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Determination of total dissolved nitrogen in seawater by isotope dilution gas chromatography mass spectrometry following digestion with persulfate and derivatization with aqueous triethyloxonium

Enea Pagliano[a,*], Beatrice Campanella[b], Lisa Shi[a], Marie-Pier Thibeault[a], Massimo Onor[b], Steven Crum[c], Jeremy E. Melanson[a], Zoltán Mester[a]

[a] National Research Council of Canada, 1200 Montreal Road, K1A 0R6 Ottawa, Ontario, Canada
[b] Consiglio Nazionale delle Ricerche (CNR), Istituto di Chimica dei Composti Organometallici, UOS di Pisa, Via Moruzzi 1, 56124 Pisa, Italy
[c] QUASIMEME, NL-6700 EC Wageningen, Bornsesteeg 10, 6721 NG Bennekom, The Netherlands

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ABSTRACT

In this study, we propose a novel approach for the determination of total dissolved nitrogen (TDN) in seawater combining high-precision isotope dilution GC–MS with persulfate digestion. A 2 mL sample aliquot was digested with an alkaline solution of persulfate to convert nitrogen containing compounds to nitrate. Digested samples were spiked with $^{15}NO_3^-$ internal standard and treated with aqueous triethyloxonium to convert the analyte into volatile $EtONO_2$. This derivative was readily separated from the matrix under gaseous form and could be sampled from the headspace before GC–MS analysis. The resulting chromatograms showed a stable flat baseline with $EtONO_2$ as the only eluting peak (retention time 2.75 min on a DB 5.625 column). Such an approach provides specificity and obviates the shortcomings of current detection methods employed to analyze seawater samples after digestion with persulfate. In negative chemical ionization mode, the method reached a detection limit of 0.5 μmol/kg TDN (7 ng/g N) and could be applied to quantify seawater samples with 1–25 μmol/kg TDN. On the upper end of the range, quantitation could be repeated within 1%, whereas on a 6 μmol/kg TDN sample repeatability was 2.3% on eight measurements. The method was employed in two proficiency testing exercises providing results in agreement with consensus values. We investigated the impact of reagent blank and we implemented a blank-matching optimal design to account for such contribution. Finally, we performed a study on the yield of persulfate oxidation for organic and inorganic nitrogen compounds typically present in seawater. Whilst nitrite and ammonium are fully converted to nitrate, more complex organic molecules showed recoveries varying from 70% to 100%.

1. Introduction

Within current monitoring schemes, accurate and precise determination of total dissolved nitrogen (TDN) in seawater is required for understating the biogeochemistry of a marine ecosystem [1,2]. Historically, such determination has always been a challenging task [3] and today's strategies still rely on methods which have limitations. Measurement of TDN requires quantitative conversion of N-compounds to a single measurable species [4].

Kjeldahl acid digestion was employed in early studies for conversion of organic nitrogen to NH_4^+ [5,6]. This method yielded total organic nitrogen and ammonia, therefore separate determination of nitrite and nitrate was required to estimate TDN. A second wet digestion approach was introduced in the late sixties by Koroleff who converted N-compounds to nitrate in aqueous alkaline persulfate ($S_2O_8^{2-}$) [7]. Despite complete orthogonality, early studies demonstrated comparability between these two strategies [5,6]. Another methodology for TDN was later introduced with high-temperature (catalytic) combustion systems (HTC) [8–12]. In this case, N-compounds were converted into NO_2^* which could be detected with a nitric oxide chemiluminescent detector. A few studies demonstrated fair comparability between HTC and aqueous oxidation with $S_2O_8^{2-}$ [10,11,13], but a recent investigation highlighted that the persulfate method yields higher recovery of nitrogen respect to HTC [12].

* Corresponding author.
E-mail addresses: enea.pagliano@nrc-cnrc.gc.ca, enea.pagliano@outlook.com (E. Pagliano).

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Within these three techniques, wet digestion with $S_2O_8^{2-}$ remains the most popular method for analysis of TDN in natural waters [3,5,6,14–25]. This approach does not require expensive apparatus, is safe to implement, and can be employed also for total phosphorous determination [3,19,25]. Published procedures using $S_2O_8^{2-}$ include batch [3,16] and flow injection methods [4] with both thermal [3,16] or UV [23,24] assisted digestion. Flow injection methods with UV mineralization allow high sample throughput [23], but the limited digestion times may result in partial conversions with interference of the unreacted $S_2O_8^{2-}$ on the Cd/Cu column which is commonly employed in nitrate analyzers [4]. Thermal digestion in batch mode is more reliable and easier to control. Reaction is typically performed at 120 °C for 30–60 min in H_3BO_3/NaOH buffer at initial pH of 9.7 [3]. In these conditions, $S_2O_8^{2-}$ oxidizes organic matter present in the sample. The theoretical amount of oxygen generated from $K_2S_2O_8$ is described by this equation [14]:

$$2K_2S_2O_8 + 2H_2O \rightarrow 4KHSO_4 + O_2$$

and complete oxidation of an organic molecule such as L-methionine follows this stoichiometry:

$$C_5H_{11}NO_2S + 19K_2S_2O_8 + 15H_2O \rightarrow 5CO_2 + HNO_3 + H_2SO_4 + 38KHSO_4$$

Detection of nitrate in digested samples has been attempted with a number of methods. Cd/Cu column $NO_3^- \rightarrow NO_2^-$ reduction followed by Griess derivatization with detection at 541 nm is the gold standard for nitrate determination [4]. However, a number of limitations affect this approach. For example, $NO_3^- \rightarrow NO_2^-$ conversion needs periodic verification [3,16] with proper maintenance/regeneration of the Cd/Cu column [5]. Furthermore, extensive use of toxic cadmium poses concerns for both the analyst and the environment. For these reasons, alternative detection of NO_3^- at 220–230 nm was proposed [21–24]. However, selectivity issues are known for direct UV analysis [4]. Ion-chromatography with suppressed conductivity was employed for NO_3^- measurement [20,25]. For this method, chlorate is a typical interference. ClO_3^- may originate from oxidation of Cl^- by $S_2O_8^{2-}$ and becomes problematic for samples with high-salinity like seawaters [20].

