Method for seepage water samples collection and phosphates (PO_4^{3-}) analysis (0.21- 26.32 µmol/dm^3)

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Table of contents

Introduction 2
The scope of application of the method 3
The principle of the method 4
Expressions 4
Substances and interfering factors 4
Analitical errors 4
Eqiupment and Supplies 5
Reagents and solutions 6
Blank 6
Calibration curve 6
Enviromental samples 6
Seepage water samples collection 6
Preperation of samples 7
Analitical steps 7
Calibration of the spectrophotometer 7
Analitycal procedure 7
Calculation 7
Calculation of dilutions for the standard curve 7
Results calculation 8
Results 8
Relative error 8
Relative standard deviation 9
Reproducibility of determination 9
Absolute error 9
Precision of the method 9
Sensitivity of the method 9

Introduction

In sea water, phosphorus occurs in four forms: dissolved inorganic, dissolved organic, suspended organic and suspended inorganic. Phosphorus is supplied to the marine environment both from external sources such as point sources and surface runoff as well as internal ones as a result of mineralization and hydrolysis of organic compounds. The main sources of phosphorus for the marine environment are untreated industrial and municipal sewage, artificial fertilizers supplied with surface runoff and groundwater inflow, rock weathering and soil erosion, and a return stream from marine sediment.
In the marine environment, phosphorus is one of the elements limiting the primary production. Low or no phosphorus concentrations reduce or stop productivity, while a slight increase results in increased phytoplankton bloom. Oxygen concentration is an important factor controlling phosphorus speciation in the marine environment. In the bottom zone, where low oxygen concentration or anaerobic conditions are often observed, the concentration of phosphates increases (to several dozen µmol/dm^3) as a result of reduction of iron and manganese, while in aerobic conditions the above-mentioned elements with phosphates fall out and accumulate in the bottom sediment.
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 sediemnts and can be a source of many chemical substances including phosphates 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 method was based on a Hach-Lange 8048 procedure, which is recommenced by HELCOM. The method is a modification of the method dedicated to fresh waters in the range from 0.02 to 2.50 mg/dm^3 PO_4^{3-}. The 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 [$dm^3 \cdot mol^{-1} \cdot cm^{-1}$] 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 additively 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 principle of the method

Colorimetric determination of orthophosphate ions in reaction with ascorbic acid is based on the reaction in an acid environment of phosphate ions with a molybdate reagent to form a phosphoromolybdate complex, which is then reduced, resulting in an intense blue colored compound. The reaction of phosphate ions with ammonium molybdate occurs in the sulfuric acid and antimony ions environment, and the reducing agent is ascorbic acid. As a result of these reactions, a mixture of heteropolyacids is formed in which the ratio of phosphorus, antimony and molybdenum is 1: 1: 12. After reduction of hexavalent molybdenum with ascorbic acid, a blue phosphoromolybdenum complex is formed. It is important that the pH of the solution during reduction does not exceed 1. The intensity of the color of the resulting blue is proportional to the content of orthophosphates and is measured by spectrophotometric, photocolorimetric methods or is assessed visually. Colorimetric methods for the determination of orthophosphate are used for drinking water, sewage and seawater - this is equivalent to the US EPA method.

Expressions

Substances and interfering factors

Determination of: aluminum above 200 mg / dm^3, arsenians interference at every level, Cr above 100 mg / dm3, copper above 10 mg / dm^3, H2S interferes at each level, Fe above 100 mg / dm3, Ni above 300 mg / dm^3 , Zn above 80 mg / dm^3, SiO2 above 50 mg / dm^3 and silicates above 10 mg / dm^3. Interference also includes: a highly buffered sample or extreme pH values <2 and> 10, turbidity, color, significant amounts of chlorides, and organic compounds. Very alkaline or very acidic water should be neutralized against phenolphthalein. The effect of silica is eliminated by diluting the sample. The effect of iron can be removed by diluting the sample or adding an equivalent amount of 0.1 M edetate solution. For large amounts of chlorides, a blue-green color develops, which is compensated by comparing the color of the sample with a standard containing chlorides of the same concentration. Turbidity is removed by centrifugation or filtration of the sample. Organic compounds, color, arsenates, depending on the type of phosphates to be determined, are eliminated by sample mineralization or by appropriate dilution.

