Development of a Size Exclusion Chromatography–Electrochemical Detection Method for the Analysis of Total Organic and Inorganic Chloramines

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Abstract

A size exclusion chromatography (SEC) followed by post-column reaction with iodide and electrochemical detection method is developed for analysis of total organic and inorganic chloramines. Ammonium chloride and a group of test compounds (Glycine, Tyrosine, DL-Alanyl-DL-Alanine, Arg-Gly-Asp-Ser, Bradykinin, Aprotinin, and α-Lactalbumin) are selected and chlorinated to represent inorganic chloramines and different sizes of organic N-chloramines. An analytical SEC column with pore size of 60Å is used and chromatographic conditions including the working electrode potential and flow rate are optimized to gain optimum resolution and sensitivity. The detection limits are estimated to be 0.12 mg/L and 0.05 mg/L, respectively, for tested inorganic and organic chloramines.

Introduction

Chlorination is the most widely-used process for drinking water disinfection because of its ability to inactivate a wide variety of pathogenic microorganisms, but understanding and controlling this process is still challenging.

Organic N-chloramines which are produced due to the interaction of organic amines with chlorine (1–5) are of special concern because they act as precursors in the formation of specific disinfection byproducts (DBPs), such as dihaloacetonitriles (6) and cyanogen chloride (7), and because their toxicity is still largely unknown. However, there is evidence that organic N-chloramines are much less bactericidal than chlorine and inorganic chloramines (8–11), and that most conventional analytical methods, including N,N-diethyl-p-diphenylenediamine ferrous ammonium sulfate (DPD-FAS) titrimetric method and DPD-FAS amperometric method, cannot be used to differentiate between inorganic and organic chloramines (12–15).

Reversed-phase liquid chromatography followed by post-column reaction with iodide and electrochemical detection has been used for the analysis of individual organic N-chloramines (16–18). However, while these methods are assets for determining the toxicity and disinfection ability of individual organic

chloramines, they do not provide data that is needed for the characterization of residual chlorine to control disinfection processes. For residual chlorine characterization, we need to quantitate the main chlorine-bearing groups, such as free chlorine, inorganic, and organic N-chloramines. Size exclusion chromatography–electrochemical detection (SEC–ED) has been used for recognition of organic Cl^+-bearing groups in wastewater based on their molecular size (19), but quantitation optimization has not been addressed.

The main goal of this study is to fill the analytical gap by developing a SEC-postcolumn electrochemical detection method for quantitative analysis of total inorganic and organic chloramines in chlorinated water. Ammonium chloride and a group of test compounds with molecular weight in parentheses [Glycine (75 amu), Tyrosine (181 amu), DL-Alanyl-DL-Alanine (160 amu), Arg-Gly-Asp-Ser (433 amu), Bradykinin (1060 amu), Aprotinin (6412 amu), and α-lactalbumin (14200 amu)] were selected and chlorinated to represent inorganic chloramine and different sizes of organic N-chloramines. Chromatographic conditions such as the working electrode potential and flow rate were optimized to gain optimum sensitivity and resolution between the inorganic and organic N-chloramine peaks. This method allows a number of different organic chloramines to be measured as a group, as observed for the tested organic chloramines and could be used for quantitative analysis of both total organic and inorganic chloramines.
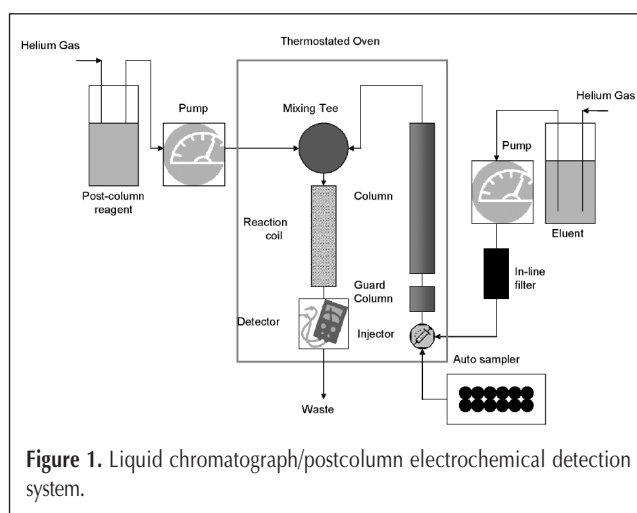

Figure 1. Liquid chromatograph/postcolumn electrochemical detection system.

