Method for seepage water samples collection and sum of nitrate and nitrite analysis (0.71–35.69 µmol/dm^3

Developed by:
Żaneta Kłostowska
Beata Szymczycha

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Introduction

Nitrogen is the main building block of cellular proteins. In the soft tissue of phytoplankton on average 16 nitrogen atoms per 1 phosphorus atom occur. Hence the ratio 16N: 1P in organic matter has been determined as the Redfield index. In the case of phytoplankton and green plants, nutrients are taken up in the form of inorganic ions, and in the case of cyanobacteria, biological binding of atmospheric nitrogen (N2) is an important process. Inorganic forms of nitrogen include: nitrates III (NO2-), nitrates V (NO3-) and ammonium ions (NH4 +). It should be noted that nitrogen compounds significantly contribute to the intensification of the eutrophication process in marine areas.

The main source of nitrogen in the marine ecosystem: emission to the atmosphere of nitrogen oxides formed during transport and in the combustion processes of biomass or minerals and, as a consequence, deposition in seas and oceans, as well as artificial fertilizers inflowing along with surface runoff and inflow or discharge of untreated sewage in the coastal zone.

Nitrogen in the marine environment undergoes many processes shown in Fig. 1. For example, nitrogen gas can be released in the process of reducing nitrates (III and V) called denitrification. Anamox, or anaerobic oxidation of ammonium ions, is another process that also causes gaseous nitrogen. On the other hand, oxygen oxidation of ammonium ions, so-called nitrification, leads to the formation of nitrates (V).

The concentration of nitrogen compounds in waters is characterized by seasonal variation, dictated by the growing season, as well as the size of loads introduced into marine areas along with river waters. In addition to seasonal variation, variation in the concentration of nitrogen compounds in the vertical profile of the water column and along the distance from the shoreline is observed. In the case of the coastal zone, an increase in concentrations compared to open sea areas is observed due to the proximity of sources of inflow of these substances.

During groundwater discharge to the marine environment, groundwater mixes with sea water in the bottom sediment. The water created as a result of mixing is seepage water, which under certain conditions comes out of the sludge and can be a source of many chemical substances including nitrates for the marine environment. This water has a composition different from both groundwater and sea water, and for this reason a dedicated methodology was prepared, with particular regard to sampling for analysis.

The scope of application of the method

The analysis of sum of nitrate ions III and V analysis in seepage water samples is developed using the spectrophotometric method prepared by Hach-Lange, which is one of the methods recommended by HELCOM. The method is a modification of the method dedicated to fresh waters for N-NO2 + N-NO3 analysis in the range of 0.7138 to 35.690 μmol / dm3. This method is based on the relationship of solution absorbance and concentration of colored substance. For the determination, the selected ion is transferred to the colored complex. Spectrophotometric techniques are defined by the Lambert-Beer law:

where:
ε - absorption coefficient [$dm^3 \cdot mol^{-l} \cdot cm^{-l}$] at wavelength λ [nm],
l - thickness of the absorbing layer,
c - concentration of analyte in the test solution.

If the test substance satisfies the above relationship, absorbance is expressed as a linear function of the concentration of the analyte.

$$A = f(c)$$

Thus, absorbance is proportional to the thickness of the absorbing layer if a monochrome radiation beam passes through a homogeneous absorbing medium.
If there are several components in the tested solution, the spectrophotometric determination can be performed correctly only if the absorbance additivity law is met, according to which the absorbance of the mixture is equal to the sum of the absorbance of individual components and the absorbance of a single component is as if only one was in the tested solution sample.
Very low concentrations of the colored substance in the solution are determined with a large error, because the permeability of the test solution is similar to that of the reference solution and usually close to 100%. In the case of intensively colored solutions, only a small part of the radiation passes through the solution, which increases the error of measurement results. In order to select the most favorable concentration of the absorbing layer, it is necessary to find such A (T) values that, for a given error ΔA (ΔT), the relative error in determining the concentration of Δc / c is the smallest.
The method described above is used to determine the trace content and to determine the purity of the main component and marked substances in environmental samples - including seepage, after being prepared for analysis.
The method range is used to measure concentrations of nitrate (III) ions 0.7138-35.69 μmol / dm3, respectively

The principle of the method

The spectrophotometric determination of the total content of nitrate ions V is based in the initial phase on the cadmium reduction reaction to nitrates III. Then, during the diazotization reaction, the nitrate ion III reacts in an acidic medium with sulfanilic acid to form an intermediate diazonium salt. The resulting salt combines with chromotropic acid to form a pink color on the test solution.
The intensity of the resulting pink color is proportional to the total content of nitrite and nitrate ions, is measured by spectrophotometric, photocolorimetric methods or is evaluated visually.

