Chemiluminescence nitrogen detection in ion chromatography for the determination of nitrogen-containing anions

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Abstract

Chemiluminescence nitrogen detection (CLND) provides equimolar response for nitrogen-containing ions such as nitrate, nitrite, cyanide, ammonium and tetradecyltrimethylammonium. Only azide yields a lower response. Nitrite, azide and nitrate are separated on a Dionex AS11 column using 5 mM NaOH as eluent with a 3 μM (1 ng N) limit of detection. Matrices, such as 1:10 diluted seawater, do not degrade these detection limits. CLND also provides equally sensitive (limit of detection 3 μM, 78 ppb) detection of weak acids such, as cyanide, which yield poor sensitivity with suppressed conductivity detection. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Chemiluminescence nitrogen detection (CLND) offers a detection method that is both specific and universal. CLND is specific in that it responds only to analytes containing nitrogen. It is universal since its response factor is solely proportional to the number of nitrogen atoms in the analyte structure. The advantages of coupling this detector to high-performance liquid chromatography (HPLC) have been clearly demonstrated in the analysis of pharmaceuticals [1], proteins [2], peptides [3], and organic synthesis products [1,4], in the determination of urinary nitrogen content [5], and in the detection of ammonium in wastewater [6]. In this paper, the application of CLND to ion chromatographic analysis is investigated.

In CLND, the sample is first combusted at high temperature ($>1000°C$) [7,8]:

$$R-N + R-H + O_2 \rightarrow CO_2 + H_2O + NO +$$

other combustion products (1)
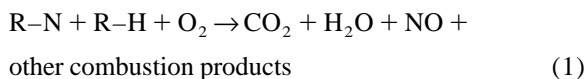
where R–N is any chemically bound nitrogen compound and R–H is any non-nitrogenous organic compound. Subsequent reaction between ozone and nitric oxide (NO) produces nitrogen dioxide in the excited state (NO_2^*):

$$NO + O_3 \rightarrow NO_2^* + O_2 + 47.8 \text{ kcal/mol} \quad (2)$$
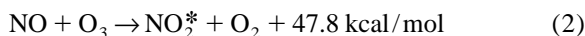
(1 cal = 4.184 J). The excited nitrogen dioxide's rapid relaxation results in the release of a photon of light, between 600 and 900 nm, which is then captured and amplified by the photomultiplier tube [7–9]:

$$NO_2^* \rightarrow NO_2 + h\upsilon \quad (3)$$

Other combustion gases such as CO_2 and H_2O do not react with ozone to produce any discernable chemiluminescence, and so do not affect the signal. So long as the ozone in Reaction 2 is in great

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excess, the reaction is pseudo first-order for NO, and so the number of photons produced are proportional to the number of NO molecules.

Thus, provided the initial combustion step (Reaction 1) is complete, this signal will be proportional to the number of nitrogen atoms originally introduced into the detector. To date, only atmospheric diatomic nitrogen has been reported to not give a chemiluminescence response [7,8]. However, it is not readily apparent that CLND would be compatible with ion chromatography (IC). That is, the post-column reaction used in CLND is a gas phase reaction. It is not apparent that inorganic anions can be effectively transferred into the gas phase. Also, as discussed above, analytes must be combusted to form NO to yield a response. It is not apparent that either oxygenated anions such as nitrite or nitrate or anions containing strong bonds such as cyanide would undergo such a decomposition. Thus, this paper investigates the effectiveness of CLND for ion chromatographic determination of nitrogen-containing inorganic anions including cyanide. The effect of sample matrix on this response is also explored.

2. Experimental

2.1. Chemicals and materials

All solutions and eluents were prepared in Nanopure ultra-pure water (Barnstead, Duburque, IA, USA). Sodium hydroxide, sodium nitrite, potassium nitrate and ammonium chloride were from BDH (Toronto, Canada). Sodium azide (99% purity) was from Aldrich (Milwaukee, WI, USA). Tetradecyl-trimethylammonium bromide (TTAB) (99% pure) and sodium cyanide were from Sigma (St. Louis, MO, USA). Potassium thiocyanate (certified A.C.S.) and oxalic acid (99.9% pure) were obtained from Fisher (Fair Lawn, NJ, USA). Concentrated hydrochloric acid (reagent A.C.S.) was from Anachemia (Montreal, Canada).

