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A rapid, precise and sensitive method for the determination of total nitrogen in natural waters

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Abstract

A rapid, automated method is described for the accurate determination of total combined nitrogen (TN) in natural waters. This methodology allows for TN analyses to be performed at three times the rate of previous techniques. The method is shown to be sensitive for a wide range of TN concentrations that may be found in various aquatic environments. The detection limits of this methodology are 2.0 μ M (freshwater) and 2.3 μ M (seawater). TN and TIN analysis of surface water samples from 300 sites inshore and offshore of south Florida, USA revealed predictable gradients in nitrogen concentrations. TN ranged from 7.56 μ M offshore to 108.87 μ M inshore. Organic forms of nitrogen constituted approximately 90% of the total nitrogen pool. © 1998 Elsevier Science B.V.

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1. Introduction

The measurement of total combined nitrogen (TN) in natural waters is becoming increasingly important. Due to the development of more sensitive methods of total combined nitrogen determination (Armstrong et al., 1966; D'Elia et al., 1977; Solorzano and Sharp, 1980; Walsh, 1989) it is now realized that organic nitrogen is the dominant form of combined nitrogen (often consisting of greater than 90% of combined nitrogen pool) in many fresh waters (Jones,

unpublished), estuaries (Haines, 1979) and near coastal and surface oceanic waters (Sharp, 1983; Jackson and Williams, 1985; Walsh, 1989). The relatively large size of this organic nitrogen pool may allow it to function as an emergency reservoir providing nitrogen during periods of low ammonium (NH_4^+) and nitrate (NO_3^-) availability (Jackson and Williams, 1985). Approximately 50% of the dissolved organic nitrogen pool consists of urea, free and combined amino acids and humic acids (Sharp, 1983) and has been shown to be biologically reactive (Paul, 1983). The other half may consist of amino sugars and fulvic acids (Sharp, 1983). The particulate organic nitrogen pool has been shown to be mostly proteinaceous (Sharp, 1983), originating from either macrophyte detritus (in estuarine and near coastal waters) or phytoplankton (in oceanic waters)

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(Paul, 1983). Historically, the importance of organic nitrogen has been greatly underestimated. This is due to the paucity of published values and serious methodological problems in the determination of organic nitrogen.

Total organic nitrogen (TON) is determined by the difference by subtracting total inorganic nitrogen (TIN) from total combined nitrogen (TN). Traditionally, the determination of aqueous total organic nitrogen was performed utilizing the wet chemical Kjeldahl digestion method (Kjeldahl, 1883; Grasshoff, 1983). The precision of this method has been shown to be variable (Smart et al., 1981; APHA et al., 1989), with coefficients of variation as high as 30.8% for total nitrogen (Ekedahl et al., 1975). This method is also expensive, not extremely sensitive and susceptible to contamination (Grasshoff, 1983; APHA et al., 1989). The time-consuming and tedious nature of this method also hinders the development of rapid automation (D'Elia, 1983).

The determination of TON has also been performed utilizing photo-oxidation with ultraviolet radiation (UV) (Armstrong et al., 1966), but some nitrogenous compounds, specifically those with N–N bonds, are refractory to this type of oxidation (Henricksen, 1970; D'Elia, 1983; Walsh, 1989).

Another wet-chemical TN method receiving more widespread use is the alkaline persulfate digestion (Koroleff, 1972; D'Elia et al., 1977). The accuracy of persulfate digestion is comparable to Kjeldahl digestions, but the precision and sensitivity of the persulfate technique is greater (Smart et al., 1981). However, failure to maintain proper pH throughout the various steps of the persulfate procedure limits recovery of more recalcitrant nitrogenous compounds and urea (Solorzano and Sharp, 1980). Highly turbid samples may also limit the accuracy and precision of the persulfate method (Nydahl, 1978).

