

**Method for seepage water samples collection and ammonium ( $\text{NH}_4^+$ ) analysis  
(1.43-178.48  $\mu\text{mol}/\text{dm}^3$ )**

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## Introduction

Ammonium ions ( $\text{NH}_4^+$ ) are a dissociated form of ammonia. Ammonia has very good solubility in water. The form in which ammonium ion occurs in the environment is largely dependent on the pH value. In the case of natural surface waters, with a pH close to 7, about 99.8% of ammonium ion is in dissolved form, while 0.2% in gaseous form. Equally, as the pH increases, gaseous form rises up to about 15%. Ammonium ions undergo the process of sorption and complexation with fine-dispersion clay material and are one of the indicators of water environment pollution. Even small amounts negatively affect the change of organoleptic properties, i.e. taste and smell. The main sources of ammonium ions in the environment include discharges of municipal or industrial sewage. In groundwater, ammonia occurs mainly as a result of the reduction of nitrates and nitrites and the mineralization of organic matter. The presence of ammonium ions in marine waters shows high concentration fluctuations throughout the year. An increase in concentrations is observed in the winter, while a decrease in concentrations is noted in the summer. Ammonium can be oxidized (nitrification), as a result of which nitrate and nitrite ions are formed.

During groundwater inflow to the marine environment, groundwater mixes with sea water in the bottom sediment. Therefore seepage water can be a source of many chemical substances including ammonium ions for the marine environment. This water has a composition different from both groundwater and sea water. Therefore, a dedicated methodology was prepared with particular regard to sampling for analysis.

### The scope of application of the method

For the analysis of ammonium ions in seepage water samples, a method was developed using the 8038 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 in the range from 1.43 - 178.48  $\mu\text{mol}/\text{dm}^3$   $\text{NH}_4^+$ . This method is based on the relationship of solution absorbance and concentration of colored substance. In order to perform the determination, the selected ion is transferred to the colored complex. Spectrophotometric techniques are defined by the Lambert-Beer law:

where:

$\epsilon$  - absorption coefficient [ $\text{dm}^3 \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ ] at wavelength  $\lambda$  [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  $\Delta A$  ( $\Delta T$ ), the relative error in determining the concentration of  $\Delta c / c$  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 scope of the method is used to measure ammonium concentrations in the range of 1.43 - 178.48  $\mu\text{mol} / \text{dm}^3$ .

### The principle of the method

Spectrophotometric determination of the total content of ammonium ions is based on direct nesslerization of the solution. It is a reaction of ammonia with Nessler's reagent (potassium tetrajodiscyrate (II)  $\text{K}_2[\text{HgI}_4]$ ) in a strongly alkaline medium, during which an amorphous, red-brown precipitate forms. In the case of low ammonium ion content, only yellow-orange (yellow-brown) coloration of the solution is observed, and the intensity of this color is proportional to the concentration of ammonium ions in the analyzed solution. This reaction is very sensitive and specific for  $\text{NH}_4^+$  ions. Determination of  $\text{NH}_4^+$  ions should be carried out in solution at an alkaline or neutral pH, as  $\text{K}_2[\text{HgI}_4]$  to  $\text{HgI}_2$  decomposes in the presence of acids.

### Expressions

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#### Substances and interfering factors

In the analysis of  $\text{NH}_4^+$  ions, interfering substances can include: chlorides (remove chlorine from a 250 ml sample by adding 1 drop of sodium thiosulfate for each  $\text{mg} / \text{L} \text{Cl}_2$ ), carbonate and magnesium hardness - interfering with concentrations above 500  $\text{mg} / \text{dm}^3 \text{CaCO}_3$  and 500  $\text{mg} / \text{dm}^3 \text{Mg}$  (if the concentration is higher than 500  $\text{mg} / \text{dm}^3$ , an additional mineral stabilizer should be added to the sample), iron - interferes at each level - causing turbidity after using Nessler's reagent, sea water - chloride ion - interferes at each level (1.0 ml (27 drops) of mineral stabilizer should be added to the sample before analysis to complex high levels of magnesium present in seawater, as a result the sensitivity of the analysis is reduced by 30% due to high chloride concentration, in order to avoid analytical errors apply calibration for chloride ion content of appropriate sea water), sulphide and - interfere at every level - causing turbidity after using the Nessler reagent, glycine, various aliphatic and aromatic amines, organic chloramines, acetone, aldehydes and alcohols - interfering at each level (may cause greenish or other color or turbidity, distil sample to removal of these compounds).

Take special care when performing the analysis !!!

When performing the marking, extreme caution should be exercised, due to exposure to mercury iodide contained in Nessler's reagent. 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 mercury iodide in the reagents used, the cuvette

and tube should be rinsed immediately after use, to remove mercury iodide particles. Measured solutions must be disposed of properly.

### Analytical Errors

The main analytical errors in determining the total content of ammonium 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

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Before proceeding with the determination, the following should be prepared:

- spectrophotometer,
- 10 cm<sup>3</sup> automatic pipette and disposable nail tips,
- screwed PTE containers with a capacity of up to 50 cm<sup>3</sup>,
- dust-free paper towel,
- MiliQ deionized water wash bottle,
- measuring cuvette,
- tripod,
- labeled chemical collection container,
- reagent sachets,
- Poly (vinyl alcohol) dispersant from Hach Lange,
- Hach Lange mineral stabilizer solution,
- Nessler's reagent from Hach Lange,
- Spreadsheet.

