water were spiked with a mixture of aliphatic amines and NH₃, chlorinated and analyzed by the on-line enrichment HPLC/EC method. The concentration of each amine in the spiking solution was equal (w/v). Chromatograms obtained from analyses of lake water samples spiked with the amino compounds at 20 ppb and chlorinated at 2 different dosages (pH 7, 1 hour contact time) are shown in Figure 39. At a chlorine dosage of 0.94 mg/l (A), singly substituted N-chloro derivatives of each of the spiked amino compounds were formed in easily detectable quantities. Relative N-chloro product distributions mimicked those observed shortly after chlorination of the model solution of Figure 27.
Figure 38. Concentration profiles of \( \text{NH}_2\text{Cl}, \text{NHCl}_2 \) and total peak area in chlorinated advanced secondary treated wastewater as determined by HPLC/EC.

![Graph showing concentration profiles of \( \text{NH}_2\text{Cl}, \text{NHCl}_2 \) and total peak area.]

Analytical conditions as described in Figure 31.

For an identical sample (spiked raw water) (B), relative product distributions changed significantly when the chlorine dose was increased to 1.67 mg/l. Levels of N-chloro primary organic amines and \( \text{NH}_2\text{Cl} \) decreased from 7 to 91 percent and 6 percent, respectively. Peak areas for N-chlorodimethylamine and N-chloropiperidine, on the other hand, remained unchanged while that for \( \text{NHCl}_2 \) increased greater than threefold. Interestingly, the peak area of N-chlorodiethylamine decreased by 46 percent. With the exception of the latter, these trends concerning changes in relative product distributions were consistent with known nitrogen-chlorine chemistry.

The HPLC/EC chromatogram obtained from analysis of a chlorinated High Rock Lake water sample spiked with the amino compounds at 4 ppb is presented in Figure 40. The
Figure 39. HPLC/EC chromatograms for High Rock Lake samples spiked by an amine mixture and chlorinated (pH 7, 1 hour contact time) at (A) 0.94 and (B) 1.67 mg/l.

Amine spike 20 ppb (w/v) for each compound. Analytical conditions and peak identities as described in Figure 27.
N-chloramines of each of the spiked amino compounds were detected at a chlorine dosage of 0.68 mg/l. Peaks detected in this figure likely resulted from only partial chlorination of each of the amines present. At lower and higher chlorine dosages fewer peaks were detected. For a similar lake water sample spiked at 2 ppb, singly substituted N-chloro derivatives of each amine were also detected.

Results obtained from these spiking experiments demonstrated the excellent sensitivity and specificity of the method and its ability to resolve components comprising the conventionally measured total residual chlorine at very dilute concentrations. By comparison with peak heights observed in these chromatograms, chromatographable N-chloramines in chlorinated High Rock Lake water, if formed, were present at lower than ppb levels (parts per trillion).

Figure 40. HPLC/EC chromatograms for High Rock Lake samples spiked by an amine mixture and chlorinated (pH 7, 1 hour contact time) at 0.68 mg/l.

Amine spike at 4 ppb (w/v) for each compound. Analytical conditions and peak identities as described in Figure 27.
Chlorination at pH 7.0 for 1 hour at ambient temperature in darkness. Chlorine residual concentrations are mean values from duplicate determinations.

Results from analyses of chlorinated Jordan Lake samples were very similar to those reported above for High Rock Lake. This water, when sampled on June 29, 1990, also was high in DOC (8.2 ± 0.1 mg/l) and failed to produce a breakpoint curve (see Figure 41) typical of systems dominated by NH₄⁺-N. Detector signal from inorganic N-chloramines again dominated the HPLC/EC chromatograms of these samples. A few small peaks were observed at chlorine dosages before the breakpoint. As seen above for the High Rock Lake samples, a peak preceding NH₂Cl was observed except that for this water its size relative to the inorganic N-chloramines was somewhat larger.

At chlorine dosages sufficient to produce measured free chlorine residuals, detection of NH₂Cl suggested that the DPD-FAS method was vulnerable to false positive interfer-
Table 13. Comparison of NH$_2$Cl concentrations measured by HPLC/EC and the DPD-FAS procedures in chlorinated Jordan Lake water

<table>
<thead>
<tr>
<th>Dose</th>
<th>Amperometric Total Residual Chlorine</th>
<th>Free Available Chlorine</th>
<th>NH$_2$Cl</th>
<th>NH$_2$Cl</th>
<th>NHCl$_2$*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DPD-FAS</td>
<td>HPLC/EC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.01</td>
<td>0.24</td>
<td>0.0</td>
<td>0.10</td>
<td>0.038</td>
<td>0.0</td>
</tr>
<tr>
<td>1.51</td>
<td>0.26</td>
<td>0.0</td>
<td>0.20</td>
<td>0.113</td>
<td>0.044</td>
</tr>
<tr>
<td>2.02</td>
<td>0.22</td>
<td>0.0</td>
<td>0.18</td>
<td>0.052</td>
<td>0.112</td>
</tr>
<tr>
<td>3.01</td>
<td>0.379</td>
<td>0.16</td>
<td>0.07</td>
<td>0.026</td>
<td>0.051</td>
</tr>
</tbody>
</table>

* Concentration based on RFs of NH$_2$Cl and NHCl$_2$ being equal on a mg/l as Cl$_2$ basis.

