Improving the Recoveries of Unstable *N*-Chloramines Determined by Liquid Chromatography–Postcolumn Electrochemical Detection*

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Abstract

Liquid chromatographic (LC) measurement of individual *N*-chloramines, which are key byproducts of wastewater and drinking water chlorination, could lead to more effective control of water disinfection. Such measurements are challenging because of analyte instability. A detector selective for *N*-chloramines is constructed based on postcolumn derivatization with iodide followed by reductive detection of the iodine product at a glassy carbon electrode. In flow injection (FIA) mode, the detector gives identical responses for a test set of four chemically diverse *N*-chloramines. In the LC mode, losses of the test compounds are observed when LC and FIA responses are compared and quantitated by introducing a relative response factor (RRF). Using the RRF, *N*-chloramine recoveries are evaluated as a function of multiple LC separation parameters. The highest recoveries are obtained using a reversed-phase (C18) column with an acetonitrile mobile phase and a pH 7.02 aqueous phosphate buffer. With these conditions, linear calibration curves are obtained for all test *N*-chloramines. The detection limits obtained are in the low 10^{-7}-mol/L range, which is nearly tenfold better than previously reported and 10–1000-fold lower than total residual chlorine concentrations typically found in disinfected water and wastewater.

Introduction

Chlorination is the most widely used method of disinfection for the roughly 39 billion gallons (150,000,000 m^3) of processed domestic wastewater discharged into waterways every day in the United States. Whether chlorine is applied as Cl_2 (g) or OCl^- (aq), naturally occurring inorganic and organic amines react with it to produce *N*-chloramines (1,2). *N*-chloramines are important byproducts of wastewater disinfection because they are precursors to mutagenic dihaloacetonitriles and trihalomethanes (3) and because chloramines may directly threaten aquatic wildlife that becomes exposed to chlorinated effluents after discharge. Inasmuch as organic *N*-chloramines, inorganic *N*-chloramines, and free chlorine differ in toxicity and disinfection ability (4–6), a measurement method that identifies individual chloramines would allow for a better understanding and control of the disinfection process and its postdischarge impact on the environment. Peptide chloramines are of particular interest in disinfected wastewaters because they resist dechlorination treatment intended to protect aquatic wildlife (7).

Although several methods have been proposed, a satisfactory method for quantitating organic and inorganic *N*-chloramines in wastewaters has proven elusive. Membrane introduction mass spectrometry has attracted recent interest (8,9) but can only determine chloramines that are low molecular weight, nonpolar, and volatile. Dansylated derivatives of chloramines have been identified by liquid chromatography (LC) with fluorescence detection (10,11), but this method has proven unsuitable for quantitation because of variable yields (12). Similar problems have been encountered with 2-mercaptobenzothiazole derivatization followed by LC with UV detection (13).

The postcolumn reaction of chloramines with iodide to produce I_2/I_3^- followed by either UV or electrochemical detection has been explored in LC (14–17). A key feature of this approach is that time-consuming precolumn preparative steps are avoided, which is a critical advantage in methods for unstable analytes such as *N*-chloramines. One study demonstrated that organic *N*-chloramines present in sewage wastewater could be separated and electrochemically detected (17) but did not address issues in quantitation.

In this study, a group of test compounds (monochloramine, *N*-chloropiperidine, *N*-chloroleucylalanine (*N*-Cl-AlaAla), and *N*-chloroalanylalanine (*N*-Cl-LeuAla)) were selected to represent, respectively, inorganic, aliphatic, and peptide *N*-chloramines. To investigate the quantitative behavior of *N*-chloramines, a flow injection analysis (FIA) mode that bypassed the analytical column was used to investigate the detector response. An equivalent

* Certain commercial equipment, instruments, or materials are identified in this paper to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

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molar response for all test compounds was noted. However, comparison of the FIA and LC molar responses revealed compound-specific *N*-chloramine losses during the separation. To investigate possible sources of the observed analyte losses, a comprehensive set of LC separation parameters was investigated. Optimized conditions were identified that provided nearly equivalent FIA and LC responses. The new method, although not providing a compound-independent molar response, can be used for quantitative determination of *N*-chloramines.

Experimental

Reagents

The peptides used in this study form *N*-chloramines for which dechlorination rates with sulfite are known (7). Alanylalanine and leucylalanine (Sigma, St. Louis, MO), as well as piperidine (Fluka, Buchs, Switzerland), were obtained and used without further purification. Ammonium nitrate (Mallincrodt, Phillips-burg, NJ) was used to prepare monochloramine solutions. All test amines were prepared to be approximately 0.2 mmol/L. A stock phosphate buffer (0.1 mol/L) was prepared to be pH 7 using sodium monobasic and dibasic phosphates (Sigma), and it was diluted to 0.02 mol/L in the peptide solutions. The buffer was made from chlorine-demand-free deionized water (from a gradient Milli-Q water system, Millipore, Milford, MA), which was prepared by the addition of approximately 2.5 mg/L of hypochlorite followed by a 24-h waiting period and 3 h of irradiation by a UV lamp. The UV treatment decomposes excess hypochlorite to oxygen and chloride. All glassware used was cleaned using a 5% nitric acid solution prepared from reagent-grade nitric acid (J.T. Baker, Phillipsburg, NJ) and thoroughly rinsed with distilled deionized water to avoid trace metal catalyzed decomposition of the chlorine species. Additionally, all *N*-chloramines were injected individually to prevent possible chlorine-transfer reactions, which would affect quantitation and, therefore, analyte calibrations.

A 5% solution of reagent-grade sodium hypochlorite (Alfa Aesar, Ward Hill, MA) was diluted to approximately 3 mmol/L and its concentration was determined regularly by amperometric titration (18). The standardized diluted solution was then used to prepare the monochloro *N*-chloramines using a tenfold stoichiometric excess of the parent amine (~0.02 mmol/L) to minimize formation of dichloramine products.

