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Nitrogen-specific liquid chromatography detector based on chemiluminescence

Application to the analysis of ammonium nitrogen in waste water

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ABSTRACT

A novel high-performance liquid chromatography–chemiluminescent nitrogen detection (HPLC–CLND) system is described. The analysis of ammonium nitrogen in metropolitan waste water by a new ion chromatographic method is presented. The significance of this novel nitrogen-specific detector is the capability for a routine and continuous operation. HPLC–CLND applications for anion and particularly reversed-phase techniques are also amenable. A nitrogen-specific detector for liquid chromatography opens a new dimension of analytical chemistry.

INTRODUCTION

Increasing environmental regulations and concerns have made it vital to analyze ammonia nitrogen in waste water systems in order to monitor and prevent unnecessary discharges. Ammonia is a product of microbiological activity and consequently present in many surface and ground waters. We describe a novel nitrogen-specific detection and analytical method by high-performance liquid chromatography–chemiluminescent nitrogen detection (HPLC–CLND) for the analysis of ammonium ions in waste water samples.

Ion chromatography (IC) has become the preferred analytical tool for the determination of ammonium ions in water [1–5]. Conductivity detectors are generally used for IC applications. However, difficulties may be encountered when analyzing complex sample matrices due to the non-selective nature of this detector. Alternatively, HPLC–CLND is highly specific for analyzing nitrogen-containing compounds. In 1970, Fontijn *et al.* [6] studied the analytical gas-phase reaction of nitric oxide and ozone which produced chemiluminescence.

A "nitrogen-specific" gas chromatography–pyrochemiluminescent nitrogen detection (GC–CLND) system was developed several years later [7]. GC–CLND has demonstrated high nitrogen specificity for nitrogen-containing pesticides [8] and a wide range of applications were previously studied including nitrosamines, pesticide residue, food-flavor, pharmaceutical and petroleum light cycle oil samples [9]. The detection mechanism for CLND is as

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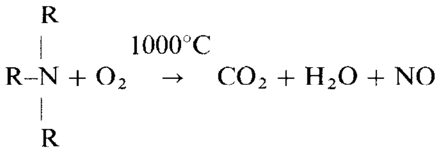
follows: as each sample component elutes from the end of the chromatographic column, it undergoes high-temperature (1000°C) oxidation. All nitrogen-containing compounds are converted into nitric oxide (NO). The resulting gases are dried and mixed with ozone (O_3) in the reaction chamber. This results in the formation of nitrogen dioxide (NO_2^*) in the excited state. Light (*hv*) is emitted by this chemical reaction and is detected by a photomultiplier tube (PMT).



$$R_2N{-}R + O_2 \xrightarrow{1000^\circ C} CO_2 + H_2O + NO$$

$$NO + O_3 \rightarrow NO_2^* \rightarrow NO_2 + hv$$

The chemiluminescence of chemically bound nitrogen is therefore detected and the resulting signal is processed by a computer. Although the above CLND mechanism is the same for HPLC and GC methods, a novel HPLC–CLND instrumentation was necessary as the analytical tool to be useful for HPLC. To date, several HPLC–chemiluminiscence detection methods based on the gas-phase nitric oxide–ozone reaction have been reported, but these have not been widely used [10]. The major limitation of previous instrumentation is the inefficient removal of water prior to the reaction chamber where the chemiluminescence is detected. Typical operation time is 2–3 h with frequent instrument downtime for drying the reaction chamber and transfer lines. One of the new design innovations incorporated in this novel HPLC–CLND instrumentation (Fig. 1) is the unique capability for continuous water removal for routine and uninterrupted operation [11]. This detector is shown in Fig. 1 and is described in the following manner. A liquid sample is injected from a sample valve (*i.e.* equipped with a sample loop) onto an HPLC column using a suitable mobile phase which is stored in a solvent reservoir. An HPLC pump is used to control the mobile phase flow-rate. A sample is chromatographed on a column and eluting compounds are introduced into the furnace of the novel HPLC–CLND system. At the furnace inlet, the inert gas carrier (helium or argon) is utilized along with the oxygen carrier for the oxidation of sample components. The resulting nitric oxide and gases pass through the membrane drying/dewatering chamber. The key feature is the continuous removal of water (generated from both the mobile phase and sample) after oxidation. The nitric oxide is then reacted with ozone in the reaction chamber. The resulting chemiluminescence is detected by the PMT. The signal(s) from the HPLC–CLND system is processed by a computer and reports are printed from a printer. Gases exit the reaction chamber and pass through the scrubber. The flow-rates of inert gas and oxygen carriers from the gas cylinders are controlled by the gas flow meters. Oxygen passing through the ozone generator is the source for the ozone supply for this detector. The description (Fig. 1) and the explanation of this HPLC–CLND system are thus provided here for the first time in a journal publication and patent is pending for this detector.