In order to address such shortcomings, we developed a method based on thermal persulfate digestion in batch mode followed by selective high-precision isotope dilution GC–MS for nitrate quantitation. Digested samples were spiked with $^{15}NO_3^-$ internal standard and reacted with aqueous triethyloxonium tetrafluoroborate to convert NO_3^- in volatile $EtONO_2$ [26,27]. This derivative was sampled from headspace and could be detected free-from-interferences in negative chemical ionization GC–MS.

2. Materials and methods

2.1. Definitions

In raw seawater samples, we can distinguish between inorganic dissolved nitrogen (IDN), organic dissolved nitrogen (ODN) and total particulate nitrogen (TPN) [4,28]. IDN is the sum of nitrite, nitrate, and ammonia. ODN is the sum of all organic species like urea, amino acids, amines, peptides and proteins which are fully dissolved in seawater. Finally, TPN is operationally defined as the nitrogen (mostly organic) contained in particulate matter which cannot go through a 0.2 μm filter. In this study, we performed method development for analysis of total nitrogen in seawater samples previously filtered at 0.2 μm. Therefore, our method yielded total ODN + IDN. This quantity is known as total dissolved nitrogen (TDN) and does not include N_2 which is present in the sample as dissolved gas.

2.2. Reagents and standards

Three batches of potassium persulfate (Sigma-Aldrich 60489; Alfa Aesar 46939; Millipore-Sigma 1.05092.0250) and boric acid (Sigma-Aldrich B7901; ACROS Organics 180570010; Millipore-Sigma 1.00165.0100) were tested for residual nitrogen. Sodium hydroxide solution 30% (Sigma-Aldrich 13171) was used for preparation of the oxidizing reagent. ^{15}N-labeled potassium nitrate (Cambridge Isotope Laboratories $K^{15}NO_3$, 99% enrichment) was employed as internal standard, whereas Fluka Nitrate Standard for IC (*Trace*CERT®, 1001 ± 4 mg/g NO_3^- in water) was used as primary standard of natural isotopic composition. Triethyloxonium tetrafluoroborate (Sigma-Aldrich 90520) was used for derivatization. All solutions/dilutions were prepared gravimetrically with ultrapure water (18.2 MΩ cm at 25 °C) or in 3.5% sodium chloride (Sigma-Aldrich S7653). For method development, several organic and inorganic compounds containing nitrogen were digested to test recovery: sodium nitrite (Fluka 67276), ammonium chloride (Carlo Erba 419417), alanine (Fluka 5150), tryptophan (Sigma-Aldrich 93659), arginine (Sigma-Aldrich 11009), proline (Sigma-Aldrich P0380), serine (Sigma-Aldrich 84959), methionine (Sigma-Aldrich M9625), urea (Sigma-Aldrich U0631), triethylamine (Carlo Erba 489553), triethanolamine (Sigma-Aldrich 90279), adenosine (Sigma-Aldrich A2002), guanosine (Sigma-Aldrich G8377), Cytidine (Sigma-Aldrich C1131), thymidine (Sigma-Aldrich T7004), uridine (Sigma-Aldrich U6375), aprotinin (Sigma-Aldrich A1153), L-glutathione oxidized (Fluka 49740). For the same purpose, NRC Certified Reference Materials (CRMs) AMET-1 (L-methionine), ALEU-1 (L-leucine), APRO-1 (L-proline) and candidates APHE-1 (L-phenylalanine), ANGII-1 (angiotensin II) were also analyzed.

2.3. Safety consideration

Triethyloxonium tetrafluoroborate is a powerful alkylating agent which is stored at −20 °C and used under fumehood with adequate protective equipment. The non-volatile nature of this reagent along with its disposition to hydrolysis mitigates potential risks.

2.4. Sample preparation

The method proposed for quantitation of TDN in seawater relies on the conversion of N-compounds to nitrate by oxidation with persulfate. The digestion procedure employed in our study was adapted from the method reported by Grasshoff et al. [3] and is consistent with the ISO 11905-1 method [16]. An oxidizing solution was prepared daily in a Nalgene bottle adding 0.5 g of $K_2S_2O_8$, 0.3 g of H_3BO_3, 50 mL of water and 375 μL of 30% sodium hydroxide solution. 2.0 mL of sample was transfered in a glass vial (Fisher Scientific 03-339-22B) with 2.0 mL of oxidizing solution. In such alkaline medium, the vials were immediately closed with PTFE/silicone septa screw caps in order to avoid losses of ammonia. The digestion was thermally assisted at 120 °C for 1 h in a heating block. After cooling to room temperature, 0.2 mL of 6.0 μg/g $^{15}NO_3^-$ internal standard was added following vortex mixing. 2 mL of digested sample was transfered to a 10 mL headspace vial (Agilent 8010-0139) and reacted with 50 μL of $Et_3O^+[BF_4]^-$ aqueous solution (Fig. 1). The last reagent was prepared by dissolving 1 g of $Et_3O^+[BF_4]^-$ in 1 mL of water at 4 °C and used within 5 min. After 30 min of reaction at room temperature, the headspace was analyzed by GC–MS as described in Section 2.6.

2.5. Calibration

An isotope dilution calibration plot was obtained for quantitation as previously described [29]. Six concentration levels in the