The postcolumn reagent solutions were prepared daily from reagent-grade potassium iodide (Fisher, Pittsburgh, PA) to be 2 mmol/L and stored in a brown bottle. These KI solutions were made from chlorine-demand-free deionized water and a 1-mol/L stock acetate buffer solution diluted to 0.1 mol/L. The 1-mol/L stock solution was prepared to be pH 4 using analytical-grade sodium acetate (Mallinckrodt) and Suprapur glacial acetic acid (Matheson, Coleman and Bell, Gibbstown, NJ). All KI solutions were also prepared using chlorine-demand-free deionized water.

Water (gradient milli-Q water system, Millipore), HPLC-grade acetonitrile (Mallincrodt), HPLC-grade methanol (Burdick and Jackson, Muskegon, MI), and sequanal-grade sodium dodecyl sulfate (SDS) (Pierce, Rockford, IL) were used for the LC and FIA mobile phases. A 1-mol/L stock solution of chloroacetic acid (Fluka) was adjusted to pH 2.75 with sodium hydroxide and used to prepare the mobile phases. The chloroacetic acid concentration in the final chromatographic solvents was 0.05 mol/L. For the neutral pH solvent conditions, a 0.5-mol/L stock solution of phosphate buffer was prepared from sodium phosphate (monobasic and dibasic) (Sigma) to be pH 7.02. The total phosphate concentration used in the final chromatographic solvents was 0.02 mol/L. For the micellar solvent conditions, the SDS solutions were prepared to be 9, 100, 200, and 300 mmol/L. The buffered SDS solutions were diluted to 200 mmol/L in SDS and 0.05 mol/L in buffer from stock buffer solutions of chloroacetic acid, acetic acid, and borate buffers. The pH of the SDS mobile phase was then adjusted with either hydrochloric acid or sodium hydroxide. The mobile phase solvents as well as the postcolumn reagent were degassed at the beginning of each day under vacuum in an ultrasonic bath.

Chromatography–FIA

The LC–FIA bypass system was set up as shown in Figure 1. A two-pump LC system with an automated gradient controller was used. A column heater was used to heat the analytical column when the separation temperature was investigated. Four different mobile phase compositions were used for both the LC and FIA experiments: (*a*) 0.05-mol/L chloroacetic acid buffer solution in water (pH 2.75) and 95% acetonitrile–5% 0.05-mol/L chloroacetic acid buffer solution in water (pH 2.75); (*b*) 0.05-mol/L chloroacetic acid buffer solution in water (pH 2.75) and 95% methanol–5% 0.05-mol/L chloroacetic acid buffer solution in water (pH 2.75); (*c*) 0.02-mol/L phosphate buffer in water (pH 7.02) and 74% acetonitrile–26% 0.02-mol/L phosphate buffer in water (pH 7.02); and (*d*) SDS solutions in water (9, 100, 200, and 300 mmol/L) and 200-mmol/L SDS solutions in a 0.05-mol/L buffer of the appropriate pH. The descriptions of

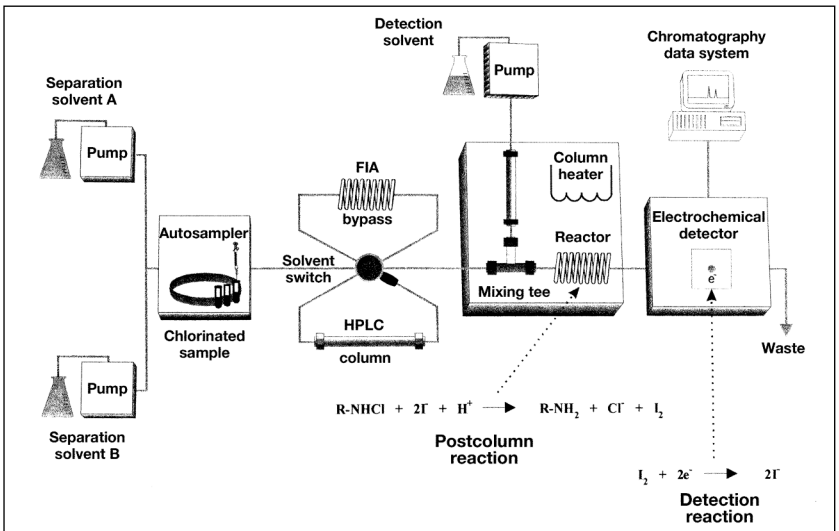

Figure 1. Liquid chromatograph/postcolumn electrochemical detection system with an FIA bypass for the detection of *N*-chloramines.

the mobile phase pH refer to the pH of the aqueous buffer prior to mixing with the organic modifier. The LC–FIA mobile phase was delivered at 1.0 mL/min. The solvent switch (shown in Figure 1) was incorporated to allow for a bypass of the analytical column to perform the FIA experiments. To correct for solvent compressibility, similar pump backpressure was afforded between the FIA and LC experiments by inserting a C18 column into the mobile phase stream prior to the sample injection point. This ensured that flow rates were identical for the comparisons of LC and FIA data.

Three analytical columns specifically designed for protein–peptide analysis were investigated. The two silica-supported Macrosphere RP columns (Alltech, Deerfield, IL) (one C18 and one C4) had a 300-Å pore size, were 250 × 4.6 mm in dimension, and packed with 5-µm spherical particles. Also, a PRP-3 column (Hamilton, Reno, NV) with a 300-Å pore size that was 150 × 4.6 mm and packed with 10-µm spherical poly(styrene-divinylbenzene) particles was investigated. These columns were all similar in that they had wide pores, which is an advantage for separations of larger-sized peptides. In addition, all columns were PEEK (polyetheretherketone) lined and fritted, and all tubing and fittings used postpump and were PEEK or polytetrafluoroethylene. To test the reactivity of the materials to chloramines, frits made of PEEK, stainless steel, titanium, and ultrahigh-molecular-weight polyethylene (UHMWPE) were obtained from Upchurch Scientific (Oak Harbor, WA).