A promising dry combustion method with chemiluminescent detection of nitric oxide was developed for the determination of nitrogen in petroleum fractions by Drushel (1977). This high temperature combustion method (HTC) was further refined and adapted for the analysis of wastewaters and seawaters (Jones and Daughton, 1985; Walsh, 1989). In the HTC method, all combined (non-elemental) nitrogen compounds are oxidized to nitric oxide. The nitric oxide (NO) is then reacted with ozone, produc-

ing an ephemeral chemiluminescent nitrogen dioxide radical (NO_{2}^{*}) which subsequently releases light energy upon degradation to NO_2 . The light emitted is stoichiometrically proportional to the amount of combined nitrogen and is measured within a photomultiplier tube (PMT). The HTC method has produced quantitative recoveries of common organic compounds (glycine, urea, EDTA) averaging 100.8% (from Walsh, 1989). Recalcitrant organic nitrogen compounds (those containing N-N and N=N bonds) has also shown high recoveries (average = 98.3%) using the HTC analysis (Walsh, 1989). The analytical system used by Walsh (1989) consisted of a pyroreactor, photomultiplier tube and a sample boat drive. In that method water samples were introduced into the system (every 3 min) by injection of 10 μ l into a quartz boat which is mechanically driven into and out of the pyroreactor.

The HTC method has been directly compared with Kjeldahl (TKN) analysis (Jones and Daughton, 1985) and with the photo-oxidation method (UV) (Walsh, 1989). For oil shale wastewaters, HTC analvsis and the Kieldahl method vielded statistically similar (P > 0.10) total nitrogen values, but the analysis of individual compounds revealed that HTC determination yielded greater recoveries than Kjeldahl determination (Jones and Daughton, 1985). Walsh (1989) compared the HTC and photo-oxidation (UV) methods for the recovery of specific free amino acids and proteins known or expected to be in seawater. Both methods produced similar values, but the HTC method yielded slightly higher recoveries (average UV:HTC ratio: 0.95 + 0.03) (Walsh, 1989). For natural seawater samples, Walsh (1989) found that the UV, persulfate oxidation and the HTC methods were directly comparable.

The adaptation of the HTC method for water samples (Walsh, 1989) is highly accurate, but is limited by reproducibility and the number of samples that can be processed. D'Elia (1983) has stated "...the most significant changes in the practice of nitrogen determination result not as much from widespread adoption of new methods as from widespread acceptance and improvement of automated methods...". It was the objective of this study to develop a rapid automated method for the precise determination of total organic nitrogen in natural waters. The method developed here is a modification of the HTC chemiluminescent method for water samples described by Walsh (1989). A commercially available Antek model 7000N total nitrogen system was adapted to accept samples from a Dynatech model GC 311H horizontal inject autosampler for gas chromatography. The horizontal inject autosampler delivers samples into the carrier stream more rapidly and under more constant conditions than the boat drive delivery described by Walsh (1989). Additional changes to the pyrolysis furnace and operating characteristics significantly increased the sensitivity and accuracy of the chemiluminescent method.

2. Methods

Aqueous TN analyses were performed utilizing an ANTEK Model 7000N elemental analyzer consisting of a pyroreactor, photomultiplier tube (PMT) and a Dynatech Model GC311H autosampler (Fig. 1). Water samples are introduced into the system by the direct injection of 5 μ l through a teflon lined sili-

cone septum and 2 cm of loosely packed quartz wool into the oxygen carrier stream (Zero grade, Trigas, 65 ml min⁻¹). The ceramic-lined quartz pyrotube is maintained at 1000°C and is operated under slight positive pressure (5-7 kPa). The positive pressure is controlled by a needle valve located immediately upstream from the pyrotube. Ceramic chips (4) are evenly spaced within the pyrotube to create turbulent flow. The combusted gaseous stream passes through a cold finger water trap, glass fiber filter (Balston, effective pore size = 0.1 μ m) and a membrane dryer (PermaPure), to remove water vapor and salt particulates prior to entering the reaction chamber. The addition of the cold finger water trap allows for the injection of larger sample volumes. The NO combustion product is reacted with ozone (15 ml min⁻¹) within the reaction chamber which is separated from the combustion chamber by a needle valve. The reaction chamber is operated under a vacuum pressure of 25 in. Hg. The resulting nitrogen dioxide radical (NO_2^*) releases light energy which is stoichiometrically proportional to the amount of chemi-