Expressions

Substances and interfering factors

In the determination of NO3- ions, interfering substances can include: calcium> 100 mg / dm3, chloride ion above 100 mg / dm3 results in a lowering of the result (the analysis can be carried out at a high concentration of chloride ions - sea water), if calibration is carried out for standards seawater), iron interferes at all concentration levels, nitrites interfere at all levels - their presence can be compensated by adding dropwise to the sample a solution of bromine water at a concentration of 30 g / dm3 until yellow color persists or by adding one drop of phenol solution at a concentration of 30 g / dm3 to remove the color. Interference also includes: a highly buffered sample or extreme pH values - should be corrected by adding appropriate reagents, turbidity, color, the presence of highly oxidizing and reducing substances.

In the case of determination of NO2 ions, the following substances and interfering factors include: antimony ions - interfere by precipitation, gold ions - interfere by precipitation, silver ions - interfere by precipitation, bismuth ions - interfere by precipitation, chloroplatinate ions - interfere by precipitation, copper understatement of the analysis result, iron ions - understatement of the analysis result and interfere by precipitation, lead ions interfere by precipitation, mercury ions interfere by precipitation, methanadanate ions interfere by precipitation, also high levels of nitrate (designated as N> 100 mg / dm3) get slightly reduced in relation to nitrites, spontaneously or during measurement, also at this level small amounts of nitrite are measurable. Strong oxidizing and reducing substances interfere at all levels.
Take special care when performing the analysis !!!
When performing the marking, extreme caution should be exercised,
due to cadmium exposure. Information on safe handling and waste disposal can be found in the safety data sheet. Observe all regulations regarding the disposal and storage of hazardous waste.
This method is sensitive to technique. Both shaking time and mixing technique affect color development. Adjust the shaking time to the sample. Sediment formed from non-oxidized metal during the reaction, remaining at the bottom of the tube, does not affect the measurement result. Due to the presence of cadmium in the reagents used, the cuvette and tube should be rinsed immediately after use,to remove cadmium particles. Measured solutions must be properly disposed.

Analytical Errors

The main analytical errors in determining the total content of nitrite and nitrate ions in seepage waters result, among others, from the basic limitations of Lambert-Beer laws, these include primarily other electromagnetic radiation interactions. In turn, chemical factors that cause deviations from the rectilinear course of absorbance include the possible occurrence in the tested solution, affecting the optical properties of the tested liquid, complexation reactions, dissociation, association, polymerization, solvation, and pH changes. Apparatus factors should also be taken into account, where in spectrophotometry it is mainly: insufficient monochromatization of radiation, and the occurrence of scattered radiation.
Analyzing the obtained results, which significantly differ by absorbance values compared to other solutions, the possibility of systematic, methodological or instrumental errors should be considered. Perform the measurement again on the newly prepared solution according to the analytical procedure,
with due care.

Equipment and Supplies

Before proceeding with the determination, the following should be prepared:
- spectrophotometer,
- 10 cm3 automatic pipette and disposable nail tips,
- screwed PTE containers with a capacity of up to 50 cm3,
- dust-free paper towel,
- MiliQ deionized water wash bottle,
- 20 cm3 syringes,
- CA membrane filters with 0.45 µm pore diameter,
- measuring cuvette,

- tripod,
- labeled chemical collection container,
- reagent sachets,
-Nitra Ver 3 and 6 by Hach Lange
- Spreadsheet.

A convenient tool for determining the total content of III and V ions in seepage water is the HACH LANGE spectrophotometer, dedicated for analyzing selected components in water and wastewater samples. Absorbance measurements were made on a HACH LANGE DR2800 spectrophotometer.

All materials used during the assay should be prepared in advance to avoid introducing the analyte into the system, which may result in an increase in absorbance. For maintenance and cleaning of materials and instruments, chemically inert agents (e.g. Extran® MA 02) should be used.

Reagents and Solutions

Blank

In order to maintain the accuracy and precision of the measurement, each time the absorbance should be measured in a given wavelength range for the blank sample. A blank sample should be prepared according to the preparation procedure as for the test solution. The blank sample solution should be MiliQ deionized water with zero content of other interfering substances and a chloride concentration similar to the sample.