An LC pump was used to deliver the postcolumn reagent at 0.5 mL/min. A column heater was used to maintain the postcolumn knitted fluoropolymer reactor and thus the reaction temperature at 23°C. The reactor coils (Supelco, Bellefonte, PA) investigated had a 0.38-m × 0.25-mm i.d. (plain tubing), 5-m × 0.25-mm i.d., 5-m × 0.50-mm i.d., and 10-m × 0.50-mm i.d., and the coils had effective reaction times of 1, 10, 31, and 79 s, respectively. Detection of the iodine formed in the postcolumn reaction was then achieved with amperometric detection via a glassy carbon electrode poised at –0.1 V (versus a Ag/AgCl reference electrode). To prevent the formation of AgI, the reference electrode was isolated from the mobile-phase– postcolumn-reagent stream using a double-junction cell filled with 3-mol/L KCl. Data were collected by computerized chromatography software.

The optimized analysis conditions were as follows: the solvent modifier was acetonitrile; the mobile phase pH was 7.02; the column was a macrosphere C18; the mobile phase flow rate was 1.0 mL/min; the postcolumn reagent was 0.002-mol/L KI in a pH 4 acetic acid–acetate buffer; the postcolumn flow rate was 0.5 mL/min; the reactor coil had an effective reaction time of 10 s; the detector potential was –0.1 V versus a Ag/AgCl reference; and the operating temperature was 23ºC. For the separation of the test compounds using these optimized conditions, the solvent composition program was 100% of the mobile phase 0.02-mol/L phosphate buffer in water (pH 7.02) (A) from 0 to 2 min. The linear gradient was then to 80% of the mobile phase 74% acetronitrile–26% 0.02-mol/L phosphate buffer in water (pH 7.02) (B) from 2 to 4 min. The linear gradient was to 100% B from 4 to 13 min and then a return to 100% A at 13.1 min.

Results

LC detector optimization

In order to optimize detector parameters, we used FIA to avoid possible oncolumn losses (14). The analyte signal was represented by the molar response, which was obtained by dividing the peak area by the analyte concentration (in units of mol/L). The following factors that might influence the postcolumn reaction of *N*-chloramines with iodide to form iodine were investigated: temperature, length of reactor coil, iodide concentration, and pH.

The detector reaction temperature was varied between 20°C and 60°C. Slight signal enhancement occurred at the higher temperatures (Figure 2A), but background currents and noise also increased. Room temperature conditions (~23°C) were therefore utilized for all experiments.

Differing lengths of postcolumn reactor coil were investigated to determine if varying the length of time allotted for the postcolumn reaction would affect the detector response. The coil with a residence time of 1 s seemed to provide insufficient time for the postcolumn reaction, but residence times greater than 10 s produced negligible improvements in signals (Figure 2B). This 10-s coil (5-m × 0.25-mm i.d.) was therefore used for all subsequent experiments. This coil produced less band broadening than longer coils.

Finally, three different iodide concentrations (0.0002, 0.002, and 0.02 mol/L) were investigated for the postcolumn reagent concentration (Figure 2C). Again, there was no advantage in the

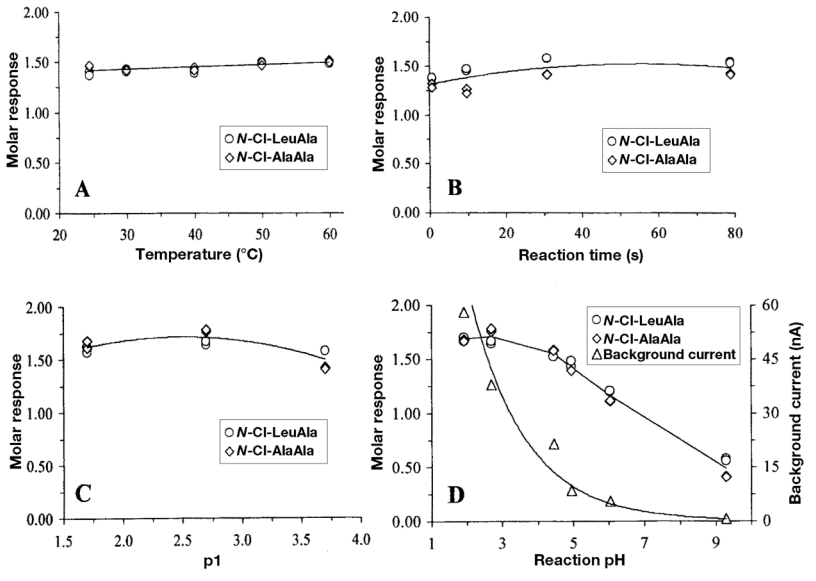

Figure 2. Optimization of molar responses as a function of various detection parameters from FIA measurments: (A) temperature, (B) reaction time, (C) iodide concentration (pI^-), and (D) reaction pH. In A, C, and D, *N*-Cl-LeuAla was 24 µmol/L and *N*-Cl-AlaAla was 23 µmol/L. In B, *N*-Cl-LeuAla was 27 µmol/L and *N*-Cl-AlaAla was 25 µmol/L. The points depicted for each *N*-chloramine represent the average of three replicate measurements. The points for the background current in D represent one measurement each.

detector response when the higher iodide concentration was used, and a higher background current and noise were produced by adventitious oxygen-generated iodine. A postcolumn reagent concentration of 0.002 mol/L was therefore used for all experiments; it provided good response as well as sufficient reagent for the determination of high *N*-chloramine concentrations.