2.2. Sample preparation

All stock solutions were made to 10 mM concentration in Milli-Q water, and then diluted to the desired concentration in water, seawater or sodium hydroxide. Stock standards were kept in Nalgene bottles. Samples were prepared fresh daily in 1.5-ml centrifuge tubes. The final concentration of the synthetic sea water [10] was: 40 mM NaCl; 2.5 mM MgCl$_2$; 3 mM Na$_2$SO$_4$; 0.75 mM CaCl$_2$; 0.9 mM KCl; 0.25 mM NaHCO$_3$; 20.08 mM KBr (20 mM greater than normal); 0.044 mM H$_3$BO$_3$; 0.009 mM SrCl$_2$ and 0.008 mM NaF.

2.3. Instrumentation

Ion chromatographic separations with CLND were carried out on a Waters 625 LC system (Waters Associates, Milford, MA, USA). Samples were injected using a Rheodyne 9125 injector (Rheodyne, Cotati, CA, USA) fit with a 5- or 20-μl injection loop. All connecting tubing was 0.005 in. I.D. polyether ether ketone (PEEK) (1 in.=2.54 cm).

All ion chromatographic separations were performed on a 250×2 mm Dionex AS11 IonPac anion-exchange column (Sunnyvale, CA, USA). In flow injection analysis (FIA) studies, the column was removed such that the injector was connected directly to the detector.

The chemiluminescence detector was an Antek 8060 nitrogen detector (Antek Instruments, Houston, TX, USA). The furnace temperature was set to 1100°C, the argon flow was 100 ml/min with an oxygen flow of 200 ml/min and the Ar make up flow at 185 ml/min. The reaction chamber pressure was maintained at 25 Torr by the vacuum pump and the ozone flow was 30 ml/min (1 Torr=133.322 Pa). The FIA studies were performed with a standard Antek quartz pyrotube, whereas all other analysis were done with a quartz tube containing a ceramic insert (Antek) to handle the higher salt content of the eluent. Data were acquired at 10 Hz using a National Instrument PC-6023E data acquisition board controlled using Measure software (version 2.0), (National Instruments, Austin, TX, USA) on a 486 microcomputer.

In some separations of the seawater sample the CLND system was replaced with a Waters 441 absorbance detector fit with a Zn lamp to monitor the absorbance at 214 nm. For the suppressed conductivity measurements a Beckman System Gold Model 125 dual piston pump (Beckman, Fullerton, CA, USA) was used to pump eluent at a flow-rate of

0.3 ml/min. Injections were performed manually with a Rheodyne 9125 (Rheodyne, Cotati, CA, USA) six-port injection valve fit with a 20-μl loop. Suppressed conductivity detection was measured with a Dionex AMMS-II suppressor and a CDM-3 conductivity detector. A constant pressure pump (<25 p.s.i.; 1 p.s.i. = 6894.76 Pa) was used to pump 50 mM sulfuric acid at a flow-rate of 5 ml/min through the suppressor. Data were collected using a Dionex AI-450 data acquisition system interfaced to the 486 microcomputer.

2.4. Column cleaning

The AS11 column was cleaned based on the manufacturer's suggested procedure [11]. The column was rinsed for 2 h at 0.6 ml/min with 1 M hydrochloric acid, followed by an hour rinse with 0.1 M oxalic acid and finally 1.5 h with 18 MΩ water. All column washings and rinses were performed in the opposite direction to the standard flow.

3. Results and discussion

3.1. Equimolar response

Previous studies demonstrated that CLND yields an equimolar response for a number of nitrogen-containing organic molecules [1,3]. That is, amines, amides and nitro functionalities all yielded a detector response proportional to the number of nitrogen in the compound within ±15%.

FIA studies were conducted to test whether such an equimolar response would be achieved for typical inorganic anions of interest in IC. Fig. 1 shows the results of these experiments. Ammonium and TTAB were included in the data set as controls. With the exception of azide (N_3^-), the standard deviation of the response factor based on peak height is 5.6%. The relative standard deviation of 10 replicate injections of 0.5 mM ammonium is 3.1%. Thus, the response for nitrite, nitrate, and cyanide is statistically equivalent to that of amines at the 95% confidence interval.

The response of cyanide is comparable in Fig. 1 is equal that of ammonium and TTAB. This was somewhat surprising as the bond dissociation energy
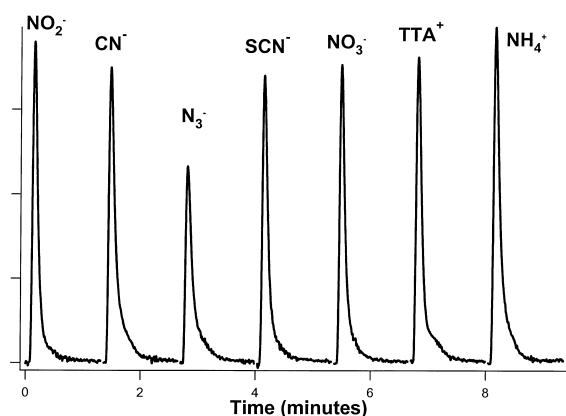