ences in this system. To investigate this further, post-column reaction detector response was calibrated for analysis of NH$_2$Cl (10 to 300 µg/l as Cl$_2$; 3 points in triplicate; $r^2 = 0.998$). Levels of NH$_2$Cl measured by HPLC/EC at various dosages were then compared with those determined by the DPD-FAS procedure. A summary of these results are presented in Table 13. The DPD-FAS procedure consistently measured higher levels of NH$_2$Cl at each of the dosages examined and appears in this limited data to be particularly susceptible to interferences by NHCl$_2$. While less prone to interferences, the HPLC-/EC method can also erroneously measure high levels of NH$_2$Cl due to coeluting compounds which may be present.
Conclusions of Applications Studies

The post-column reaction electrochemical detector and on-line enrichment technique described in this report provide a very significant improvement upon the analytical methods currently available for the analysis of organic N-chloramines in chlorinated waste and surface waters. The method offers high specificity and detection limits that are on the order of $10^9$ M for injected compounds. In addition, samples injected directly require no preparatory work or pH adjustments and separations employ the mild conditions of HPLC. Thus, potential disturbances of the relatively labile N-chloramine distributions are minimized. As such, this method provides a valuable tool for studying aspects of chlorine-nitrogen chemistry that heretofore have been inaccessible due to lack of suitable analytical methods.

When applied to the organically enriched matrix of a chlorinated primary wastewater, detector selectivity was demonstrated and a number of peaks corresponding to organic N-chloroalkylamines were tentatively identified. In this matrix, however, the abundance of inorganic N-chloramines hampers the separation and detection of the much more dilute organic N-chloramines. Analyses of chlorinated surface waters showed the clear dominance of the inorganic N-chloramines among the combined residual chlorine pool in these much more dilute systems. Though a few minor peaks were observed, these results demonstrated that part-per-trillion sensitivity will be required to detect organic N-chloramines in these systems.
LIST OF REFERENCES


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U. S. Environmental Protection Agency. 1978. *Organic compounds identified in U.S. drinking water.* Health Effects Research Laboratory Cincinnati, OH.


LIST OF PUBLICATIONS

Manuscripts


Papers Presented

Jersey, J. A. and J. Donald Johnson. 1990. Direct HPLC analysis of N-chloramines employing post-column reaction with iodide and electrochemical detection. Environmental Electrochemistry Symposium, Federation of Analytical Chemistry and Spectroscopy Societies, October 7-12, Cleveland, OH.

Jersey, J. A. and J. Donald Johnson. 1990. Sub-nanogram N-chloramines by HPLC-electrochemical analysis. Division of Environmental Chemistry, American Chemical Society, April 22-27, Boston, MA.

GLOSSARY OF TERMS AND ABBREVIATIONS

Ag/AgCl - silver/silver chloride reference electrode
Au - gold electrode
CDFW - chlorine demand free water
CP - carbon paste electrode
CV - cyclic voltammetry
DMACl - N-chlorodimethylamine
E_{applied} - applied potential to working electrode
EC - electrochemical
E_{1/2} - the half wave potential, where the measured current is one-half the diffusion limited value
E_{pa} - potential of peak anodic current
E_{pc} - potential of peak cathodic current
FAC - free available chlorine
GC - glassy carbon working electrode
HAc - acetic acid
HPLC - high-performance liquid chromatography
i_d - inner diameter
K_h - hydrolysis equilibrium constant
KI - potassium iodide
KOT - knitted open tubular reactor
MACl - N-chloromethylamine
ml - milliliter
mM - millimolar, 10^{-3} molar
mV - millivolt
M - molar
NaAc - sodium acetate
namps - nanomap, 10^{-9} amp
ng - nanogram, 10^{-9} gram
NH_2Cl - monochloramine
nm - nanometer, 10^{-9} meter
picoamps - 10^{-12} amp
pg - picogram, 10^{-12} gram
pH - solution pH
pK_a - negative log of the acid dissociation equilibrium constant
Pt - platinum electrode
RF - response factor, instrument response per unit concentration
RRT - relative retention time
RSD - relative standard deviation
SCE - saturated calomel reference electrode
SHE - standard hydrogen electrode
THMs - trihalomethanes
μg/l - concentration in ppb
μl - microliter
μm - micrometer
μM - micromolar
UV - ultraviolet
V - volt
APPENDIX A

GERMICIDAL PROPERTIES OF N-CHLORAMINES

Several factors make it difficult to draw comparisons among data in the literature concerning the disinfection strengths of various forms of free and combined chlorine. Problems typically encountered include pH control, tester strain purity and inconsistent efforts undertaken to account for levels of chlorine demand. To further complicate matters, different studies use a variety of methods for residual chlorine measurements. Despite these limitations, there are widely held beliefs that free chlorine, especially HOCl, is a much stronger disinfecting agent than inorganic and organic N-chloramines.

The disinfection strength of monochloramine has been reviewed in detail elsewhere (Wolfe 1984 et al.; NRC 1980 and 1987). Data in Table 1 show the approximate disinfecting strengths of inorganic monochloramines relative to HOCl. From this limited data one can clearly see that inorganic N-chloramines are weaker disinfecting agents than FAC. Nonetheless, monochloramine is widely used as a residual disinfectant in drinking water distribution systems due to its stability. With the current concerns regarding the health effects associated with chlorination by-products, and in particular the trihalomethanes (THM), use of monochloramine will likely increase in the near future since research thus far has shown that it produces lower THM levels.

The disinfection strength of organic N-chloramines has been little studied. Feng (1966) was one of the first to show that the N-chloramines of glycine, methionine, taurine and gelatin were much weaker bactericides than monochloramine at neutral pH. Marks and Strandsksy (1950) demonstrated the weakness of N-chlorosuccimide and N-chloro-piperidine as disinfectants relative to hypochlorous acid. Chloramine-T, a compound commonly used for swimming pool disinfection (N-chloro-p-toluenesulphonamide), has been shown to perform poorly as a bacterial (E. coli; Ortenzio and Stuart 1964) and viral (Gowda et al. 1981, 1986) disinfecting agent.