We also investigated the effect of postcolumn reaction pH, which was measured following the reactor coil. Figure 2D shows the pH effect of decreasing molar response with increasing pH, identifying a favorable pH region plateauing between pH 2 and 4. This result is not surprising because the postcolumn reaction consumes H^+, and the reaction between the iodide and *N*-chloramines becomes kinetically slow at higher pHs (2). Although pH 4 is near the sloping end of the optimal region, it was chosen over pH 2 for all subsequent experiments because there was less detector background current.

Reactivity of system materials

The FIA setup was used to explore chloramine reactivity to common materials used in LC systems. This was done by inserting frits of different compositions into the flow path before adding the postcolumn reagent. Two test compounds (*N*-Cl-LeuAla and monochloramine) were investigated with frits made of PEEK, stainless steel, titanium, and UHMWPE. Frits were chosen to have similar porosity and dimensions. Tests were performed with two different mobile phases: 0.01-mol/L phosphate buffer in water (pH 7.02) and 0.05-mol/L chloroacetic acid buffer in water (pH 2.75). The results are shown in Table I. All peak areas fall within the expected measurement variation without any observable trend. This set of experiments suggests that the material an LC system is made from is not a critical issue in the determination of chloramines. In an experiment in which an OCl^- solution was in contact with stainless steel frits for an extended period of time, losses were observed. Therefore, we employed PEEK-lined columns and PEEK frits in this work; PEEK is biocompatible and likely to be less reactive toward chlorinated analytes than metals.

Chromatographic optimization

Effect of mobile phase composition and residence time

The effect of an organic component in the mobile phase on LC recovery using the four test compounds was evaluated. The results for a mobile phase consisting of acetonitrile and a pH 2.75 aqueous component are shown in Figures 3A and 3B. Figure 3A is the figure for the FIA experiment. It may be observed that, although there is some loss of signal with increasing acetonitrile in the mobile phase, the data for all compounds delineate the same curve, which can be fit by a simple quadratic equation (presented in the figure). This slight dependence on acetonitrile content may reflect the solvent effect on the postcolumn reaction/detection efficiency.

In order to isolate LC column effects from small changes in the detector response that are caused by varying mobile phase compositions, a new parameter, the relative response factor (RRF), is used to depict the LC data and is defined as:

$$RRF = MR_{LC} / MR_{FIA} \qquad \text{Eq. 1}$$

where MR_{LC} is the molar response obtained for an analyte in an LC experiment, and MR_{FIA} is the molar response obtained for the analyte at the same mobile phase composition in the FIA experiment.

In the RRF data, MR_{LC} represents the average of three replicate measurements, and MR_{FIA} is obtained from the quadratic equation representing the FIA data for that solvent composition.

Figure 3B plots the relative response factor data collected for an acetonitrile–water mobile phase. Figure 3B reveals drastically differing responses for each of the four analytes, especially at low percentages of acetonitrile. It should be noted that the two peptides (*N*-Cl-LeuAla and *N*-Cl-AlaAla) have the most FIA-like behavior in that their responses are relatively consistent over a wide range of elution conditions. Monochloramine and *N*-chloropiperidine, on the other hand, show a trend of increasing molar response with increasing acetonitrile. At first glance, these results suggest that large water concentrations in the mobile phase (i.e., low acetonitrile) result in loss of the analytes oncolumn, with monochloramine and *N*-chloropiperidine being particularly susceptible. However, it must be realized that retention time varies with changing mobile phase composition (Figure 4).

To evaluate whether the observed analyte losses are affected by differing retention times, the oncolumn time was artificially extended by stopping the flow. Figure 5 is a plot depicting the changes in the RRF that occurred with time for two of the analytes. The linearity observed in this log plot implies that both analytes experienced a first-order decay process during their time on the column. Furthermore, when the lines were extrapolated to zero time, they did not reach an RRF of 1.0. This implies that there was an additional loss process that has a magnitude that is independent of the time an analyte spends on the column, and it varies for different analytes.

Monochloramine had virtually the same retention time for all acetonitrile–water ratios (Figure 4), yet its RRF varied with mobile phase composition (Figure 3B). Because the effect of mobile phase composition on the detector was normalized using the RRF values, all of the variation in the monochloramine RRF values noted was dependent upon mobile phase composition. Higher monochloramine losses are found for mobile phases containing larger percentages of water.

Table I. Measurement of Peak Areas When Various Frit Materials Are Placed in the FIA Flow Path*

Compound	Mobile phase pH	Peak area (no frit)	Peak area (PEEK)	Peak area (SS†)	Peak area (UHMWPE)	Peak area (titanium)
Monochloramine	2.75	32.2	31.8	31.1	31.7	31.2
Monochloramine	7.02	29.0	29.8	28.9	29.6	29.0
N-Cl-LeuAla	2.75	37.9	36.9	37.3	37.1	37.4
N-Cl-LeuAla	7.02	29.3	28.6	28.4	28.6	28.5

* All *N*-Chloramines were 15 µmol/L. The peak area units were µV·s (× 10^{-6}).
† SS, stainless steel.

Effect of organic component of the mobile phase

Three organic modifiers were investigated to determine whether the *N*-chloramine losses were related to the nature of the organic solvent used in the mobile phase. The first (acetonitrile) was discussed in the previous section, and the results for varying the acetonitrile concentration in the mobile phase are shown in Figure 3B. The results for the analogous experiment with methanol as organic modifier are shown in Figure 6. It may be observed that, even though the RRFs for the analytes followed the same trend as with acetonitrile, the RRF values were much lower, reaching a maximum of only 0.70. This figure suggests that even though most of the analyte decomposition occurs when the aqueous content of the mobile phase is high, the analyte losses nonetheless depend on the choice of organic component, with methanol providing relatively poorer responses.