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Materials and Methods

Reagents

Ultrapure water was prepared from deionized tap water using a Milli-Q ion exchange and activated carbon cartridge treatment system (Millipore, Milford, MA). Phosphate buffer (0.02M, pH = 6.9) was prepared using potassium phosphate monobasic (for liquid chromatography, J.T. Baker) and potassium phosphate dibasic (for liquid chromatography, J.T. Baker). The buffer solution was used directly as the HPLC mobile phase and also for the preparation of chloramine solutions. Ammonium chloride (99.5% BDH Inc., Toronto, Canada) was used for monochloramine preparation. Glycine (J.T. Baker, ultrapure bioreagent, 99.5%), Tyrosine (Fluka, ≥ 99%), DL-alanyl-DL-alanine (Aldrich, St. Louis, MO), Arg-Gly-Asp-Ser (≥ 95% Sigma, Busch, Switzerland), Bradykinin (≥ 97%, Calbiochem, La Jolla, CA), Aprotinin from Bovine Lung (Calbiochem, ≥ 95%), and α-Lactalbumin from bovine milk (Sigma, ≥ 85%) were used to represent different-sizes of organic amines. Sodium hypochlorite solution (6%, VWR, Philadelphia, PA) was diluted to 0.1mM, and its concentration was determined by the DPD colorometric method (Clesceri et al., 1995). The standardized solution was used for the preparation of N-chloramine solutions. The postcolumn reagent solution containing potassium iodide (EMD, 99.0%) 0.09M, sodium acetate (J.T. Baker, 99.9%) 0.294M, and glacial acetic acid (EM Science, Gibbstown, NY HPLC grade) 1.4% was prepared daily and used fresh.

Chlorine-demand-free glassware was used for making and storing all chlorine and chloramines solutions. The glassware was prepared by adding 1 mL of a 1:10 dilution of commercially available chlorine solution (Javex, Toronto, Canada), filling with deionized water, and allowing it to stand overnight followed by rinsing with deionized water immediately before use.

System setup

The SEC–ED system set up for the analysis of chloramines is shown in Figure 1. A two-pump LC system (Dionex DX500 Sunnyvale, CA) with an automated eluant gradient controller was employed. Separation was achieved with a Macrosphere GPC analytical column (60 Å 7.5 × 300 mm) along with a Macrosphere GPC (300 Å 4.6 × 7.5 mm) guard column (Grace Discovery Sciences, Deerfield, IL). This column material consists of a diol-modified porous silica packing that was developed for peptide and protein separation. It is capable of resolving molecules in the exclusion range of 250–28,000 Daltons, enabling the separation of inorganic chloramines from organic chloramines, which include larger molecules. As well, with this column there is minimal loss of analyte during separation because of the absence of high-energy sorptive forces relative to other LC methods.

The separation and guard columns were stainless steel lined and fritted. All other tubing and fittings used post-pump were PEEK (polyethyletherketone).

A postcolumn pump (PC 10 Pneumatic controller, Dionex) was used to deliver the postcolumn reagent. The reactor was a 375 μL knitted fluoropolymer coil. Detection of iodine formed in the post-column reactor was achieved with amperometric detection via a thin-layer glassy carbon electrode (Dionex ED 40 elec-

trochemical detector). The injector, guard and separation columns, mixing tee, reaction coil, and detection cell were kept in a thermostated oven. Samples were introduced into the LC system by direct injection with a 100 μL sample loop. Separation was achieved by isocratic elution using 0.02M phosphate buffer, pH = 6.9. PeakNet 5.1 software was used for data acquisition and processing.

It has been shown that of the different parameters affecting the detection signal of organic chlormines (such as temperature, reaction time, iodide concentration, and reaction pH), only the reaction pH is significant and the maximum signal value is obtained at a pH lower than 5.0 (16). Therefore, for this study the detector parameters were adjusted as follows: temperature, 35°C; reaction time (R), 15 s; iodide concentration, 0.09M; and reaction pH, 4.5.