The quantitative analysis of ammonium nitrogen in metropolitan waste water by the novel HPLC–CLND nitrogen-specific detection in the IC mode is discussed. The method, data, linearity of detector response and sensitivity are presented. Also demonstrated are the HPLC–CLND methods for anion and reversed-phase techniques.

EXPERIMENTAL

Apparatus

HPLC analysis was performed on a Micromeritics Model 760 HPLC pump with a micro flow pump head purchased from Alcott Chromatography (Norcross, GA, USA), Antek HPLC–CLND nitrogen-specific detector from Antek Instruments (Houston, TX, USA) and Delta chromatography software (Digital Solutions, Margate, Australia) run on an IBM 286 compatible computer. Sample injection was performed on a Rheodyne (Cotati, CA, USA) Model 8126 injection valve (5-μl sample loop). The HPLC columns used in this study were obtained from Keystone Scientific (Bellefonte, PA, USA). All HPLC mobile phases were filtered through a Millipore (Bedford, MA, USA) HV filter with a pore size of 0.45 μm.

Reagents and standards

Ammonium sulfate standard and methanol were obtained from Fisher Scientific (Fair Lawn, NJ, USA). L-Tartaric acid was purchased from Aldrich

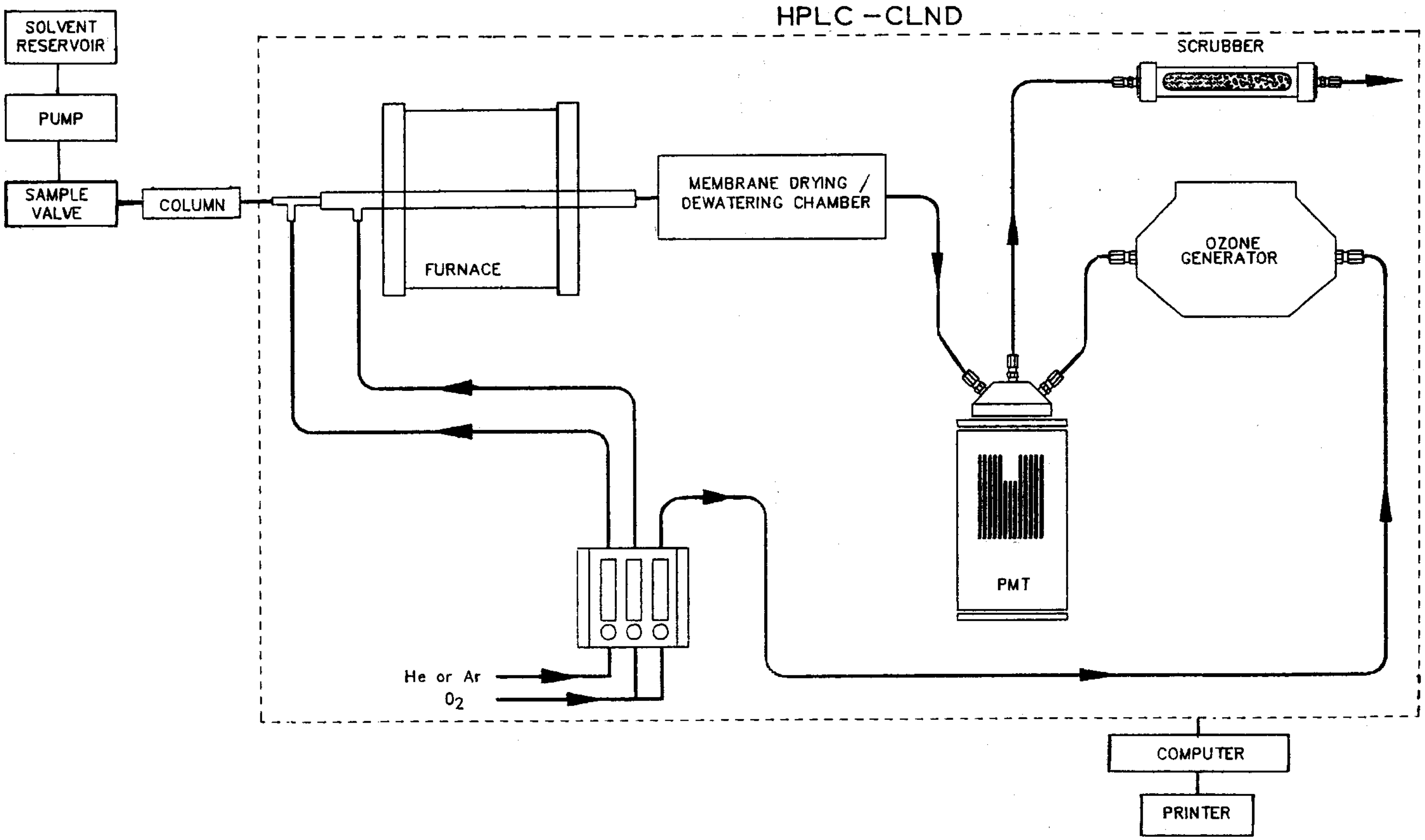


Fig. 1. Schematic diagram of the HPLC–CLND system. © Antek Instruments Inc.

(Milwaukee, WI, USA). Sodium nitrate, sodium nitrite and urea standards were obtained from J. T. Baker (Phillipsburg, NJ, USA). All reagents and standards were greater than 99% pure and were used without further purifications. Distilled water used for making standard solutions and mobile phases was obtained from Ozarka Drinking Water Company (Houston, TX, USA).

Standard preparation and analytical method