A micellar eluent (SDS) was also investigated. In an FIA experiment in which concentrations of SDS were varied from 9 to 300 mmol/L, the responses obtained were significantly lower than those that were obtained with acetonitrile and methanol, especially at higher SDS concentrations. Also, experiments in which the pH of the SDS mobile phase was varied from 3.15 to 9.16, an LC experiment revealed substantial losses over all pHs, even though the analytes were not significantly retained on the analytical column. For example, one of the test *N*-chloramines (*N*-Cl-AlaAla) had a maximum RRF of only 0.55. Because of these two limitations, SDS is not useful as a mobile phase modifier for

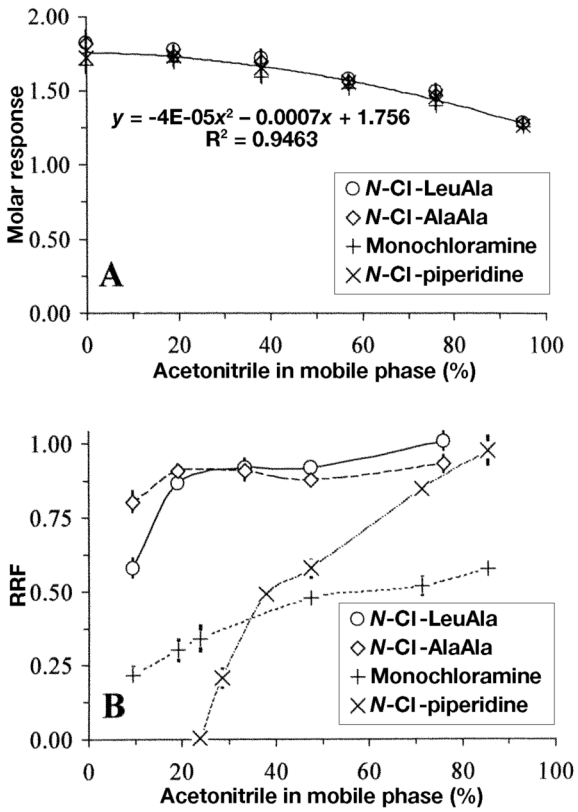

Figure 3. Effect of varying acetonitrile in the mobile phase on molar responses for test *N*-chloramines in FIA and LC: (A) FIA data and (B) RRF data for *N*-chloramines on a C18 LC column. All *N*-chloramines were 18 µmol/L.

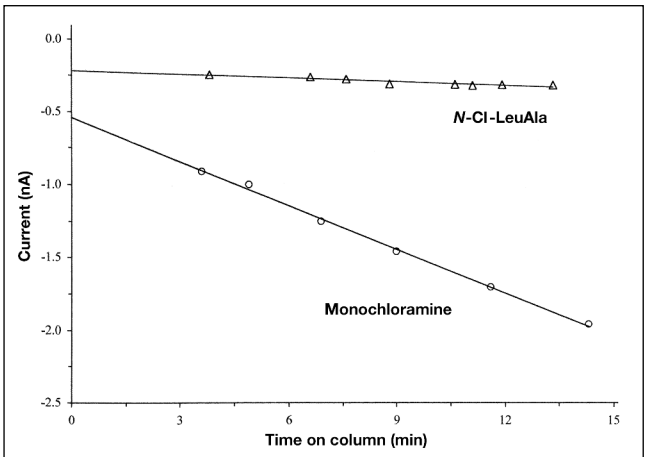

Figure 5. Effect of oncolumn time on the RRF for *N*-Cl-LeuAla and monochloramine on the C18 analytical column with the pH 2.75 water–acetonitrile mobile phase. Both chloramines were 20 µmol/L.

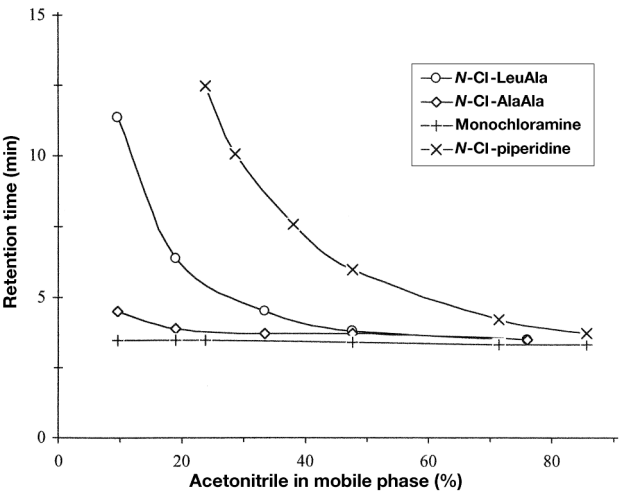

Figure 4. Effect of varying acetonitrile in the pH 2.75 mobile phase on retention time for the test set of *N*-chloramines for the experiment shown in Figure 3B.