Wolfe et al. (1985) conducted an interesting set of experiments in which the disinfection strength of monochloramine was evaluated as it was affected by glycine additions and position of chlorination in a water treatment scheme. Their results showed significant reductions in disinfection of E. coli when glycine was present in waters where ammonia was added prior to and simultaneously with chlorine additions. If preformed monochloramine was added, no significant decreases in disinfection were observed. The authors postulated two possible mechanisms to explain the observed decreases in disinfection: (1) glycine successfully competed kinetically with ammonia for added chlorine to form non-biocidal N-chloroglycine; and (2) chlorine transfers occurred from biocidal monochloramine to glycine to form the weak disinfectant N-chloroglycine.
Table 1. Relative disinfection strengths of free and combined chlorine

<table>
<thead>
<tr>
<th>Chlorine Species</th>
<th>E. coli</th>
<th>Poliovirus 1</th>
<th>G. muris cysts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free and Inorganic Chloramines&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOCl</td>
<td>1</td>
<td>1</td>
<td>~1</td>
</tr>
<tr>
<td>OCl</td>
<td>0.04</td>
<td>0.19</td>
<td>~1</td>
</tr>
<tr>
<td>NH&lt;sub&gt;2&lt;/sub&gt;Cl</td>
<td>6 x 10&lt;sup&gt;-4&lt;/sup&gt;</td>
<td>1.1 x 10&lt;sup&gt;-4&lt;/sup&gt;</td>
<td>~0.2</td>
</tr>
<tr>
<td>N-chloro derivatives of&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.015</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>Chloramine T</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3,5,5-trimethyl-hydantoin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>piperidine</td>
<td>6 x 10&lt;sup&gt;-5&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Based on c x t for 99% inactivation.
<sup>b</sup> Data summarized from that presented in NRC, 1987.
<sup>c</sup> Marks et al., 1945; assuming 99% c x t of 0.04 mg/l-min from Olivieri 1983.

Ram and Malley (1984) conducted a similarly interesting set of experiments wherein disinfection by measured free chlorine residuals in solutions containing a wide variety of organic-N compounds, each present at 1.0 mg/l as N, was examined. Three disinfection schemes were evaluated using E. coli as the indicator organism and DPD-colorimetric and amperometric titration as the free chlorine measurement techniques. Preliminary experiments were used to determine the quantities of added chlorine needed to produce measured free chlorine residuals of a specific concentration for each organic nitrogen...
compound and reaction time. Surprisingly, in only one of the many experiments conducted was disinfection found to be inadequate in the presence of apparent free chlorine residuals of 0.2 and 0.05 mg/l.
APPENDIX B

INTERFERENCES IN THE MEASUREMENT OF FREE AVAILABLE CHLORINE

Research examining interferences in measurement of free available chlorine has been basically of two types: model compound work and studies of treated waters. This research can be further subdivided into studies designed to examine interferences from inorganic monochloramine and organic N-chloramines. Jensen and Johnson (1989b) and Gordon et al. (1987) have recently reviewed the specificity of standard methods for free available chlorine analysis. Potential interferences due to inorganic monochloramine will be considered first in the discussion presented here.

Due to its abundance in most treated wastewaters and drinking waters, inorganic monochloramine is the interferant most likely to cause problems in the measurement of free chlorine residuals. Several research groups have measured monochloramine interferences in FAC measurements performed by the commonly applied DPD methods (The chemistry of the monochloramine-DPD interaction has been discussed in detail by Moore and colleagues, 1984). Results from these studies are summarized in Table 1. The greatest weakness of the DPD based methods is break-through of monochloramine into the FAC fraction. Measured time dependent rates of interference by monochloramine were found to range from 1.3 percent to 6.1 percent per minute. Omitting mercuric chloride from DPD buffers, additions of which are recommended by Standard Methods (APHA et al., 1985), has been shown to increase the rate of interference (Strupler 1979 and 1985; Fiquet 1982 as cited in Fiquet 1985). The form of added DPD reagents was found to have a significant effect in the work by Snead and coworkers (1981). Larger interferences were observed when DPD was used in the powder rather than tablet form.

For the amperometric membrane electrode, Keeslar (1975) found 4 percent monochloramine breakthrough into the FAC fraction at +200 mV applied potential. Levels of interference decreased with increasingly positive applied potential. Johnson and coworkers (1978) measured monochloramine interferences at 3 percent with a Delta electrode held at +337 mV. Similarly, Snead et al. (1981) found monochloramine interferences to range between 2 percent and 5 percent with a prototype Orion amperometric membrane electrode.

In our laboratory Jensen (1988) and Jensen and Johnson (1990a) recently examined interferences by monochloramine in measurement of FAC by the amperometric titration procedure. Not surprisingly, the level of interference was found to rise with increasingly negative applied potentials. It was estimated that a free available chlorine residual of 0.2 mg/l would be measured in a monochloramine solution of 3.9 mg/l (as Cl₂) under standard conditions. This level of interference was stated to represent an overestimation.
Table 1. Interferences by monochloramine in the DPD procedure for free chlorine.