Analytical ammonium ion standards (10, 15, 20 and 80 ppm nitrogen) were prepared from a 1000-ppm nitrogen aqueous ammonium sulfate stock standard solution and used for the quantitative determination of ammonium nitrogen in waste water samples by HPLC–CLND in the IC mode. The waste water samples were analyzed without preliminary sample clean-up, by a 2-μl partial filled injection into a 5-μl sample loop. Ammonium ions were analyzed using an HPLC (150 mm × 1 mm I.D., 10 μm particle size) Hamilton PRP-X200 column. The mobile phase, 0.1 *M* L-tartaric acid–water (20:80, v/v), was utilized at a flow-rate of 30 μl/min. The CLND pyrolysis temperature was 1037°C.

A mixed nitrate–nitrite standard solution containing 40 ppm nitrogen from sodium nitrate and 80 ppm nitrogen from sodium nitrite was prepared from the 1000-ppm nitrogen stock standard (aqueous) solutions. The anion standards were analyzed by HPLC–CLND in the IC mode. The IC trace was achieved by the same injection technique using the HPLC (150 mm × 1 mm I.D., 10 μm particle size) Hamilton PRP-X100 column. The mobile phase, 4 m*M* potassium hydrogenphthalate (pH 4.5), was utilized at a flow-rate of 40 μl/min.

The urea standard (76 ppm nitrogen in methanol) was prepared and analyzed by reversed-phase HPLC–CLND. The HPLC analysis was performed by the same 2-μl injection on a Partisil ODS 3 (250 mm × 1 mm I.D., 5 μm particle size) column. The mobile phase, methanol–water (95:5, v/v), was used at a flow-rate of 40 μl/min.

RESULTS AND DISCUSSION

HPLC detection with high nitrogen specificity may be obtained by using the capabilities of the novel HPLC–CLND system. The chromatographic separations of ammonia and a mixture of several