Fig. 1. Equimolar response for N-containing inorganic anions using CLND. Experimentation conditions: flow injection analysis; 5 μl injections of 10 mM of each ion; 0.3 ml/min distilled water.

for C≡N (891 kJ/mol) is much greater than that for an amine (N–H, 393 kJ/mol) and almost comparable to that of nitrogen gas (N≡N, 941.4 kJ/mol) [12] to which CLND does not respond [7,8]. Literature studies indicate that under the conditions of the pyrotube, CN will react with O_2 to produce NCO and O radical [13]. The O radical and NCO then react to yield CO and NO [13]. These species are carried into the reaction chamber where the NO can react with O_3 and produce a signal [7].

In Fig. 1, nitrite and nitrate were also demonstrated to yield a response equivalent to that of ammonium and TTAB. The mechanism by which nitrite and nitrate are decomposed or reduced to NO is unknown. However the results in Fig. 1 are consistent with previous studies in which signals for nitrite and nitrate were noted but the response factor was not quantified [6]. Sodium nitrite was even used as a standard in the determination of urinary nitrogen [5].

All other nitrogen-containing analytes, which have bond dissociation energies significantly below that of cyanide [12], would be expected to yield a similar response. Thus, the low response for azide (N_3^-) which contains N=N bonds (418 kJ/mol) [12] was initially somewhat surprising. Azide contains three nitrogen atoms. Other analytes tested by FIA demonstrated responses equivalent to the number of nitrogen atoms per molecule. For instance, caffeine with four nitrogen gave 4.1 times the response of an equal

concentration of nitrite. Thus it was expected that 10 mM azide should yield a response three times that of 10 mM of the other single N-containing analytes. Yet in Fig. 1, the response for azide was only 58% that of ammonium. Interestingly at lower azide concentrations, such as in Fig. 3, the response for azide becomes comparable to that of single N-containing anions. In retrospect the low response for azide is not as surprising, as one of the chief decomposition products of azide is nitrogen gas (N_2) [14]. Certainly it is because of its rapid decomposition to N_2 that sodium azide is used in emergency air bags in automobiles [15]. The pyro-chemiluminescence detector does not respond to nitrogen gas [7,8]. Nevertheless, some response was observed for azide. Studies of the thermal decomposition of azide containing compounds (i.e., explosives) such as poly[bis(3,3′-azidomethyl)oxetane] [–($C_5H_8N_6O$)–] and poly(glycidylazide) [(–$C_3H_5N_3O$–)] indicate that 31.7 to 37.4% of the nitrogen is converted to ammonia, hydrogen cyanide and/or other nitro products [16], to which CLND would respond. At higher concentrations, such as in Fig. 1, some gas-phase recombination to form N_2 occurs, thereby lowering the CLND response for azide. Thus, while the response of CLND to azide is not equimolar to that of other nitrogen-containing compounds, it is consistent with the expected decomposition behavior.

3.2. Determination of cyanide

Studies were performed to determine the sensitivity of the CLND system for cyanide. Hydrogen cyanide is a very weak acid (pK_a=9.22). Thus, under the acidic eluent conditions (pH<7) [17] which exist after suppression, cyanide is largely protonated. As a consequence, the limit of detection for cyanide with suppressed conductivity is much lower than for other anions. Recently, Caliamanis et al. [18] demonstrated that the detection limits for cyanide using suppressed conductivity could be improved to 50 μM by using a second anion micromembrane suppressor to convert HCN into NaCN, much in the manner of Sjogren and Dasgupta [19].

The separation conditions of Caliamanis et al. [18] (Dionex AS11 column and 5 mM NaOH as eluent) were used herein. Initially an extremely tailed peak

was observed. The efficiency of this peak (N=313 based on the tangent method) was comparable to that of Caliamanis et al., but much poorer than the 5000 plates achieved for other anions. Further, the elution behavior of cyanide was irreproducible. Cleaning of the column as described in Section 2.4 yielded much more efficient peaks (N=1200) for cyanide, as shown in Fig. 2.