<table>
<thead>
<tr>
<th>Interference % per min.</th>
<th>Temperature °C</th>
<th>Conc. NH₂Cl Reference (M)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.7</td>
<td>18</td>
<td>4.2 x 10⁻⁵</td>
<td>Palin 1957</td>
</tr>
<tr>
<td>3.6</td>
<td>25</td>
<td>--</td>
<td>Johnson and Overby 1969</td>
</tr>
<tr>
<td>3.7</td>
<td>--</td>
<td>1.1 x 10⁻⁵</td>
<td>Fiquet 1982</td>
</tr>
<tr>
<td>3.8</td>
<td>20</td>
<td>1.4 x 10⁻⁴</td>
<td>est. from data of Snead et al., 1981¹</td>
</tr>
<tr>
<td>4.5</td>
<td>20</td>
<td>10⁻⁴ - 10⁻⁵</td>
<td>Cooper et al. 1982²</td>
</tr>
<tr>
<td>5.8</td>
<td>20-22</td>
<td>3.7 x 10⁻⁵</td>
<td>est. from data Canelli 1980</td>
</tr>
<tr>
<td>6.1</td>
<td>35</td>
<td>6.9 x 10⁻⁵</td>
<td>est. from data Johnson 1978</td>
</tr>
<tr>
<td>2.7 to 4.0</td>
<td>--</td>
<td>7.1 x 10⁻⁶</td>
<td>Nicholson 1965</td>
</tr>
<tr>
<td>1.3</td>
<td>10</td>
<td>10⁻⁴ - 10⁻⁵</td>
<td>Stupler 1985</td>
</tr>
<tr>
<td>2.6</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.3</td>
<td>26</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. Interference for DPD in tablet form. Rate for powder form 29%/min.
2. Interference of 5 min. contact times.
of true free chlorine concentration by a factor greater than 2000. Snead et al. (1981), using a bacterial virus for specific detection of free chlorine, found the amperometric titration procedure gave false free chlorine positives in the presence of inorganic N-chloramines.

Selected data from the literature examining interferences from organic N-chloramines in free chlorine measurements by the DPD and amperometric titration procedures are summarized in Table 2. Wajon and Morris (1980) conducted an extensive study of this problem by measuring free available chlorine levels in chlorinated solutions of amino acids and heterocyclic nitrogen compounds. Interference was inferred to occur if free available chlorine readings were non-zero. They compared observed free available chlorine measurements with calculated values and concluded that none of the methods evaluated could measure free chlorine in the presence of cyanuric acid without significant interference. In addition, three N-chloroamino acids (sarcosine, creatine and proline chlorinated at a molar nitrogen to chlorine ratio of 1:1) were found to interfere in free chlorine measurements by the DPD procedure. The more acidic N-chloro heterocyclic compounds were found to interfere to a greater extent. None of the methods examined were found to be free of interferences in the majority of solutions studied.

In more recent work conducted by Jensen (1988) and Jensen Johnson (1990b), the time dependency of FAC interferences associated with the DPD method and organic N-chloramines was examined. For ten compounds examined, first order interference rate constants ranged from $4.6 \times 10^{-4}$ s$^{-1}$ for N-chloroalanine to 0.29 s$^{-1}$ for N-chloro-5,5-dimethylhydantoin. For these compounds, false positive responses at one minute ranged from 1 to 11 times true free chlorine concentrations. Preliminary data suggested that iodine impurities in buffer salts may catalyze the interference reactions. Jensen (1988) and Jensen and Johnson (1990a) also examined interferences by organic N-chloramines in free chlorine measurements performed by the amperometric titration procedure. For both model compounds and chlorinated process waters, interferences were found to increase as applied potentials became more negative.

Cooper and colleagues (1982) provided indirect evidence for interference by organic N-chloramines in measurements of free chlorine residuals in a treated drinking water. In their work, the amperometric membrane electrode measured values for free chlorine which averaged 21 percent less than values measured on identical samples by amperometric titration. Evidence supporting the role of organic nitrogen in the chlorination chemistry of this treated groundwater was found in the form of measured haloacetoni-triles following chlorinations. Though many possibilities exist to explain the observed data, the authors suggested that possibly the amperometric titrator was not specific for free chlorine. Wolfe et al. (1985) observed similar findings in studies of the inhibition of chlorine disinfection in the presence of organic-N. In their work, the amperometric and DPD methods were unable to distinguish between organic and inorganic N-chloramine.
Thus, in this case organic N-chloramines were found to interfere in measurement of monochloramine.
### Table 2. Summary of interferences observed in measurement of free available chlorine by organic N-chloramines.

<table>
<thead>
<tr>
<th>N-chloramino Compound</th>
<th>Analytical Method</th>
<th>DPD</th>
<th>Amperometric Titration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Amino Acids</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sarcosine</td>
<td></td>
<td>(1)</td>
<td></td>
</tr>
<tr>
<td>proline</td>
<td></td>
<td>(1)</td>
<td></td>
</tr>
<tr>
<td>creatine</td>
<td></td>
<td>(1)</td>
<td></td>
</tr>
<tr>
<td><strong>Heterocyclic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cyanuric acid</td>
<td></td>
<td>(1,2,4)</td>
<td>(1)</td>
</tr>
<tr>
<td>succimide</td>
<td></td>
<td>(1)</td>
<td></td>
</tr>
<tr>
<td>creatinine</td>
<td></td>
<td>(1)</td>
<td></td>
</tr>
<tr>
<td>uracil</td>
<td></td>
<td>(1)</td>
<td></td>
</tr>
<tr>
<td>thymine</td>
<td></td>
<td>(1)</td>
<td></td>
</tr>
<tr>
<td>purine</td>
<td></td>
<td>(1)</td>
<td></td>
</tr>
<tr>
<td>caffeine</td>
<td></td>
<td>(1)</td>
<td></td>
</tr>
<tr>
<td>piperidine</td>
<td></td>
<td></td>
<td>(3)</td>
</tr>
<tr>
<td>urea</td>
<td></td>
<td>(4)</td>
<td></td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>m-aminophenol</td>
<td></td>
<td>(1)</td>
<td></td>
</tr>
<tr>
<td>halazole</td>
<td></td>
<td></td>
<td>(2)</td>
</tr>
</tbody>
</table>

References: 1. Wajon and Morris 1980  
2. Whittle 1970  
3. Marks and Joiner 1948  
4. Canelli 1980
APPENDIX C

TOXICITY OF INORGANIC AND ORGANIC N-CHLORAMINES

Progress in evaluating the toxicity of organic N-chloramines has been hampered by the lack of methods for organic N-chloramine analysis and their incompatibility with the current microbiological assay techniques. Until methods become available to identify and measure specific N-chloramine compounds, it will not be possible to properly and completely assess the toxicity of chlorination and the resulting chlorine produced oxidants.