The same setup with minor adjustments (including elimination of postcolumn reaction) was used for the direct detection of the non-chlorinated forms of the tested amino acids, peptides, and proteins. Electrochemical detection is conventionally used for detecting amino acids including tyrosine, tryptophan, cysteine, and peptides containing these amino acids (20–22).

Experimental procedures

Tested compounds (ammonium chloride, glycine, tyrosine, DL-alanyl-DL-alanine, Arg-Gly-Asp-Ser, bradykinin, aprotinin, and α-lactalbumin) were separately chlorinated by combining equal volumes of a 1.0mM solution of a test compound (in 0.02M phosphate buffer pH = 6.9) and a 0.1mM sodium hypochlorite solution (in 0.02M phosphate buffer pH = 6.9). They were mixed in amber bottles and allowed to equilibrate for 1 h. This was expected to yield stock solutions containing 0.1mM of each organic-N-monochloramine.

A 0.1mM solution of monochloramine and 0.05mM N-chloroglycine (N-Cl-Gly) solution was prepared by diluting the stock solutions in Milli-Q water. These solutions were used as standard samples to represent inorganic and organic chloramines in experiments to optimize the mobile phase flow rate and detector working potential. They were also used for testing the reproducibility of response signals.

The reproducibility of response signals was tested using 10 injections of each of the monochloramine 0.1mM and N-chloroglycine 0.05mM solutions under the following conditions: mobile phase flow rate, 1.0 mL/min; post column pressure, 40 psi; working electrode potential, 0.1 V.

The working potential for the electrochemical detector was optimized by preparing and analyzing a hydrodynamic voltamogram of monochloroglycine for a working potential range of –0.5 to 0.7 V.

For flow rate optimization, each monochloramine 0.1mM and N-chloroglycine 0.05mM solution was analyzed at flow rates in the range of 1–2.5 mL/min. The flow rate, which provided optimum sensitivity and resolution, was determined. Resolution of monochloramine N-chloroglycine peaks (R_S) at each flow rate was calculated using following equation (23):

$$R_s = 1.18 \frac{(t_G - t_M)}{(w_G + w_M)} \qquad \text{Eq.1}$$

where t_G and t_M are the retention times of N-Cl-Gly and monochloramine in min w_G and w_M are the band widths of N-Cl-

Gly and monochloramine peaks at half-height in min.

Solutions of monochloramine (NH$_2$Cl), N-Cl-Gly, N-chlorolanylalanine (N-Cl-AlaAla), and N-chloroarginilglycilaspartilserine (N-Cl-ArgGlyAspSer) in the concentration range of 0.1 to 10 mg/L as Cl$_2$ were prepared for calibration by diluting the stock solutions in Milli-Q water. All N-chloramines were injected individually to prevent possible chlorine-transfer reactions. Because of the unstable nature of chloramines, the stock and standard solutions were made fresh every day.

The limits of detection were determined using a statistical method from conventional linear regression of data used for the calibration (24). It was defined as the analyte concentration giving a signal equal to blank signal, y_B, plus three times the standard deviation of the blank, s_B (Equation 2). The terms y_B and s_B are obtained from conventional linear regression of data used for the calibration. The intercept value was used as the blank signal (y_B), and the standard deviation of the residuals as the standard deviation of the blank (s_B).

$$y = y_B + 3s_B \qquad \text{Eq. 2}$$

Results and Discussion

Qualitative analysis

Figure 2 shows typical chromatograms of 0.005mM solutions of tested organic N-chloramines in comparison with monochloramine 0.005mM and sodium hypochlorite (free chlorine) 1mM. These chromatograms were obtained under the following analytical conditions: mobile phase flow rate 1.0 mL/min; post column pressure 40 psi, detector potential 0.1 V versus Ag/AgCl refrence. All tested organic chloramines appear at a retention time of approximately 10 min and are successfully separated from monochloramine, which appears at a retention time of 12.5 min. Small differences in the retention times exhibited by the organic chloramines shown in Figure 2 mainly resulted from the runs being obtained on several different days. Free chlorine appears as a separate peak at a retention time of approximately 15 min.