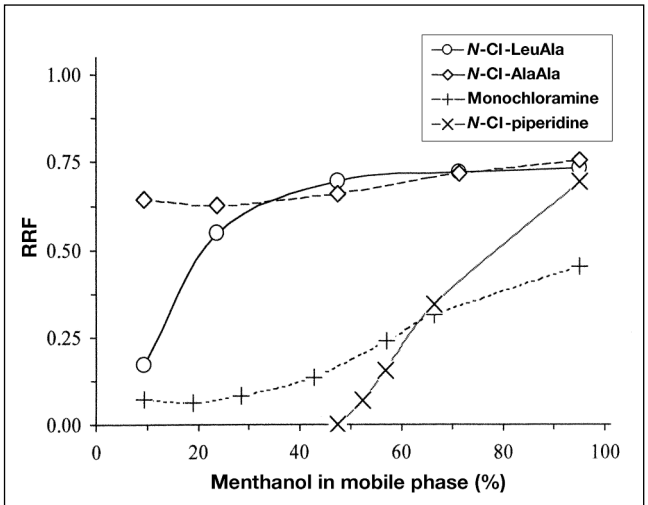

Figure 6. Effect of varying methanol in the pH 2.75 mobile phase on the RRF for test *N*-chloramines on the C18 analytical column. All *N*-chloramines were 20 µmol/L.

N-chloramine determinations. Because both SDS and methanol did not improve *N*-chloramine recoveries, acetonitrile was chosen as the organic solvent for all further experiments.

Effect of mobile phase pH

The experiments described to this point utilized a mobile phase containing a pH 2.75 aqueous component. Figure 7 presents data obtained with a pH 7.02 aqueous component. The higher pH mobile phase produces somewhat higher RRF values for all analytes (compare Figures 7 and 3B). Unfortunately, there is some ambiguity in this comparison; quantitating peak areas at high concentrations of acetonitrile proved difficult because broad multiple peaks were obtained. This may account for the RRF values having been greater than one.

One variable that was not well controlled in this experiment was the pH in the knitted coil reactor after the mobile phase had mixed with the pH 4 postcolumn reagent. For the lower pH mobile phase, a final pH in the reactor was 3.63, though for the higher pH mobile phase the final pH was 4.77. As can be discerned in Figure 2D, the molar response begins to be dependent upon the reactor pH in the vicinity of pH 4. Another experiment was run with the pH 7.02 mobile phase but with a chloroacetic acid buffered (pH 2.75) postcolumn reagent. In this case, the final pH in the reactor was 3.27. The results for this experiment (not shown) reveal that the lower reactor pH did not substantially improve the responses for each of the test analytes. It is therefore evident that the higher pH, phosphate-buffered mobile phase produces somewhat lower oncolumn losses. However, it should be noted that the retention of peptide chloramines was decreased by increasing the pH from 2.75 to 7.02, as a result of the deprotonation of the amino acid carboxylic acid group. Ultimately, the choice of the LC mobile phase pH may depend on the resolution required for the separation of *N*-chloramine mixtures.

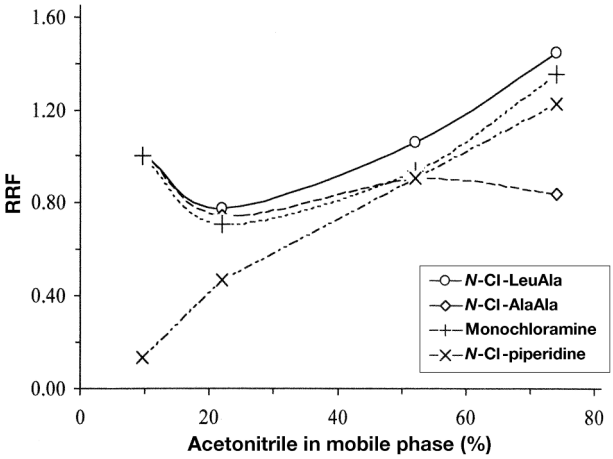

Figure 7. Effect of varying acetonitrile in the pH 7.02 mobile phase on the RRF for test *N*-chloramines in a chromatography experiment with the C18 analytical column. All *N*-chloramines were 20 µmol/L.

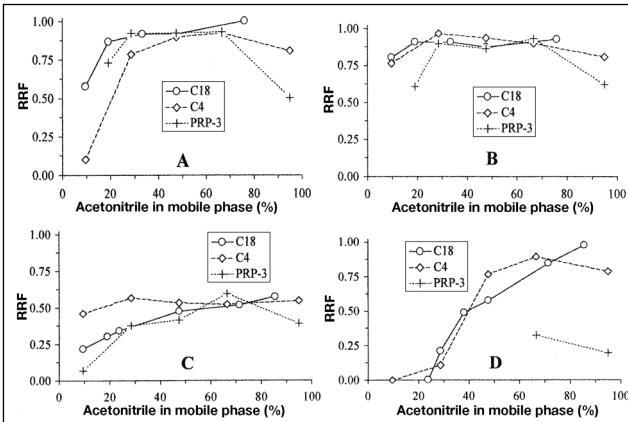

Figure 8. Comparison of RRF values obtained for each *N*-chloramine on three different columns: C18, C4, and PRP-3. (A) *N*-Cl-LeuAla, (B) *N*-Cl-AlaAla, (C) monochloramine, and (D) *N*-Cl-piperidine. The mobile phase used was pH 2.75. For the C18 column, all *N*-chloramines were 18 µmol/L. For the C4 and PRP-3 columns, the *N*-chloramines were 20 µmol/L.

Effect of column temperature

Two experiments determined whether oncolumn losses were affected by changing the column temperature. A water–acetonitrile mobile phase (pH 7.02) was used. In the first experiment, the mobile phase components and the column were cooled with ice prior to sample injection. This resulted in a mobile phase that was 17°C as measured prior to mixing with the postcolumn reagent. The temperatures of the postcolumn reagent as well as the knitted coil were held at 40°C with a column heater to counteract the effect of cooling the mobile phase. No measurable differences were observed in peak areas obtained at 17°C versus room temperature (~23°C). Evidence that we successfully lowered the column temperature was observable in the slightly longer LC retention times obtained.