Human health effects associated with free chlorine and inorganic monochloramine have been thoroughly reviewed (NRC 1987; Moore and Calabrase 1980). Based upon positive evidence for hepatocellular changes, decreased body weights and liver toxicity, the Safe Drinking Water Committee of the National Research Council proposed suggested no adverse effect levels (SNARL) of 581 and 166 μg/l for monochloramine in adults and small children, respectively.

The N-chloramines possess a highly electrophilic nitrogen capable of participating in nucleophilic substitution reactions. Miller (1978) believed electrophilic nitrogen to be the "ultimate carcinogen" responsible for alkylation of DNA in the mechanism of aromatic amine carcinogenesis. It has become increasingly clear in recent years that a shared characteristic of many "ultimate" carcinogens is their electrophilicity. Cheh and coworkers (1980a and 1980b) studied the electrophilic nature of direct acting mutagens produced by chlorination of drinking water. In their work dechlorination was found to significantly reduce mutagenicity. Potencies of mutagenic extracts were estimated by titrating concentrates with various nucleophiles. Wilcox and Denny (1985) and others (Treby et al., 1986 and 1988; Croue and Reckhow 1989;) have since reported similar findings for chlorinated drinking water. These observations support the postulate that organic N-chloramines could be potentially of toxicological significance.

In studies conducted by Scully and Bempong (1980 and 1982) and Bempong and Scully (1980), N-chloropiperidine has been found to give a positive mutagenic response in the Ames Assay as a direct acting mutagen and to modify in-vitro cell transformation processes in Chinese hamster ovarian cells. In addition, this compound was found to induce structural aberrations in chromosomes at levels proportional to its concentration. Toxicological studies for other specific N-chloro compounds have not to date appeared in the literature.

Hall (1981) provides a thorough review of the toxicity of chlorination to freshwater organisms and illustrates the difficulties which complicate experiments designed to perform such assessments. The inability of laboratory studies to simulate field situations
and the avoidance abilities of many species make accurate assessments very difficult. The reviewed data showed the importance of acclimation temperature, chlorine speciation, exposure times and elevated temperature as variables influencing toxicity of aqueous chlorinations. In the broad sense, total residual chlorine exposures required to cause excessive mortality were generally higher than those typically found in chlorinated wastewater discharges.
APPENDIX D

HYPOCHLOROUS ACID AND N-CHLORAMINES AS MICROBIAL TOXINS IN ISOLATED NEUTROPHILS

There is a significant body of evidence supporting the functioning of hypochlorous acid as a potent microbial toxin that is produced intracellularly in stimulated systems of isolated neutrophils. Neutrophils are the body's primary white blood cells and their chief function is the destruction of invading bacteria. The enzyme myeloperoxidase, in neutral to mildly acid media, catalyzes the oxidation of chloride to hypochlorous acid in the presence of peroxide (Thomas 1979; Grisham et al., 1984; Hurst et al., 1984; and Hurst 1988). Researchers have shown in isolated neutrophil systems that residual oxidant concentrations increase with time and are quite stable and primarily hydrophilic in nature (Grisham et al., 1984 and Thomas 1979). Involvement of N-chloramines was suggested based upon observations of UV absorbance spectra, the ability of the residual oxidant to chlorinate added ammonia, oxidant solubilities in organic solvent, reaction with added thiol and high intracellular concentrations of primary amino compounds. In such systems free chlorine would be short lived. The authors hypothesized that N-chloramines serve two functions in these cells. First, they may protect neutrophil cells from self attack by highly reactive HOCl. Secondly, the cells may use N-chloramines as a reservoir of biocidal chlorine in a less reactive form.
APPENDIX E

EFFECT OF POST-COLUMN REAGENT COMPOSITION UPON DETECTION OF N-CHLORAMINES

The objective of this experiment was to examine the effects of post-column reagent composition upon detection of model N-chloramines. Specifically, whether detection selectivity could be controlled by post-column reagent composition was evaluated. Compositions were selected to closely mimic those employed by Standard Methods (APHA et al., 1985) to resolve free chlorine and inorganic monochloramine from the total combined chlorine pool. Analyses of model compounds were performed over a broad concentration range to fully explore the effects of reagent composition upon the reaction kinetics and thus specificity of post-column reaction. Experimental methods and materials employed in these measurements were identical to those described earlier in this report.

N-chlorodimethylamine and NH₂Cl were the model compounds selected for study. N-chlorodimethylamine was chosen to be representative of the organic N-chloramines. Monochloramine was selected since it is oftentimes the most dominant component within the combined residual chlorine pool. In addition, NH₂Cl retains significant biocidal activity and likely will be used increasingly as the primary disinfectant in drinking water treatment practices. Little if any data are currently available regarding the specificity of Standard Methods (APHA et al., 1985) for analysis of monochloramine.