Calibration curve

In order to properly calibrate the spectrophotometer, the calibration curve method should be used, which is the dependence of absorbance on the concentration of the reference substance.
In the case where the dependence of absorbance on concentration is determined by a linear function, a minimum of three calibration points should be performed. Performing two points is necessary to run a straight equation and read the coefficients a and b for the standard curve. The linearity of the measuring system is confirmed by the third point, which also illustrates the increase in analyte concentration. Good laboratory practice indicates that a minimum of three measurements have been taken for each point. This allows the removal of outliers and the correct delimitation of absorbance depending on the concentration of the analyte. It is necessary to prepare from 3 to 10 standard solutions with known, strictly determined concentrations of the analyte, selected so that they differ by about 30% and include in their range the concentration of analytes in the determined solutions. Each time before making a series of measurements, a calibration curve should be prepared. Changing the reagent lot or the room temperature can affect the curve slope or its shift. The results obtained should be presented in the coordinate system: signal - absorbance - analyte concentration. A specific function must be adapted to the data obtained. Measurements should be made at a specific wavelength that corresponds to the maximum absorption of the substance being determined, and relative to the reference solution. Obtaining a rectilinear course of the relationship A = f (c) indicates that the studied system satisfies the Lambert-Beer law. Then, on the basis of a straight line, determine the direction factor from which the absorption coefficient of the determined substance should be determined.

In order to prepare standard solutions for the calibration curve with known concentrations, the standard solution of nitrite (in relation to SRM with NIST $NaNO_2$ in H_2O 1000 mg / l NO_2 Certipur®) and nitrates (in relation to SRM with NIST $NaNO_3$ in H_2O 1000 mg / l NO_3 Certipur®) to obtain the room temperature recommended for working solutions. The main solution should be a certified reference material, stored in accordance with the manufacturer's instructions, which minimizes the occurrence of systematic errors.

Enviromental samples

Seepage water samples collections

In order to collect seepage water samples in the first stage, an active seepage area in the coastal zone should be selected. Place the lance in the sludge at a depth of 10 cm (the depth of location of the lance in the sediment should be read from the scale). Then place the syringe in the adapter at the top of the lance with a capacity of 50 cm3. Generate the first batch of water by creating a vacuum using the piston. Empty the syringe, repeat the procedure. Flush the 50 cm3 PTE container with the water sample. Then take another water sample, transfer into the container. The sample should be poured slowly along the wall to prevent oxygenation of the test material. After filling the container to a volume of 50 cm3, place in it the electrodes: pH, PSU, Eh and oxygen optode. Perform in situ measurements using a multi-parameter meter. The electrodes used for the measurement should be previously calibrated in accordance with the manufacturer's instructions. The determinant of the occurrence of freshwater seepage is the value of PSU in relation to sea water. A value lower by 0.5 PSU than sea water indicates the presence of seepage water in the studied area. After in situ measurements, a Teflon hose connected to the peristaltic pump should be connected to the lance in place of the fitting. Check the sample for suspensions and turbidity before sampling. If present, filter the sample using a cellulose syringe filter with a 0.45 µm pore diameter connected to the hose. The sample should be taken using a low flow value. Take 50 cm3 samples, seal the container tightly and place in the refrigerator for transport to the laboratory. If it is not possible to perform the determination immediately, the sample should be filtered and the samples stored below 6 ° C for a period of 48 hours. The sample can also be preserved by freezing at -20 ° C until the sum of nitrate and nitrite ions is determined.

Preperation of samples

If the water sample has been preserved by freezing, it should be moved to a room with room temperature for thawing for 12 hours prior to analysis.

Analitycal steps

Calibration of the spectrophotometer

In order to correctly perform absorbance measurements, before starting the analysis, the device should be turned on for two hours before taking it. To assess the correct operation of the spectrophotometer in a given wavelength range, a series of measurements should be made for samples of known concentration. Before measuring the absorbance of a colored solution

taking into account the optical path, a suitable cuvette should be selected for the Hach Lange 10 ml spectrophotometer.