Calibration curves based on peak area were linear (R=0.998) over the range studied (5–100 μM) with an intercept statistically equal to zero. The precision measured at 25 μM was 9% RSD. The detection limit for cyanide at the 95% confidence interval was 3 μM (78 μg/l), as determined using the US Environmental Protection Agency (EPA) procedure [20]. This corresponds to a mass detection limit of 0.8 ng N, which is close to that typically quoted for CLND [8]. The concentration detection limit using CLND is more than an order of magnitude lower than that recently reported for suppressed conductivity with an ion-exchange reactor [18], and comparable to that achievable using direct conductivity detection [17] or suppressed conductivity after oxidation of cyanide to cyanate [21]. However, detection limits achieved with derivatization [22,23] and amperometry [24] are significantly lower, albeit the latter is prone to interference by chloride [25].

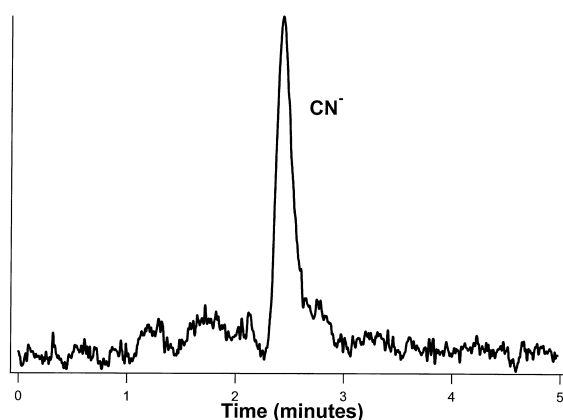Using the AS11 column, cyanide was separated from other nitrogen-containing anions such as azide

Fig. 2. Determination of cyanide using IC–CLND with a cleaned column. Experimental conditions: Dionex AS11 column after cleaning procedure (Section 2.4); 5 mM NaOH at 0.3 ml/min; 20 μl injection of 10 μM cyanide.

and nitrate, but co-eluted with nitrite. Thus, a different column and/or eluent would be needed to quantify cyanide in the presence of nitrite.

3.3. Separation and determination of other nitrogen-containing anions

Separations of nitrite, azide and nitrate was achieved using the AS11 column with a 5 mM sodium hydroxide eluent. A typical chromatogram is shown in Fig. 3. Detection limits for these anions in distilled water were about 3 μM (0.8 ng N), which was comparable to that achieved for cyanide above. However one of the advantages of CLND is that it is insensitive to non-nitrogen-containing species. Thus it should be ideal for determination of nitrite, azide and nitrate in complex matrices. Fig. 4 shows chromatograms for 50 μM of nitrite, azide and nitrate in a 1:10 dilution of synthetic seawater (Section 2.2). Azide and nitrate are detectable at this level using suppressed conductivity (Fig. 4a). Some overlap with the bromide peak is apparent. However the level of Br$^-$ in the synthetic seawater (20 mM) is significantly higher than would normally be observed, and so this overlap would not normally cause a problem. The nitrite peak however is completely lost in the tail of the large chloride signal.

Direct UV absorbance is often used for detection of nitrite and nitrate [26]. Fig. 4b shows the chromatogram observed when UV detection at 214 nm
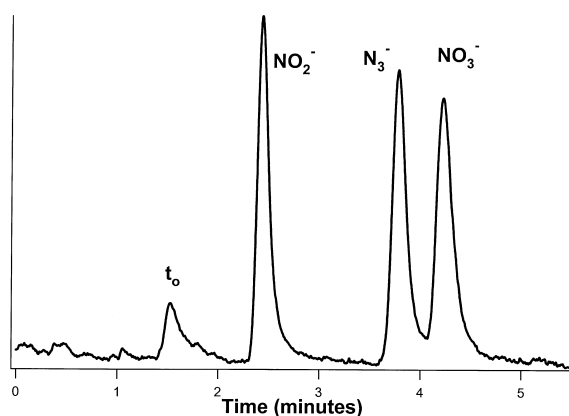