A convenient apparatus for determining the total content of ammonium ions in seepage waters is the HACH LANGE spectrophotometer, dedicated to the analysis of selected components in water and sewage samples. Absorbance measurements were made on a HACH LANGE DR2800 spectrophotometer (photometric accuracy:

3 mAbs at 0.0 to 0.5 Abs, 1% at 0.50 to 2.0 Abs; repeatability  $\pm 0.1$ nm).

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.

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## Reagents and solutions

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### 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 other interacting substances and similar chloride content.

### 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 a minimum of five measurements per 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 5 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 calibration solutions for the calibration curve with known concentrations, the ammonium ion calibration solution (SRM) must first be left with NIST  $\text{NH}_4\text{Cl}$  in  $\text{H}_2\text{O}$  1000 mg / l  $\text{NH}_4$  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

## Environmental samples

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### Seepage water samples collection

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 cm<sup>3</sup>. Generate the first batch of water by creating a vacuum using the piston. Empty the syringe, repeat the procedure. Flush the 200 cm<sup>3</sup> 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 cm<sup>3</sup>, 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 PSU value. A value lower by 0.5 PSU compared to dark water indicates the occurrence of seepage 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. Turn on the pump, put the end of the hose in the PTE container, flush the entire volume and pour out. Before sampling, the sample should be checked for the presence of suspensions and turbid substances. 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 cm<sup>3</sup> of the filtered sample, seal the container tightly and place in the refrigerator for transport to the laboratory. The sample should be preserved by freezing at - 20 ° C until the determination of ammonium ions.

## Preparation of sample

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.

## Analytical steps

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### *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, you must select the appropriate cuvette.

### **Analytical Procedure**

After thawing the samples, 15 cm<sup>3</sup> of test material should be taken into a PTE container, and then diluted with 10 ml of MiliQ water. In the next step, add 3 drops of mineral stabilizer, screw the tube tightly and mix gently by turning. In the next stage, add 3 drops of polyvinyl alcohol, tightly seal the tube and mix gently by swirling. Unscrew the tube and add 1 cm<sup>3</sup> Nessler's reagent. Wait a minute, then transfer the sample to the measuring cuvette, place in the spectrophotometer and measure the absorbance at  $\lambda = 425$  nm

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.

## Calculation

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### **Calculation of dilutions for the standard curve**

On the basis of the following data and the formulas used.  
Add 10 ml of main standard to 90 ml of MiliQ water.

Standard (W):  $C [\text{NH}_4^+] = 1000 \text{ mg NH}_4^+ \text{ l}^{-1} = 55437.1 \text{ } \mu\text{mol} \cdot \text{dm}^{-3}$

Main Standard ( $\text{SD}_G$ ):  $10 \text{ ml W} - 100 \text{ ml} = 5543.71 \text{ } \mu\text{mol} \cdot \text{dm}^{-3}$

Working standard 1 ( $\text{SD}_{R1}$ ):  $0.01 \text{ ml SD}_G - 50 \text{ ml} = 1.108742 \mu\text{mol} \cdot \text{dm}^{-3}$

### I - W

$M_{\text{NH}_4^+} = 18.03846 \text{ g} \cdot \text{mol}^{-1}$

$C = 1\text{g}/18.03846 \text{ g} \cdot \text{mol}^{-1} = 0.0554371 \text{ mol} \cdot \text{dm}^{-3} = 55437.1 \text{ } \mu\text{mol} \cdot \text{dm}^{-3}$

### II - $\text{SD}_G$

$C_1 \cdot V_1 = C_2 \cdot V_2$

$55437.1 \text{ } \mu\text{mol} \cdot \text{dm}^{-3} \cdot 10 \text{ ml} = C_2 \cdot 100 \text{ ml}$

$C_2 = 5543.71 \text{ } \mu\text{mol} \cdot \text{dm}^{-3}$

### Results calculation

To determine the concentration of the analyte in the sample, the corresponding signal (in the form of absorbance) is recorded and referred to the calibration curve.

Pattern transformation

$A_{425} = 35.021 C_{\text{NH}_4^+} + 0.5267$

$A_{425}$  - absorbance at 425 nm

$C$  -  $\text{NH}_4^+$  ion concentration ( $\mu\text{mol} \cdot \text{dm}^{-3}$ )

$C_{\text{NH}_4^+} (\mu\text{mol} \cdot \text{dm}^{-3}) = (A_{425} + 0.5267) / 35.021$

### Results

Tabel 1. Example of calculation

Sample ID	Concentration [ $\mu\text{mol dm}^{-3}$ ]	abs <sub>1</sub>	abs <sub>2</sub>	abs <sub>3</sub>	abs <sub>4</sub>	abs <sub>5</sub>	abs <sub>śr</sub>	abs <sub>śr</sub> - abs <sub>śr</sub> Śl 1
<b>SDR1</b>								
<b>SDR2</b>								
<b>SDR3</b>								
<b>SDR4</b>								
<b>SDR5</b>								
<b>SDR6</b>								
<b>SDR7</b>								
<b>SDR8</b>								

### Relative error

It refers the value of the absolute error to the actual value ( $\mu$ ) 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  $\sigma$  (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 ( $\epsilon$ ). The molar absorption coefficient may not exceed  $1.5 \times 10^5$ . 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  $\epsilon = 104$  (molar absorption coefficient of a medium sensitive spectrophotometric method).