The second experiment involved heating the column to 50°C. For this experiment, the temperature of the postcolumn reagent and knitted coil were also held at 50°C. Again, no significant differences in peak areas for *N*-Cl-LeuAla could be measured. These experiments indicate that oncolumn losses are essentially independent of temperature.

Effect of other stationary phases

Several analytical columns were tested to explore whether column composition was a factor in oncolumn losses of analytes. The first alternative column investigated was a C4 column having the same silica base as the C18 column. The second alternative

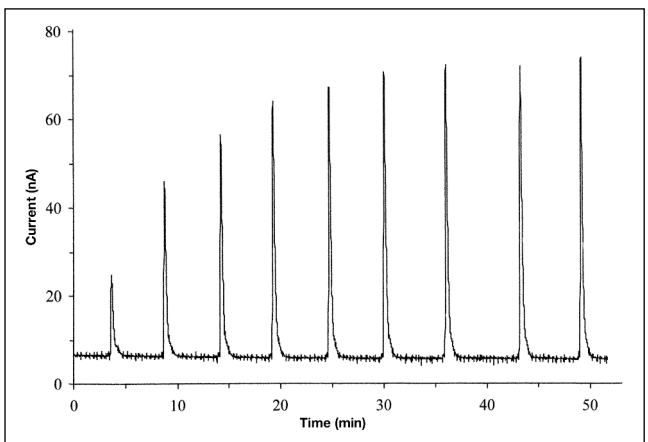

Figure 9. Chromatogram of successive monochloramine injections after the C18 column was left overnight with a mostly aqueous mobile phase.

was a column packed with spherical poly(styrenedivinylbenzene) particles and containing no silica support phase. Results for these and the C18 column are compared in Figure 8. The polystyrene column provides lower responses for each of the four analytes under most conditions. Furthermore, we found it unsuitable for the separation of *N*-chloramines because complete analyte decomposition was associated with solvent compositions, providing retention longer than the column void volume. On the other hand, the curves for the C18 and C4 columns were similar for each analyte. Although there are instances in which the C4 column provides a slightly higher RRF for an analyte than the C18 column, the C18 column offers a better prospect of chromatographic resolution of the analytes.

Possibility of reactive contaminants in the mobile phase

The observation that higher *N*-chloramine losses occurred with predominantly aqueous mobile phases suggested the possibility that the aqueous component contains a chloramine-consuming contaminant that may accumulate on the column. Using the C18 column, we tested this idea in several ways. In the first approach, potential contaminants were accumulated by pumping a mostly aqueous mobile phase (15% acetonitrile, pH 7.02) for 1 h and allowing overnight equilibration. Subsequently, nine monochloramine injections were made over a short period of time. Figure 9 shows that the signal increases as expected if a certain amount of reactive contaminant had built up on the column during the conditioning period. However, the demand caused by this contaminant was quickly exhausted when the chloramine injections began. This led us to routinely reject the first five injections of the day. Even after reproducible signals were achieved for the last four injections in Figure 9, the monochloramine RRF was still less than unity, with a value of 0.7. When the analogous experiment was performed by conditioning overnight with a mostly organic mobile phase (70% acetonitrile, pH 7.02), the signal increase in Figure 9 was not observed.

Secondly, we replicated an experiment performed by Furness-Green et al. (14) by injecting a large amount of hypochlorite into the system prior to analyte injection. Hypochlorite is both a stronger and more labile oxidizing agent than chloramines and would be expected to preoxidize any chloramine-reducing contaminants. No improvement in *N*-chloramine peak areas were achieved with this approach.

Finally, we evaluated pumping the column with a very dilute hypochlorite solution for several hours to allow for the more complete removal of accumulated contaminants. Again, no improvement in *N*-chloramine peak areas was achieved. We concluded that oxidizable contaminants are no more than a minor problem and do not account for RRF values of less than 1 in our LC experiments with chloramines.

Analytical performance

Linear calibration curves for all analytes in LC and FIA were noted for both mobile phase pH conditions investigated. Using the optimized conditions, linear regression data for the four model compounds over the concentration range of 0.7 to 25 µmol/L are shown in Table II. All curves show correlation coefficient values (R^2) greater than 0.996. Nearly equivalent slopes were obtained for monochloramine, *N*-Cl-AlaAla, and *N*-Cl-LeuAla, though *N*-Cl-piperidine gave a significantly lower response.

Excellent reproducibility was achieved provided that the first 5 injections of the day are rejected (see Figure 9), the coefficient of variation of peak height is 2%, and the coefficient of variation of peak area was 0.9% (n = 11).

Using the optimum conditions, detection limits for the four test compounds were determined as the intersection of the calibration lines over a 0.7–18-µmol/L concentration range, with the value of the peak-to-peak baseline noise evaluated over a time interval equivalent to the peak width. The detection limits were estimated to be 1.2×10^{-7}, 1.3×10^{-7}, 2.0×10^{-7}, and 6.3×10^{-7} mol/L for *N*-Cl-AlaAla, *N*-Cl-LeuAla, monochloramine, and *N*-chloropiperidine, respectively, using a 20-µL injection volume. These limits improved on our previous work (17) by roughly a factor of 10. Of particular significance, total residual chlorine in disinfected wastewaters and drinking waters is commonly in the 10^{-5}–10^{-4}-mol/L range. Thus the LC method described here now has the capacity to quantitate chloramine components that comprise a few percent or less of the typical total residual chlorine concentration. Because the detection system is selective for iodide-oxidizing components, interferences from the myriad of organic compounds in wastewaters should be minimal.