No response signal was detected for the larger chlorinated peptides that were tested (bradykinin, aprotinin, and α-lactalbumin) regardless of the N/Cl ratio employed. To ascertain that all potential organic chloramines in the molecular weight range
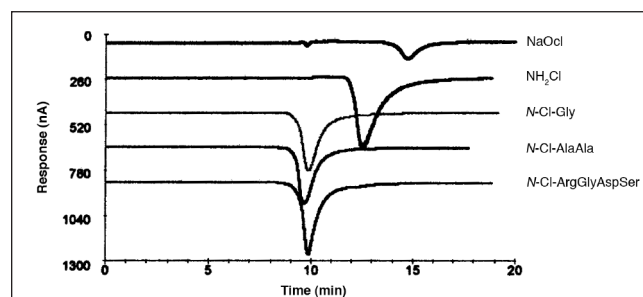
of approximately 100–14000 amu appear at the same retention time to be measured as total organic chloramines, the retention time of tyrosine and two large peptides (aprotnin and α-lactalbumin) were determined by direct electrochemical detection. As SEC separates compounds based on their molecular size, it was expected that each organic amine would have approximately the same retention time as its corresponding chloramine. The chromatograms of aprotnin and α-lactalbumin are compared with the chromatogram of tyrosine in Figure 3. The analysis conditions were as follows: the mobile phase flow rate, 1.0 mL/min; detector potential, 0.9 V versus Ag/AgCl reference. As expected, the tested non-chlorinated amino acids and peptides have the same retention time as the tested organic N-chloramines.

Response signal reproducibility

The results of the signal reproducibility experiment (Figure 4A) show that monochloramine signal increased significantly (about 3 times) from the first injection to the fifth whereas the last six injections produced equivalent signals. The relative standard deviation (RSD) of last six injections was 3.1%. It seems that there is an initial reaction between the column material and monochloramine that diminishes its response signal, but once this initial oxidant demand is satisfied, the system is stable for
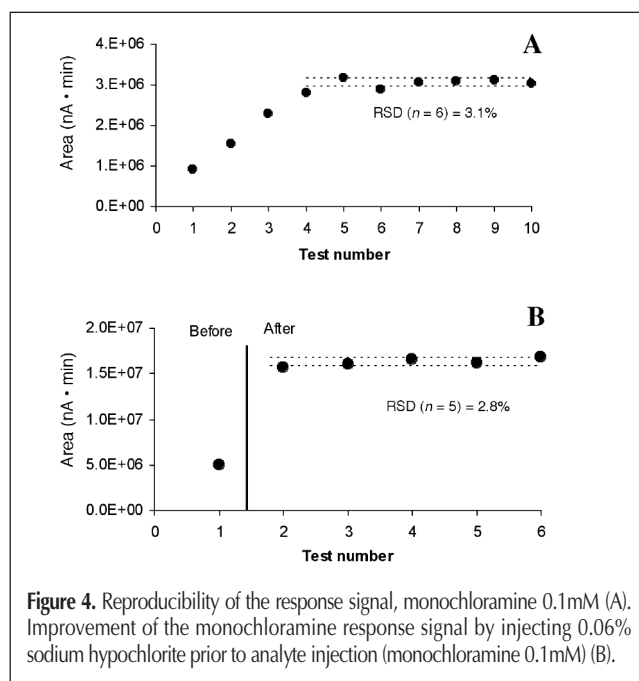

Figure 4. Reproducibility of the response signal, monochloramine 0.1mM (A). Improvement of the monochloramine response signal by injecting 0.06% sodium hypochlorite prior to analyte injection (monochloramine 0.1mM) (B).
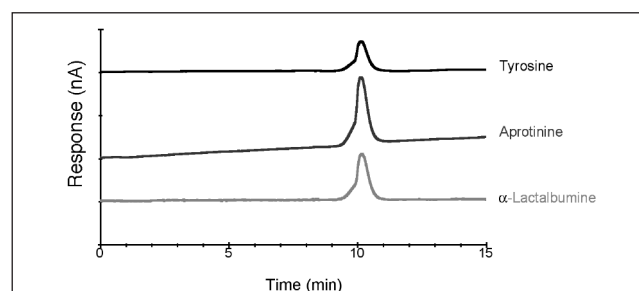

Figure 2. Comparison of the chromatograms of tested organic chloramines with free chlorine and monochloramine. Chromatograms from top to bottom: chlorine, monochloramine, N-chloroglycine, N-chloroAla-Ala, and N-chloroArg-Gly-Asp-Ser. Mobile phase flow rate 1.0 mL/min, detector potential versus Ag/AgCl reference 0.1 V, temperature 35°C, iodide concentration 0.09M, reaction pH 4.5, reaction time 15 s.