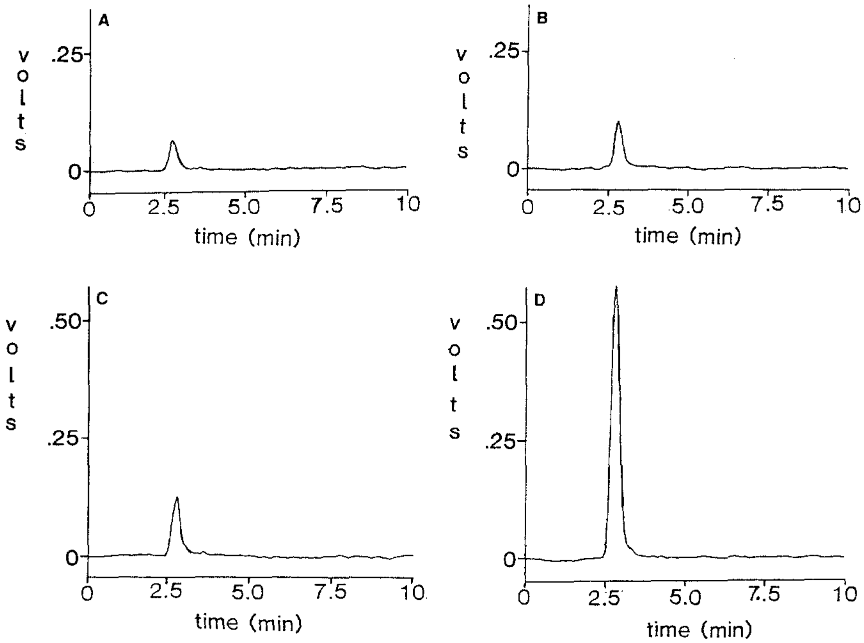


Fig. 2. HPLC–CLND tracings of the four ammonium ion standard solutions: (A) 20 ng, (B) 30 ng, (C) 40 ng and (D) 160 ng nitrogen.

volatile organic amines have been achieved by GC–chemiluminescence detection and reported in the literature [12,13]. However, by using nitrogen-specific detection (HPLC–CLND) in the IC mode, it is also possible to utilize an additional mode of selectivity (for molecular ions). This is accomplished by using the optimized IC mobile phase. In this way, two modes of selectivity (detector and mobile phase) are available resulting in greater specificity than obtained with GC–CLND. To demonstrate the utility of this powerful tool, ammonium nitrogen in metropolitan waste water samples were quantitatively analyzed by HPLC–CLND in the IC mode. A sensitivity down to 5 ng nitrogen was observed in this experiment. Lower ammonium nitrogen levels (picograms) would be achievable by sample concentration techniques such as solid-phase extraction (SPE). The linear detector response with the ammonium nitrogen calibration was obtained and the results from the linear regression analysis are presented in Table I (where r = correlation coefficient, m = slope and b = y-intercept). The four-point standard curve was obtained by the HPLC–CLND ammonium ion method using the L-tartaric acid mobile phase and the Hamilton PRP-X200 HPLC column. The standard chromatograms representing the ammonium ion standards (20, 30, 40 and 160 ng nitrogen) are given in Fig. 2. The HPLC–CLND trace of the metropolitan waste water samples A and B are provided in Fig. 3. The chromatograms showed only a single peak, at a retention time (t_R) of 2.8 min, and the samples were quantitated from the ammonium ion standard calibration curve. Ammonium nitrogen levels of 16.8 and 18.0 ppm nitrogen, respectively, were found in these samples. Calculations were performed by linear regression analysis with a correlation coefficient of 0.99952.

Nitrogen-specific detection of anions by HPLC–CLND in the IC mode is amenable. An HPLC–CLND profile (nitrite t_R = 6.1 min; nitrate t_R = 7.2 min) of a mixed nitrate–nitrite standard (80 and 160 ng, respectively) is shown in Fig. 4. Studies of using inorganic salts as mobile phase modifiers for prolonged usage still need to be determined and is currently under investigation.

A reversed-phase HPLC–CLND trace of urea standard in methanol was obtained, with t_R = 4.7 min (Fig. 5). The scope for methods and applications using this detector at this time is primarily in the reversed-phase mode. The amount of water in the mobile phase is unlimited (100% water may be used if desired). The organic portion of the mobile phase is limited to non-nitrogen-containing solvent mixtures. The use of nitrogen-containing compounds well below the detection limit as mobile phase modifiers is yet to be investigated. Alternative solutions would be directed to stationary phase specialists to develop HPLC columns for the separations of various classes of nitrogen-containing molecules which are compatible for nitrogen-specific detection. The preferred flow-rates for optimum detector response are 1 to 5 µl/min for micro HPLC and up to 60 µl/min for microbore HPLC. Microbore HPLC columns with 1–2 mm I.D. are preferred. Other HPLC–CLND applications are currently underway and will be reported elsewhere.

TABLE I

HPLC–CLND ANALYSIS OF AMMONIUM NITROGEN IN METROPOLITAN WASTE WATER

$(NH_4^+)_2SO_4$ standard was prepared in distilled water and the ammonium nitrogen in waste water samples were calculated as parts per million nitrogen (ppm N) by linear regression analysis: r = 0.99952; m = 0.00001; b = −0.00687.