Analitycal procedure

The analytical procedure has two stages. In the first stage, measure a 15 cm3 sample with a calibrated pipette into a previously prepared container, then pour NitraVer 6 into the solution. Close the container tightly and mix for 3 minutes. When shaking, not all of the reagent volume needs to dissolve. Return the tube to the rack and wait 2 minutes to react the reagent in the solution. Then draw the solution from the tube into the syringe. Insert a CA syringe filter with a 2.5 cm diameter membrane and 0.45 µm pore diameter, dedicated to aqueous solutions. Remove air bubbles, then measure out into a clean test tube 10 cm3 of solution. To this sample, add NitriVer 3 reagent and mix gently for 30 seconds. Return the tube to the rack for 15 minutes, then transfer the volume of 10 cm3 of the sample to the cuvette and measure absorbance at 520 nm for seepage water.
In the event of intense coloring, the sample should be diluted with a certain amount of MiliQ water and then considered at a later stage in calculation of the dilution concentrations.

Calculations

Calculation of dilutions for the standard curve

Standard (W): C [NO_3^-] = 1000 mg NO_3^- l^{-1} = 16128 µmol·dm^{-3}

Main standard (SD_G): 10 ml W - 100 ml = 1612,8 µmol·dm^{-3}

Working standard 1 (SD_{R1}): 0,04 ml SD_G – 50 ml = 0,32256 µmol·dm^{-3}

Dillution calculation:

I -W

M NO_3^-= 62,0049 g·mol^{-1}

C= 1g/62,0049 g·mol^{-1} = 0,0161277576 mol·dm^{-3} = 16128 µmol·dm^{-3}

II - SD_G

$C_1 \cdot V_1 = C_2 \cdot V_2$

16 128 µmol·dm^{-3} · 10 ml = C_2 · 100 ml

C_2= 1612,8 µmol·dm^{-3}

Results calculations

A_{520}=35,021 C NO_2^-+NO_3^- – 0,5267

A_{520} – absorbancja przy długości fali 520 nm

C – stężenie sumy jonów NO_2^-+NO_3^- (µmol·dm^{-3})

C NO_2^-+NO_3^- (µmol·dm^{-3})=(A_{710} +0,5267)/35,021

Tabel 1. Example of calculation

Opis	Stężenie [µmol dm^{-3}]	abs_1	abs_2	abs_3	abs_4	abs_5	$abs_{śr}$	$abs_{śr}$ – $abs_{śr\ Ślep\ 1}$
SDR1								
SDR2								
SDR3								
SDR4								
SDR5								
SDR6								
SDR7								
SDR8								

Relative error

It refers the value of the absolute error to the actual value (µ) and thus determines its meaning for the determination. The relative error for high concentration samples should not exceed 0.1%, for determination of trace amounts it may exceed even 20%.

$$E_{wzg} = \frac{E_{abs}}{\mu} = \frac{x - \mu}{\mu}$$

$$\%E_{wzg} = \frac{x - \mu}{\mu} \cdot 100$$

Relative standard deviation

RSD (relative standard deviation), independent of measurement units. It is expressed as the quotient of the standard deviation and the mean of the measured values:

RDS is a number less than one and is often expressed as a percentage as the coefficient of variance CV%.

Reproducibility of determinations

Statistical treatment of results gives RDS reproducibility. The accuracy of the determinations depends on the concentration of the analyte in the sample. It is assumed that the developed method meets the requirements when:

where:
c - mass concentration of the analyte.

Precision alone is not a sufficient parameter to obtain and evaluate accurate results.

Absolute error

It is defined as the difference between the measured value of x and the actual value. It can be positive or negative and is usually given in absolute value. For the average value of the measurements it is the difference of this value and the actual value.

Precision of the method

The precision of the method is the degree of agreement between the results obtained by the same method and on the same sample with repeated assays. It can be defined as the dispersion of individual results with repeated experiments relative to the average result of the determinations. The greater the precision, the smaller the spread. With repeated experiments, we never get two identical results, and the correct results are always arranged according to the normal distribution in the shape of a bell curve. The best measure of precision is the standard deviation σ (or its approximation s). Random error is responsible for the accuracy of the markings.

Sensitivity of the method

In spectrophotometric measurements, the parameter determining the method sensitivity is the molar absorption coefficient (ε). The molar absorption coefficient may not exceed 1.5 x 105. The lowest concentration of the substance (mol / l) spectrophotometrically measurable can be calculated using the Lambert-Beer formula. Assuming that A = 0.02 (minimum absorbance that can be measured), l = 2 cm (cuvette thickness), and ε = 104 (molar absorption coefficient of a medium sensitive spectrophotometric method).