Figure 3. Chromatograms of tested proteins in comparison with tyrosine. Chromatograms from top to bottom: tyrosine, aprotnine, and α-lactalbumin, mobile phase flow rate 1.0 mL/min; detector potential Ag/AgCl versus 0.9 V reference.

subsequent monochloramine injections. No evidence of consumption of N-chloroglycine due to the reaction with column material was observed, which might be explained by the relatively weaker oxidative behavior of organic chloramines. Monochloramine is a better proton acceptor and, consequently, more active than organic chloramines in reduction reactions.

In order to eliminate the chlorine demand of the separation column, a 0.06% solution of sodium hypochlorite was injected into the column, and the reproducibility of the response signals was evaluated for five subsequent injections of a 0.1mM monochloramine solution. As illustrated in Figure 4B, the monochloramine response signal (peak area) increased by approximately three times with this approach and signals were very reproducible. The relative standard deviation (RSD) of last six injections was 2.8%. It is recommended that this approach be applied every day before injection of samples to eliminate the column chlorine demand and achieve high reproducibility. Such applications of oxidant were not observed to have any adverse effect on the long-term stability of the column in that there was no evidence of material degradation during the 1.5 year continuous operation of the equipment during these method evaluation efforts.
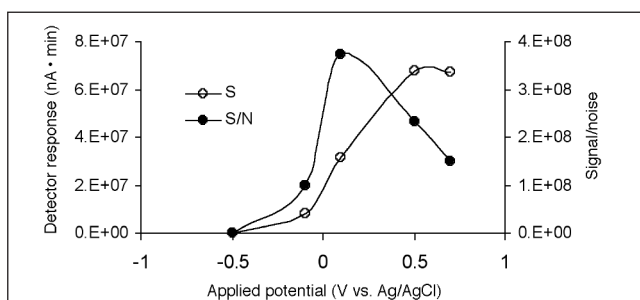

Figure 5. Hydrodynamic voltamogram of N-chloroglycine, clear circles represent the signals (peak areas) and full circles represent the signal to noise ratios.
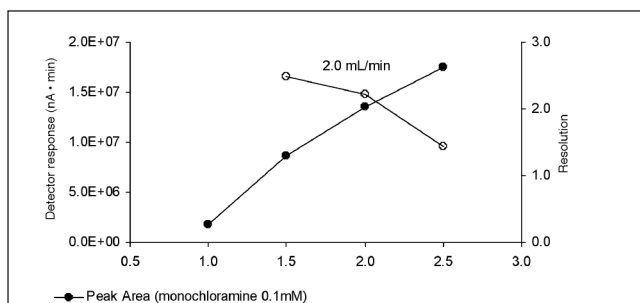

Figure 6. Variation of the response signal of monochloramine 0.1mM and its resolution with N-chloroglycine 0.005mM by changing the mobile phase flow rate.

Detector optimization

In order to ascertain the optimum working potential for the electrochemical detector, the hydrodynamic voltamogram of N-chloroglycine was run. A solution of 0.005mM N-chloroglycine was injected at working potentials of –0.5, –0.1, 0.1, 0.5, and 0.7 V, beginning with the highest voltage. Once stabilized, the peak responses were measured for repeat injections at successive reductions in the applied potential (25). The peak response (peak area) in nA.min and the peak-to-peak baseline noise in nA.min was measured at each voltage, and the output signals and the signal-to-noise ratios (S/N) were plotted in a voltamogram. The voltamogram (Figure 5) confirmed that a voltage of 0.1 V provides a proper balance between sensitivity and S/N ratio.