Analytical Errors

Main analytical errors when determining the content of orthophosphate ions,
in exudative waters, they result, among others, from the basic limitations of Lambert-Beer laws, including 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 considered, 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.

Eqiupment 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,
- measuring cuvette,
- tripod,
- labeled chemical collection container,
- reagent sachets,
- Phos Ver 3 - Hach Lange reagent
- Spreadsheet

A convenient spectrophotometer by HACH LANGE (photo 1), dedicated to the analysis of selected components in water and sewage samples, is a convenient tool for determining orthophosphate ions in seepage waters. 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, use chemically inert agents (e.g. Extran® MA 02).

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, zero interfering substance and chloride ion concentration similar to the samples.

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. 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 phosphate standard solution (reference solution with reference to SRM from NIST KH_2PO_4 in H_2O 1000 mg / l PO_4 Certipur®) must be left in advance.
d = 0.998 g / cm3 at 20 ° C). 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

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 the lance's position in the sludge should be read from the scale). Then in the fitting,
place a 50 cm3 syringe in the upper part of the lance. Generate the first batch of water by creating a vacuum using the piston. Empty the syringe, repeat the procedure. Flush the 200 cm3 PTE container with the water sample. Then take another water sample, transfer to a 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 PSU value. A value smaller by about 0.5 than sea 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 cm3 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 orthophosphate 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 to defrost.

Analytical 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, you must select the appropriate cuvette. For the Hach Lange spectrophotometer, the cuvette volume is 10 ml.

Analytical Procedure

After mixing the sample to obtain a homogeneous solution, transfer with an automatic pipette 10 cm3 of the sample to a previously prepared PTE tube. Open the PhosVer 3 reagent sachet, transfer to the sample tube. Close the tube tightly and mix for 20-30 seconds, using a swinging motion. The reagent does not completely dissolve. Return the tube to the rack, wait 120 seconds, until the reaction is complete. The absorbance should be measured two to eight minutes after the reagent has been added. Transfer the sample to the cuvette, place in the spectrophotometer measuring cell, after selecting the appropriate program or wavelength [nm]. The wavelength for measuring the concentration of orthophosphate in seepage water is λ = 710 nm.

Calculation

Calculation of dilutions for the standard curve

Standard (W): C [PO43-] = 1000 mg PO43- l-1 = 10 529 µmol • dm-3
Main standard (SDG): 10 ml W - 100 ml = 1052.9 µmol • dm-3
Working standard 1 (SDR1): 0.02 ml SDG - 50 ml = 0.21058 µmol • dm-3
Dilution calculations

I

$M_{PO4\ 3-}$= 94,9714 g·mol^{-1}
C= 1g/94,9714 g·mol^{-1} = 0,01052948572 mol·dm^{-3} = 10529 µmol·dm^{-3}

II - SD_G

$C_1 \cdot V_1 = C_2 \cdot V_2$
10 529 µmol·dm^{-3} · 10 ml = C_2 · 100 ml
C_2= 1052,6 µmol·dm^{-3}

III-SD R1

$C_1 \cdot V_1 = C_2 \cdot V_2$
105,26 µmol·dm^{-3} · 0,01ml = C_2 · 50 ml
C_2 = 0,21058 µmol·dm^{-3}

Calculation of the determination result

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. The following is an example calibration curve for orthophosphate ions.

A_{710} = 35,021 C PO_4^{3-}- 0,5267

A_{710} – absorbancja przy długości fali 710 nm

C – stężenie fosforanów (µmol·dm^{-3})

C PO_4^{3-} (µmol·dm^{-3}) = (A_{710} +0,5267)/35,021

Results

Tabel 1. Example of calculation

Sample ID	**Concentration [µmol dm^{-3}]**	**abs_1**	**abs_2**	**abs_3**	**abs_4**	**abs_5**	**$abs_{śr}$**	**$abs_{śr}$ – $abs_{śr\ Śl\ 1}$**
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).