The ability of the optimized conditions to separate a mixture of the four test *N*-chloramines is shown in Figure 10. The mixture was prepared to have equimolar concentrations of the four

Table II. Linear Regression Data for Four Test *N*-Chloramines

Compound	Slope (nA•s•(µmol/L)$^{-1}$)	*y*-Intercept (nA•s)	R^2
N-Cl-LeuAla	59.6	7.35	0.9983
N-Cl-LeuAla	54.0	28.2	0.9962
Monochloramine	54.3	35.6	0.9977
N-Cl-piperidine	17.4	35.9	0.9972

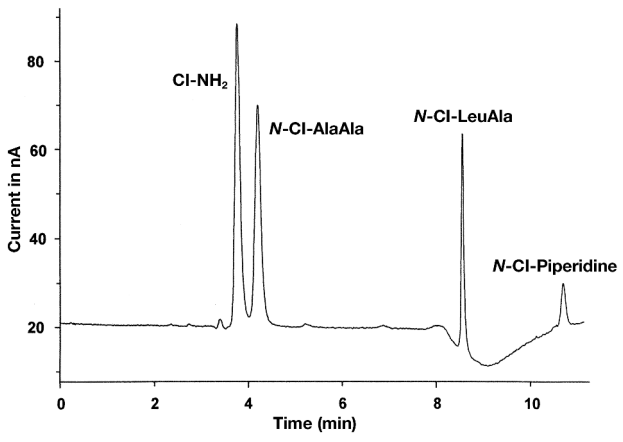

Figure 10. Separation of the four test *N*-chloramines using the optimized conditions. The sample was prepared by mixing an equivalent amount of each of the *N*-chloramines, individually prepared as 250-µmol/L amine with 20-µmol/L OCl^- added, using a 0.5-mmol/L phosphate buffer (pH 7).

N-chloramines from individual solutions, each containing an excess of free amine. However, the peak areas for the four components were not equal. Two factors contributed to the differences in the peak areas: equivalent molar responses were not obtained between the four analytes, and *N*-chloramines in the presence of excess free amines redistributed the active chlorine with time (15).

Discussion

A goal of this research has been to develop an LC system that provides an equivalent molar response for unstable chloramines in complex matrices. As judged by identical signals for all of the test compounds using FIA (Figure 3A), the postcolumn detection system has been optimized to the point that equivalent molar responses are achieved. Unfortunately, the introduction of the LC separation column results in analyte-dependent signal losses that are consistent with the known reactivity of the different chloramines. Losses are most severe with monochloramine and *N*-Cl-piperidine. These chloramines are better proton acceptors and are consequently more reactive than the *N*-chloropeptides in many reactions, including reduction reactions (6). Greater losses at lower pH (Figure 3B versus 7) and at comparable concentrations of methanol versus acetonitrile (Figure 6 versus 3B) are consistent with Brønsted acid catalysis, which is common in chloramine reactions.

For all analytes except *N*-chloropiperidine, the first step in reactive losses may proceed through chloramine disproportionation:

$$2RNHCl \rightarrow RNCl_2 + RNH_2 \qquad \text{Eq. 2}$$

This disproportionation is favored by acid catalysis at low pH and inhibited by excess amine (19,20). In order to minimize this reaction, the chloramine samples were always prepared at neutral pH and with excess free amine. In the FIA mode, in which the analyte and excess amine are not separated, this reaction is not favored, resulting in maximum detector responses. In the LC mode, the separation of the chloramine from the parent amine may initiate disproportionation, particularly with the pH 2.75 mobile phase. Lower detector signals in the LC mode would occur after disproportionation for two reasons. Dichloramines formed as the chloramines traverse the column and would be dissipated and "lost" to the chromatographic peak area because of greater retention, thereby contributing only to the background detector current. Relative to monochloramines, dichloramines also could show enhanced reactivity toward reducing contaminants or exhibit autodecomposition. Organic dichloramines autodecompose by dehydrohalogenation, which produces chlorimines and nitriles with the loss of one and both equivalents of oxidizing capacity, respectively (21,22). *N*-chloropiperidine also autodecomposes to an imine (23). In contrast, $NHCl_2$ autodecomposes primarily to N_2 (20,24).

Two objections can be raised to the hypothesis that disproportionation is involved in the primary loss initiation step, based on the known behavior of chloramines in free solution. First, disproportionation is kinetically relatively slow, and second, the rate law that describes disproportionation is second order in chloramine concentration, at least in the case of $NHCl_2$ (19). The linearity of the calibration curves (Table II) and the linearity of the ln(chloramine) versus time plot (Figure 5) would appear to rule out a second order loss process. Thus, if oncolumn losses occur through disproportionation, a special mechanism, probably involving catalysis by a column component, must be at work.

Defining a clear source of chlorine-consuming reductants in the LC experiment has proven elusive. Experiments with different frit materials, columns, and NaOCl preoxidation of columns seem to exonerate the LC column as the direct source of the reductants. Although organic modifiers in the mobile phase might reasonably be suspected as consumers of oxidizing analytes, this hypothesis is contradicted by the observations that the amount of loss diminished as the amount of modifier increased, and that losses occurred with chemically diverse modifiers.

Fortunately, we have identified a set of LC operating conditions in which the losses of monochloramine, *N*-Cl-AlaAla, and *N*-Cl-LeuAla are minimized and linear calibration curves are obtained. The nearly identical slopes indicate that a calibration based on any of these chloramines could be used to estimate active chlorine in a wastewater sample. Calibration of analytes exhibiting higher losses (such as *N*-chloropiperidine) would not be amenable to such an approach. If it were desirable to determine *N*-Cl-piperidine, it could be calibrated independently. However, in dechlorinated wastewaters, most residual chloramines are expected to be peptide and protein chloramines (6,7,17); these should display the favorable behavior we have found for *N*-Cl-AlaAla and *N*-Cl-LeuAla.

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