Fig. 3. Analysis of nitrogen-containing anions in water with IC–CLND Experimental conditions: Dionex AS11 column; 5 mM NaOH at 0.3 ml/min; 20 μl injection of 50 μM each of NO$_2^-$, N$_3^-$ and NO$_3^-$.
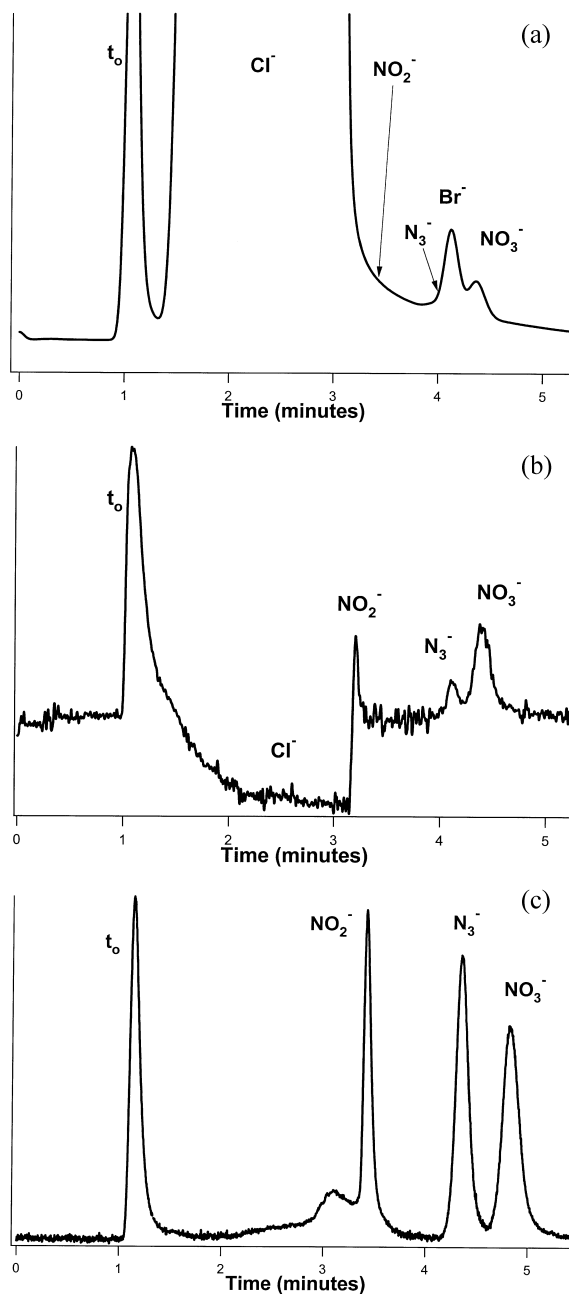

Fig. 4. Analysis of nitrogen-containing anions in 1:10 seawater by IC with: (A) suppressed conductivity detection; (B) UV absorbance detection at 214 nm and (C) CLND. Experimental conditions as in Fig. 3.

was used to determine these anions in 1:10 diluted seawater. Some slight shift in retention times is evident due to differences in the length of tubing connecting the column to the various detectors. More importantly however is that this chromatogram is far from appealing. The chloride causes a large negative peak, caused by the displacement of the absorbance background created by the hydroxide. This negative peak almost completely obscures the nitrite peak. The azide and nitrate peaks are still visible but disproportionate due to their differing absorptivities and are potentially masked by other UV absorbing anions such as bromide.

In contrast, CLND yields a chromatogram (Fig. 4c) that is almost indistinguishable from that in distilled water (Fig. 3). The chloride matrix has caused a slight shift to longer retention times, but the peak efficiencies and sensitivity are essentially as they were for the cleaner matrix, with the exception of nitrite. The overlap of the large chloride peak with the nitrite results in a splitting of the nitrite peak.

3.4. Limitations of CLND for ion chromatography

Some limitations in the use of CLND for ion chromatography were noted. Of primary concern to ion chromatography is the restriction on the amount of salt that can be used in the eluent. Salt can deposit on the pyrotube. The manufacturer's literature states that a pure quartz pyrotube can be used with up to 25 mM salt concentrations [14]. However, after only 2 weeks use with a 5 mM buffer, our quartz pyrotube shattered upon cooling. Use of a quartz pyrotube with the ceramic insert has rectified this problem, but we cannot yet speak to the long term stability of this solution. A better long-term solution would be to use a suppressor to convert the eluent (e.g., NaOH) into a more volatile product (H_2O). This should extend the life of any pyrotube to that obtained with non-electrolyte eluents, and might allow for the use of even greater salt concentrations.

Secondly, the restriction that the eluent includes no nitrogen-containing components causes limitations. In reversed-phase liquid chromatography, acetonitrile can readily be replaced with methanol. However in ion interaction chromatography, ion pair reagents such as cationic surfactants are often used to dynamically coat a reversed-phase stationary phase

to provide some ion-exchange retention. As shown in Fig. 1, cationic surfactants such as tetradecyltrimethylammonium will yield a response with CLND. Thus, until non-nitrogen-containing ion pair reagents are developed, the use of CLND for ion chromatography is limited to fixed anion exchangers.

4. Conclusions

CLND yields low micromolar concentration detection limits and sub-ng N mass detection for nitrogen-containing anions such as nitrite, nitrate, azide and cyanide. With the exception of azide, the response of these anions is proportional to the amount of nitrogen in the anions. Thus universal calibration using a single analyte is possible. The selectively of the detector to nitrogen-containing species makes it extremely effective in analysis of complex matrices such as seawater.

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