Results from this experiment are shown graphically in Figure 1. The pH 4.0, 0.09 M KI post-column reagent is the standard composition selected from earlier post-column reaction detector optimization experiments. The composition of this solution is very similar to that used in both the DPD and amperometric titration procedures for total residual chlorine. The pH 7.0, 0.018 M post-column reagent was selected to provide conditions mimicking those of Standard Methods (APHA et al., 1985) for selective analysis of NH₂Cl. The final post-column reagent examined was identical to the HPLC mobile phase used in these experiments. This solution, having no iodide, provides reaction conditions similar to those used by the DPD and amperometric titration methods for free chlorine analysis.

For both N-chloramines examined, detector sensitivity and dynamic range were greatest for the pH 4.0, 0.09 M post-column reagent as expected. Use of this reagent allows subnanogram detection limits and calibrations in excess of three orders of magnitude to be performed with minimal error. Upon switching to pH 7.0, 0.018 M post-column reagent, detection limits increased by greater than ten to twentyfold for monochloramine and N-chlorodimethylamine, respectively. In addition, for both compounds the kinetics...
Figure 1. Effect of post-column reagent composition upon detection of model N-chloramine compounds. (A) N-chlorodimethylamine and (B) NH₂Cl. Conditions as follows: (a) mobile phase 30:70 CH₃CN/H₂O pH 6.2 water at 1.0 ml/min; (b) post-column reagent as described at 0.5 ml/min; (c) working electrode glassy carbon at -0.100 V vs Ag/AgCl; (d) inj. volume 50 μL.; (e) lines connecting data points predicted values by first and second order regression models $R = C^n$ and $R^2 = C^{2n}$, respectively.
of post-column reaction clearly become concentration dependent. A simple linear model no longer is adequate to describe detector response for either compound under these conditions. In the absence of any added iodide, detection limits increased one-hundredfold for both model compounds. Under these conditions detector response exhibited a degree of linearity for monochloramine while for N-chlorodimethylamine response was markedly a function of concentration.

These results support the choice of reagent selected as the standard post-column reagent. Under the reaction conditions it provides, which duplicate those used in Standard Method DPD and amperometric titration methods (APHA et al., 1985) for total chlorine residual analysis, detector response is nearly identical for NH$_2$Cl and N-chlorodimethylamine over the broad concentration ranges examined. There is no evidence of discrimination exhibited among forms of combined chlorine.

Surprisingly, when switching to the pH 7.0, 0.018 M post-column reagent only marginal discrimination among NH$_2$Cl and N-chlorodimethylamine was observed. Under the carefully controlled conditions provided by the post-column reactor, the extent of reaction between iodide and these two N-chloramines was very similar for this reagent. This is of particular interest since the kinetics of this reaction provide the basis for selective detection of NH$_2$Cl in the presence of organic N-chloramines in both the DPD and amperometric titration Standard Methods (APHA et al. 1985). The only difference in conditions between these experiments and those of Standard Methods is the presence of 20 percent (V/V) HPLC grade acetonitrile. This should have negligible effects upon the relative kinetic rates of these reactions. Based on these limited observations, significant interferences by organic N-chloramines in measurement of NH$_2$Cl by the DPD and amperometric titration procedure are very probable. The degree of interference likely increases for the N-chloramines of less basic amino compounds.

Detector response observed for both N-chloramines in the absence of post-column iodide addition is somewhat confusing. Under these conditions, detection should occur by direct reductions at the electrode surface. For both compounds, the applied potential (-0.100 V vs Ag/AgCl) is considerably less than that required to produce diffusion limited currents under these conditions. The observed results may be attributable to iodine produced by reactions with trace iodide impurities in the phosphate buffers used (Jensen 1988). This is consistent with the more marked concentration dependence of currents observed for N-chlorodimethylamine.
Kinetic Considerations of N-Chloramine Reaction with Standard Post-Column Reagent

If we assume a simplified reaction between N-chloramines and I⁻ as shown below,

$$2 \text{H}^+ + 2 \text{RNR}'\text{Cl} + 2 \text{I}^- \rightarrow 2 \text{RNR}'\text{NH} + \text{I}_2 + 2 \text{Cl}^-$$

with a rate determining step of (Gray and Workman, 1983):

$$\text{H}^+ + \text{RR'NCl} + \text{I}^- \rightarrow \text{RR'NH} + \text{I}^+ + \text{Cl}^-$$

then in the presence of excess I⁻ and a pH 4 buffer, pseudo first order conditions exist where the rate of reaction is equal to:

$$-\frac{d[\text{RR'NCl}]}{dt} = \frac{d[\text{I}^+]}{dt} = k_{\text{obs}}[\text{RR'NCl}]$$

where $k_{\text{obs}} = k[I^-][\text{H}^+]$

By knowing the residence time within the delay tube we can calculate a minimum $k_{\text{obs}}$ for a specified degree of reaction completeness between an N-chloramine and iodide. Under standard HPLC and post-column flows of 1.0 and 0.5 ml/min, respectively, the residence time within the 10 ft. delay coil used in this work was approximately 24 seconds. Assuming a reaction completeness of 99 percent, solution pH of 4, a 24 s residence time, and complete and rapid mixing within the delay reactor, necessary rate constants can be derived for various post-column iodide concentrations. Estimates derived in this manner are listed below.

<table>
<thead>
<tr>
<th>post-column [I⁻] (M)</th>
<th>k (M⁻²s⁻¹) 10 ft delay tube</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>2.3 E4</td>
</tr>
<tr>
<td>0.015</td>
<td>1.5 E5</td>
</tr>
<tr>
<td>0.01</td>
<td>2.3 E5</td>
</tr>
<tr>
<td>0.001</td>
<td>2.3 E6</td>
</tr>
</tbody>
</table>
Examination of the literature has not revealed specific references addressing the rates of these reactions. The only pertinent reference obtained is that of Gray and Workman (1983). They cite kinetic work done by Huffman (Huffman 1976) in which the rates of reaction between iodide and monochloramine and dichloramine were examined. Estimates of $k$, based on the rate determining step shown above, were reported to be $1.81 \times 10^{10}$ and $8.5 \times 10^{5} \text{M}^{-2}\text{s}^{-1}$ for monochloramine and dichloramine, respectively. Lister and Rosenblum (1963) reported a rate constant of $3.3 \times 10^8 \text{M}^{-1}\text{s}^{-1}$ for the oxidation of iodide by hypochlorite ion. Oxidation rates by the N-chloramines of strongly basic organic amines could conceivably be slower than that of monochloramine.