Fig. 1. Flow diagram of ANTEK nitrogen system.

cally bound nitrogen. Integration counts are produced from the PMT signal (voltage = 800 V). Each injection cycle takes 65 s, requiring 15 s to rinse sample needle and 50 s for combustion and reaction/detection. Three injections per sample produce precise determinations. The resulting sample analysis rate, including standards and blanks, is 18.5 samples h^{-1} .

As shown by Walsh (1989), KNO₃ is a suitable HTC standard, even for recalcitrant compounds. As such, total nitrogen standards were prepared from reagent grade KNO₃, and deionized water (DI). Standards were prepared from a 35.73 mM solution of KNO₃ in DI and made as standard additions to either DI or to filtered (5.0 μ m) sea water collected in the Atlantic Ocean and stored in the laboratory in plastic jugs (SW). Background total nitrogen values (assumed to be residual dissolved organic nitrogen) in the DI or SW were subtracted before the generation of standard curves. Standard additions consisted of nitrogen concentrations of between 0 and 700 μ M. The combustion efficiency of this HTC methodology was tested with standard additions of glycine (50 μ M, 50 μ M-N) and urea (50 μ M, 100 μ M-N). These organic nitrogen compounds were chosen because they are representative of the dissolved organic nitrogen compounds known to be present in natural waters (Sharp, 1983).

Some modifications were made to sample preparation and pyrotube temperature. These modifications prevent clogging of the injection needle and extend the life of the combustion column and are worthy of discussion. Each 2 ml sample was prepared in a 2 ml crimp top vial and acidified with 10 μ l of 3 N HCL. This preparation step prevents the formation of oxide and carbonate salts inside the injection needle when the needle enters the pyrotube. Another modification is the maintenance of pyrotube temperature at 1000°C as opposed to 1100°C as suggested by Walsh (1989). Combustion efficiency was not compromised by the lowering of the combustion temperature but the 100 C° reduction in temperature drastically reduced the rate of vitrification of the quartz column.

The precision of this HTC methodology was tested using surface water samples collected in March and April 1995 from six sites inshore and offshore of the southern tip of Florida, USA. These sites are situated



Fig. 2. Location map of 300 surface water sampling sites inshore and offshore of the southern tip of Florida and the Florida Keys, USA.

along an onshore to offshore gradient and are representative of the various aquatic environments found in tropical and subtropical regions. Additionally, as part of long-term water quality monitoring efforts (Boyer et al., 1997; Jones, unpublished data), surface water samples were collected during July 1996 from 300 inshore and offshore sites within this same region (see Fig. 2). Two unfiltered surface water samples were collected from each location and were analyzed in triplicate. Total nitrogen (TN) was determined by HTC with KNO₃ standards (143 μ M-N) run every 20 samples. For the 300 monitoring sites. two filtered (25 mm glass fiber GF/F) surface water samples were analyzed for total inorganic nitrogen (TIN = nitrate + nitrite + ammonium). Nitrite + nitrate and ammonium analyses were performed on a rapid flow autoanalyzer (Alpkem model RFA 300). Total organic nitrogen (TON) was determined by difference (TN - TIN).

3. Results and discussion

A series of blanks and standards prepared in DI and SW (18–714 μ M) shows good precision (Figs. 3



Fig. 3. Integration counts versus added KNO₃ concentration in DI (freshwater) and sea water. Regression lines are represented by the following equations: TN (μ M) = 3006 * counts - 11,130, r^2 = 0.998 (freshwater); TN (μ M) = 2676 * counts - 6460, r^2 = 0.998 (seawater). Error bars represent ± one standard error of the mean determined from five or six injections. PMT voltage = 800 V.