Sample	Standard $(NH_4^+)_2SO_4$ (ppm N)	Sample size (µl)	Integration of peak area	NH_4^+ (ng N)	NH_4^+ (ppm N)
	10	2	2279.18	20	
	15	2	2875.11	30	
	20	2	3452.41	40	
	80	2	13039.02	160	
A		2	3150.92	34	16.8
B		2	3351.79	36	18.0

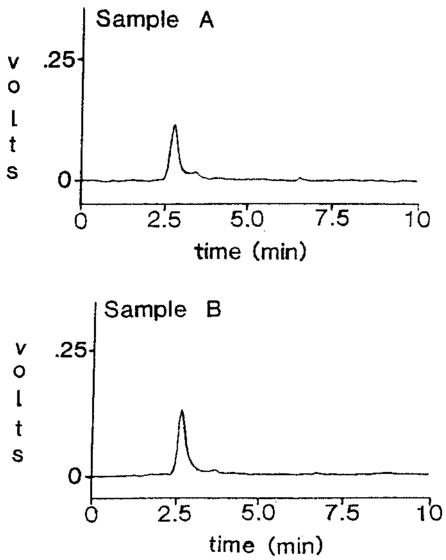


Fig. 3. HPLC–CLND tracings of the metropolitan waste water samples A and B.

HPLC method development can be facilitated using nitrogen-specific detection in many areas of agricultural chemistry and environment studies. The technique may also be used to generate confirmatory methods for pharmaceutical and environmental studies. Other potential use of this detector may extend into the area of biotechnology research in the analysis of proteins as well as into biochemistry method development where amino acids may be analyzed without derivatization.

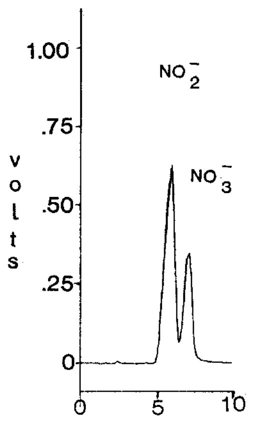


Fig. 4. HPLC–CLND tracing of nitrite and nitrate ions.

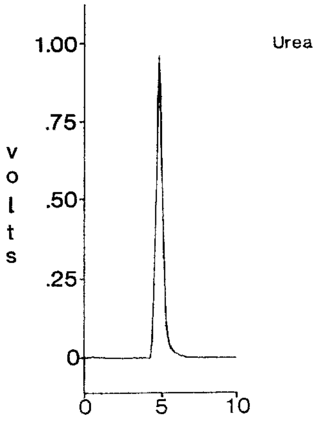


Fig. 5. Reversed-phase HPLC–CLND tracing of urea in methanol.

CONCLUSIONS

A novel HPLC–CLND system is presented and described for the first time. The quantitation of ammonium nitrogen in metropolitan waste water in the IC mode is demonstrated.

HPLC–CLND has many advantages. A nitrogen-specific detector can simplify the analysis of complex samples and often shorten the analysis time by resolving only the nitrogen-containing components. This novel HPLC detector provides a sensitive method with excellent linear detector calibration and is a tool for routine and continuous operation. There is a wide range of analytical applications for which the detector can be used. HPLC–CLND applications for reversed-phase techniques are particularly amenable. Investigation of prolonged usage of inorganic salts as mobile phase modifiers is still underway. The instrumental method with nitrogen specificity for HPLC provides a new dimension of analytical chemistry and is just the beginning of a new trend in HPLC.

REFERENCES

1 L. R. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, Wiley, New York, 2nd ed., 1979, p. 438.
2 James M. Miller, *Chromatography: Concepts and Contrasts*, Wiley, New York, 1988, p. 182.
3 P. Kolla, J. Kohler and G. Schomburg, *Chromatographia*, 23 (1987) 465.
4 S. A. Bouyoucos, *Anal. Chem.*, 49 (1977) 401.
5 H. Small, T. S. Stevens and W. C. Bauman, *Anal. Chem.*, 47 (1975) 1801.
6 A. Fontijn, J. Sabadell and R. J. Ronco, *Anal. Chem.*, 42 (1970) 575.
7 R. E. Parks and R. L. Marietta, *U.S. Pat.*, 4 018 562 (1975).
8 A. J. Britten, *Res. Dev.*, 31 (1989) 76.
9 L. O. Courthaudon and E. M. Fujinari, *LC·GC*, 9 (1991) 732.
10 A. Robbat, Jr., N. P. Corso and T.-Y. Liu, *Anal. Chem.*, 60 (1988) 173.
11 E. M. Fujinari and A. J. Britten, Patent pending.
12 N. Kashihira, K. Makino, K. Kirita and Y. Watanabe, *J. Chromatogr.*, 239 (1982) 617.
13 D. P. Rounbehler, S. J. Bradley, B. C. Challis, D. H. Fine and E. A. Walker, *Chromatographia*, 16 (1982) 354.