Based upon the limited data cited above, estimates of required rate constants and the standard conditions employed (pH 4.0, 0.09 M KI), post-column reactions between iodide and N-chloramines should very closely approach completion. Results of the post-column reagent composition studies reported above and the detector responses observed for a variety of N-chloramines presented in earlier work support this statement. Though not clearly specified, the iodide concentration recommended by Standard Methods (APHA et al., 1985) for total residual chlorine analysis by the amperometric titration procedure is approximately 0.015 M.
APPENDIX F

ANALYSIS OF N-CHLOROGLYCylGLYCINE BY HPLC WITH POST-COLUMN REACTION DETECTION

The objectives of this experiment were twofold. First, the feasibility of quantifying N-chloroglycylglycine and thus N-chloro peptides in general was examined. Assuming success in this experiment, the objective of the next experiment was to measure concentrations of N-chloroglycylglycine in solutions chlorinated across the breakpoint curve. Methods and procedures used here have been described fully in preceding sections of this report.

Though the concentration range examined was quite limited (1.1 x 10^{-6} to 1.6 x 10^{-5} M), quantitative analysis of N-chloroglycylglycine was successful. A linear regression analysis of data gathered over an approximate tenfold concentration range resulted in an $r^2$ value of 0.997. Reaction conditions used to form N-chloroglycylglycine standards in this experiment (pH 8, [N]:[Cl] molar ratio of 10) strongly favored formation of the singly substituted N-chloro peptide.

Results of the breakpoint experiment shown in Figure 1 are difficult to interpret on the basis of known nitrogen chlorination chemistry. As in breakpoint experiments described previously, chlorinations were performed at pH 7.0 and reactions were allowed to proceed for 1 hour in darkness at ambient temperature. As expected, levels of N-chloroglycylglycine increased with increasing chlorine dose to a maximum at a reduced nitrogen to chlorine molar ratio of one. At this point approximately 80 percent of the reduced peptide nitrogen was chlorinated. With increasing chlorine dosages beyond this point, however, the concentration profile for N-chloroglycylglycine failed to follow the concentration profile expected of substrates possessing two exchangeable protons.

Why the concentration profile for the singly substituted derivative (as measured by HPLC/EC) failed to go to zero at chlorine dosages past a molar Cl-to-reduced-N-ratio of 1 remains unknown. Decreases in concentration of N-chloroglycylglycine were very gradual at dosages exceeding a Cl to reduced N molar ratio of one. Under the conditions of this experiment the peptidic nitrogen should be unreactive towards aqueous chlorine. Chlorination of the singly substituted N-terminal N-chloro peptide, while considerably slower than the first chlorination, is nonetheless favorable. Surprisingly this solution exhibited no chlorine demand, as evidenced by the overlap of dose and total residual chlorine plots. Unfortunately free chlorine measurements were not performed in this experiment. On the basis of these limited data and accepted nitrogen-chlorine chemistry, the concentration profile observed for the chlorination of this dipeptide remains unexplainable.
Figure 1. Concentration of N-chloroglycylglycine and total chlorine residual in solutions of glycylglycine chlorinated at various dosages. Conditions as follows: (a) Zorbax C$_{18}$ analytical column (25 x 46 cm I.D.), 30:70 CH$_3$CN:pH 6.2 water at 1.0 ml/min; (b) standard post-column reagent at 0.5 ml/min; (c) glassy carbon working electrode at -0.100 V vs Ag/AgCl; (d) inj. volume 50 μl; (e) glycylglycine concentration 1.0 x 10$^{-5}$ M.
APPENDIX G

RETENTION TIME SUMMARY FOR MODEL N-CHLORAMINES ANALYZED BY POST-COLUMN REACTION ELECTROCHEMICAL DETECTION

Presented below are summaries of retention times for singly substituted N-chloro amino acids and aliphatic amines analyzed by reversed-phase HPLC with post-column reaction detection. Summaries are presented for conditions selected to provide both maximum chromatographic resolution and rapid analyses. N-chloramines were synthesized under conditions strongly favoring formation of only singly substituted products (pH ≥ 8, [N]:[Cl] molar ratios > 10). Retention times were determined for each compound individually to ensure product purity and to correctly identify retention times. Mean retention times of duplicate mixture determinations are presented in Tables 1 and 2 for different gradient separations. Reproducibility of retention times by gradient HPLC/EC were less than 1 percent RSD. An HPLC post-column reaction detection chromatogram of N-chloroamino acids and N-chloro aliphatic amines is shown in Figure 1.
Table 1. Summary of N-chloro amino acid and aliphatic amine retention times under conditions favoring maximum chromatographic resolution