Fig. 4. Integration counts versus added KNO₃ concentration in DI (freshwater). The regression line is represented by the following equation: TN (μ M) = 5077 * counts – 229, r^2 = 0.995. Error bars represent ± one standard error of the mean determined from four to six injections. PMT voltage = 900 V.

and 4). DI and SW blanks which were subtracted from the standard additions were 13.1 and 20.5 μ M, respectively. The relationship between added nitrogen concentration and integrator counts is similar between freshwater and seawater (Fig. 3). The standard addition curves are linear up to nitrogen concentrations of 143 μ M-N. Under the flow rates and operating conditions described previously, detector response becomes saturated at higher nitrogen concentrations and does not allow for accurate determinations.

If the detection limit is defined as 2 times the SD of the blanks (Willason and Johnson, 1986), then the detection limit of this method is 2.0 μ M (n = 10, 13.1 μ M-N blank (DI)) and 2.3 μ M (n = 10, 20.5 μ M-N blank (SW)).

Elevating the PMT voltage to 900 V increases the sensitivity of this method. A series of blanks and low-level KNO₃ standards prepared in DI (3.6–14.3 μ M) also shows good precision (Fig. 4). This demonstrates the applicability of this method for measuring low nitrogen concentrations such as those that may be encountered in the open sea. Standard addition analysis of glycine and urea, both of which

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Table 1

Total nitrogen concentrations (μ M) of surface water samples collected along a gradient from onshore to offshore during March and April of 1995

Mean	C.V. (%) ^a	n ^b
97.60	4.2	6
74.39	1.4	6
34.27	4.1	6
9.18	7.2	6
7.20	5.5	6
9.94	9.9	6
	Mean 97.60 74.39 34.27 9.18 7.20 9.94	Mean C.V. (%) ^a 97.60 4.2 74.39 1.4 34.27 4.1 9.18 7.2 7.20 5.5 9.94 9.9

^aC.V. determined as standard deviation/mean.

^bTwo samples collected and analyzed in triplicate.

^cSamples filtered through 5 μ m filter.

are known to occur in the dissolved organic nitrogen pool of natural waters (Sharp, 1983), resulted in recoveries of 99.5 and 94.3%, respectively.

Analysis of natural surface water samples revealed a predictable gradient of decreasing total combined nitrogen concentrations from onshore to offshore (Table 1). Concentrations of TN ranged from 7.20 μ M at 100 m offshore of Molasses Reef

to 97.60 μ M in the Florida Everglades. These results demonstrate the utility of this method for examining nitrogen dynamics in a wide range of aquatic environments.

Analysis of surface water TN and TIN concentrations from 300 sampling sites inshore and offshore of the south Florida and the Florida Keys (Figs. 5 and 6) reveals large gradients in the distribution of



Fig. 5. Contour plot of total nitrogen concentrations. Crosses indicate sampling sites.



nitrogen. The distribution of TIN is similar to that of TN, with nitrogen concentrations of TN and TIN highest within the center of Florida Bay. TN concentrations ranged from 7.56 μ M offshore of the northern Florida Keys to 108.87 μ M within central Florida Bay, while TIN ranged from 0.13 to 25.54 μ M along the same gradient. Organic forms of nitrogen dominated the nitrogen pool consisting of approximately 90% of the total nitrogen concentrations. The generation of these TN and TIN contour plots of nitrogen concentrations illustrate the ability of this HTC methodology to discern fine-scale differences in the distribution of nitrogen across an estuarine gradient. The volume of sampling sites necessary to generate these detailed distributions necessitates a fast and efficient means of total nitrogen analysis. This is provided by the automated HTC methodology without a need for expensive reagents and without chemical waste to dispose of. Neither Kjeldahl nor alkaline persulfate digestions can provide these features.

This method is simple, automated and rapid (approximately one minute per injection cycle). The method is also highly accurate and precise for a wide range of total nitrogen concentrations that may be encountered in various aquatic environments. The simplicity and rapidity of this method will hopefully lead to the widespread acceptance the chemiluminescent method of combined nitrogen determination.

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