Flow rate optimization

The influence of the flow rate on the system performance was investigated in order to determine the optimum flow rate to provide appropriate sensitivity and peak resolution. Monochloramine and N-chloroglycine solutions with concentrations of a 0.1mM and 0.005mM were analyzed at flow rates in the range of 1–2.5 mL/min. These concentrations were selected because they produce similarly sized peaks for monochloramine and N-chloroglycine. Because changing the flow rate effects the iodine concentration and pH of the mixture in the reaction coil, the pressure of the post-column pump was adjusted to keep the pH of the mixture at 4.5 at all tested flow rates.

The response signal of monochloramine increased significantly by increasing the flow rate but no significant change in response signal was observed in the case of N-Cl-Gly. A relative standard deviation (RSD) of 14.1% was achieved for response signals of N-Cl-Gly measured at four tested flow rates.

For flow rate optimization, the response peak of monochloramine and its resolution with N-Cl-Gly were compared at the tested flow rates (Figure 6). Although, even at a flow rate of 2.5 mL/min, the peaks were satisfactorily separated and a resolution of 1.5 was obtained, a flow rate of 2.0 mL/min was selected as the optimum flow rate to prevent peak overlap if the concentration of inorganic chloramines was to be much higher than organic chloramines.

Analytical performance

Nearly equivalent responses were obtained for all tested organic chloramines with same concentration under optimized conditions (flow rate 2.0 mL/min, detector working potential of 0.1 mV, postcolumn pressure 70 spi), but monochloramine exhibited a significantly lower response.

Using the optimized conditions, linear regression data for the four model compounds over a concentration range of 0.1 to 10 mg/L as Cl_2 are shown in Table I. All curves show correlation coefficient values (R^2) greater than 0.99. The identical responses for tested organic chloramines indicate that a calibration based on any of the tested chloramines could be used to estimate total organic chloramines.

Detection limits were estimated to be 0.12 mg/L for monochloramine and 0.05 mg/L for the tested organic chloramines. Because the total residual chlorine in drinking waters and

Table I. Linear Regression Data for Model Compounds

Chloramine	Limiting range (mg/L Cl_2)	Number of points (–)	Slope [(nA.min)/(mg/L Cl_2)]	Intercept (nA.min)	Regression Coefficient R^2
Monochloramine	0.1–10	7	(6 ± 0.5) E+06	(–1.5 ± 0.3) E+06	0.999
N-Cl-Gly	0.07–7.1	10	(28 ± 1) E+06	(–1.4 ± 3.2) E+06	0.999
N-Cl-AlaAla	0.07–7.1	10	(34 ± 1) E+06	(0.0 ± 4.8) E+06	0.999
N-Cl-ArgGlyAspSer	0.07–7.1	10	(36 ± 1) E+06	(0.4 ± 2.1) E+06	1.000

disinfected wastewaters is commonly in the range of 0.5–10 mg/L, the method described here is sensitive enough for detection of the total organic chloramines in low-chlorine waters if they comprise one tenth or more of the total residual chlorine concentration. Although not included herein due to space limitations, this method has been used successfully for drinking water disinfection studies for which manuscripts are in preparation.

Conclusions

An SEC followed by post-column reaction with iodide and electrochemical detection method has been developed for analysis of chloramines. The developed method fills an analytical gap, which existed between measuring total residual chlorine and individual chloramines by allowing the direct analysis of total organic and inorganic chloramines. It allows the separation of organic and inorganic chloramines and quantitation of them as total organic and inorganic chloramines.

Equivalent molar signals were observed for all tested organic chloramines, and no evidence of decomposition or consumption of organic *N*-chloramines during elution was observed. Any of the tested peptides or amino acids could be used as a standard compound for the quantification of total organic chloramines.

Evidence of monochloramine loss during analysis was observed. The monochloramine loss decreased with NaOCl pre-oxidation of the column and also by decreasing the on-column time of monochloramine (increasing the flow rate). These results could be evidence of the loss of the monochloramine by a mechanism involving its reaction with the column material.

SEC and postcolumn detection systems were optimized and a set of operation conditions were identified in which the loss of monochloramine was minimized and linear calibration curves were obtained for all of the chloramines tested.

This analytical method could be used in studies to estimate the disinfection efficiency of residual chlorine in chlorinated water and wastewaters based on the relative concentrations of total organic and inorganic chloramines.

Acknowledgments

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