<table>
<thead>
<tr>
<th>N-chloramine&lt;sup&gt;b&lt;/sup&gt;</th>
<th>RT (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>asp, glu</td>
<td>5.64</td>
</tr>
<tr>
<td>ser, thr, asn</td>
<td>6.29</td>
</tr>
<tr>
<td>gly, gln</td>
<td>6.85</td>
</tr>
<tr>
<td>ala</td>
<td>7.25</td>
</tr>
<tr>
<td>B-ala</td>
<td>7.95</td>
</tr>
<tr>
<td>NH&lt;sub&gt;3&lt;/sub&gt;, alpha amino butyric</td>
<td>8.65</td>
</tr>
<tr>
<td>lys, sarcosine</td>
<td>9.03</td>
</tr>
<tr>
<td>gamma amino butyric, arg</td>
<td>9.82</td>
</tr>
<tr>
<td>val</td>
<td>12.29</td>
</tr>
<tr>
<td>norv</td>
<td>15.09</td>
</tr>
<tr>
<td>methylamine</td>
<td>18.83</td>
</tr>
<tr>
<td>tyr, ile</td>
<td>21.39</td>
</tr>
<tr>
<td>leu</td>
<td>22.59</td>
</tr>
<tr>
<td>norleucine</td>
<td>24.35</td>
</tr>
<tr>
<td>phe, ethylamine</td>
<td>29.29</td>
</tr>
<tr>
<td>trp</td>
<td>31.27</td>
</tr>
<tr>
<td>dimethylamine</td>
<td>37.84</td>
</tr>
<tr>
<td>isopropylamine</td>
<td>41.54</td>
</tr>
<tr>
<td>propylamine</td>
<td>46.33</td>
</tr>
<tr>
<td>tert-butylamine</td>
<td>52.62</td>
</tr>
<tr>
<td>butylamine</td>
<td>62.21</td>
</tr>
<tr>
<td>diethylamine</td>
<td>63.50</td>
</tr>
<tr>
<td>piperidine</td>
<td>65.58</td>
</tr>
</tbody>
</table>

<sup>a</sup> Analytical conditions as follows: (a) mobile phases A 0:100 and B 50:50 CH<sub>3</sub>CN/pH 6.2 water, respectively; gradient 0% to 100% B in 60 minutes, linearly; flow 0.5 ml/min; (b) Econosil 25 x 0.46 cm I.D. C<sub>18</sub> column; (c) standard post-column reagent at 0.3 ml/min; (d) inj. volume 20 μl; (e) glassy carbon working electrode at -0.100 V vs Ag/AgCl; (f) mean RT from duplicate runs.

<sup>b</sup> Identity of precursor amines, standard 3 letter abbreviations used for essential amino acids.
Table 2. Summary of N-chloro amino acid and aliphatic amine retention times under conditions favoring analysis time

<table>
<thead>
<tr>
<th>N-chloramine(^b)</th>
<th>RT (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>asp, glu</td>
<td>3.18</td>
</tr>
<tr>
<td>ser, thr, asn</td>
<td>3.48</td>
</tr>
<tr>
<td>gly, gln, ala</td>
<td>3.74</td>
</tr>
<tr>
<td>B-ala</td>
<td>4.08</td>
</tr>
<tr>
<td>gamma and alpha amino butyric acid and sarcosine</td>
<td>4.58</td>
</tr>
<tr>
<td>NH(_3)</td>
<td>4.93</td>
</tr>
<tr>
<td>arg</td>
<td>7.29</td>
</tr>
<tr>
<td>val</td>
<td>7.45</td>
</tr>
<tr>
<td>norv</td>
<td>8.90</td>
</tr>
<tr>
<td>methylamine</td>
<td>11.21</td>
</tr>
<tr>
<td>ile</td>
<td>12.19</td>
</tr>
<tr>
<td>leu, tyr</td>
<td>12.56</td>
</tr>
<tr>
<td>norleucine</td>
<td>13.57</td>
</tr>
<tr>
<td>phe, ethylamine</td>
<td>16.15</td>
</tr>
<tr>
<td>trp</td>
<td>18.36</td>
</tr>
<tr>
<td>dimethylamine</td>
<td>20.62</td>
</tr>
<tr>
<td>isopropylamine</td>
<td>22.26</td>
</tr>
<tr>
<td>propylamine</td>
<td>24.73</td>
</tr>
<tr>
<td>tert-butylamine</td>
<td>28.26</td>
</tr>
<tr>
<td>butylamine</td>
<td>33.68</td>
</tr>
<tr>
<td>diethylamine</td>
<td>34.53</td>
</tr>
<tr>
<td>piperidine</td>
<td>35.80</td>
</tr>
</tbody>
</table>

\(^a\) Analytical conditions as follows: (a) mobile phases A 0:100 and B 70:30 CH\(_3\)CN/pH 6.2 water, respectively; gradient 0% to 70% B in 32 minutes, linearly; flow 1.0 ml/min; (b) Econosil 25 x 0.46 cm I.D. C\(_{18}\) column; (c) standard post-column reagent at 0.5 ml/min; (d) inj. volume 20 \(\mu\)l; (e) glassy carbon working electrode at -0.100 V vs Ag/AgCl; (f) mean RT from duplicate runs.

\(^b\) Identity of precursor amines, standard 3 letter abbreviations used for essential amino acids.
Figure 1. Gradient HPLC post-column reaction detection chromatogram of N-chloroamino acids and N-chloro aliphatic amines.

Analytical conditions as described in Table 1. Peak identities as the N-chloro derivatives of the following compounds: 1-asp, glu; 2-ser, thr, asn; 3-gly, gln; 4-ala; 5-B-ala; 6-NH$_3$, alpha amino butyric acid; 7-lys, sarcosine; 8-gamma amino butyric acid, arg; 9-val; 10-norvaline; 11-methylamine; 12- tyr, ile; 13-leu; 14-norleucine; 15-phe, ethylamine; 16-trp; 17-dimethylamine; 18-isopropylamine; 19-propylamine; 20-tert-butylamine; 21-butylamine; 22-diethyl-amine; 23-piperidine.