SAFE SWIMMING
Measuring phosphates and nitrates in water

Author
F.Bosman, H. Hotsma, R. Van Iterson, S. Noorda, Drenthe College, van Schaikweg 94 Emmen, the Netherlands; f.bosman@drenthecollege.nl
Languages available
English, Hungarian

Summary
In this project, a team determines the degree of water pollution from the nutrients nitrate and phosphate. Nitrate and phosphate are water-soluble ions, which cause excessive growth of algae and other water plants. These ions come from fertilisers (or from old-type washing powders containing phosphate).
The team determines the phosphate and nitrate content and draws a conclusion about the acceptability of the water for swimming. Resource and budget management is part of the project.

Activity type D
Use of scientific knowledge and understanding to solve problems
Working in teams to solve problems
Resource/ budget management

Techniques
Visible spectrophotometry
Fluorescence spectroscopy

Field
Natural water, lake or canal

Time
Practical lessons: 305 minutes
Theory lessons: 70 minutes
Out of class: 40 minutes

StandardBase procedures
Determination of phosphate in diet Coca Cola using visible spectroscopy

StandardBase techniques
UV-VIS spectrophotometry

Other resources
Technomatch, Emmen, Netherlands; www.technomatch.nl
http://www.umanitoba.ca/institutes/fisheries
http://www.standardbase.com

Student′s document

SAFE SWIMMING: Measuring phosphates and nitrates in water

In many countries there are pools, lakes and canals which have a beautiful green colour. It is very tempting to go for a swim - you might have an image of an emerald green ocean. Is the green water ′ocean water‵ or is the colour caused by green water plants that are growing rapidly due to the availability of nutrients? Suppose you work at a laboratory that regularly checks the quality of swimming water. In the local newspaper there is an article where a reporter questions the water quality of a small stream where you used to swim very frequently. The people staying at the *Green Oasis* camp site, nearby, are getting nervous about all negative publicity and articles concerning the suspicious green colour of the water. The owner of the site, Mr. Gregory Reen, gives you a call as he knows you from childhood and remembered that you work at a laboratory. He asks you and your team to investigate the situation and do the necessary analyses.
You will also need to establish the total cost of the analyses.

Your brief
1. Calculate the cost (chemicals, personnel and building rent) of each analysis. After the determinations, investigate whether these were the actual costs.
2. Investigate the water quality in respect of nitrate using two different analytical methods.
3. Compare both methods of nitrate determination.
4. Determine the phosphate concentration in the water.
5. Investigate the guidelines in your country for nitrate and phosphate content in swimming water.
6. Draw a conclusion as to whether the water is acceptable as swimming water in your country.

You will work in three groups:
Group 1: Determination of phosphate in water
Group 2: Determination of nitrate in water using UV-VIS
Group 3: Determination of nitrate in water using fluorescence spectroscopy

Your investigation
Your teacher will tell you when the analyses are to be carried out.

All groups
Task
Make a plan for all activities and book the necessary instruments and chemicals.
List:
- chemicals needed for the analysis
- prices of the chemicals
- time needed for the analysis
- the average cost of personnel
- the hire cost of instruments
- the building rent

Gather information about the techniques you will use.
Distribute the three determination methods between groups 1, 2 and 3.

Discuss the plan with your teacher, including instruments and chemicals.

A risk assessment must be carried out before starting practical work.

Group 1 (phosphate by VIS)
Task 1
Use www.standardbase.com: *Determination of phosphate in diet Coca Cola using visible spectrophotometry*
Prepare the standard phosphate solution.

Task 2
Prepare the calibration curve.

Task 3
Adjust the quantities in the method to suit your water sample. Measure the calibration curve and sample.

Group 2 (nitrate by VIS)
Task 1
Using **Student sheet***: Determination of nitrate in water with VIS spectrophotometry*, prepare the standard nitrate solution.

Task 2
Prepare the calibration curve.

Task 3
Adjust the quantities in the method to suit your water sample.
Measure the calibration curve and sample.

Group 3 (nitrate by fluorescence photometry)
Task 1
Using **Student sheet***: Determination of nitrate in water with fluorescence photometry*, prepare the nitrate solution.

Task 2
Prepare the calibration curve.

Task 3
Adjust the quantities in the method to suit your water sample. Measure the calibration curve and sample.

All groups
Task 1
Calculate the costs of each method for the determination of nitrate and phosphate content in water.

Task 2
Compare the results of the nitrate content analyses by groups 2 and 3 and decide which is the best method for precision and accuracy.

Task 3
Gather the results for the phosphate content of the water.

Task 4
Summarise the groups' results of the determination of NO_3^- and PO_4^{3-} and write a report on the acceptability of the water for swimming.
Calculate the total cost of the analyses of nitrate and phosphate in a water sample (using the fluorescence method as well as the xylenol method).

Your findings
Write a report with your results and conclusions for the head of the laboratory (teacher). Use your data to explain which method you would recommend to analyse nitrate, based on precision and accuracy. Also write a report for the head of the laboratory and for the owner of the camp site about the acceptability of the water for swimming.

Student self assessment
Use the file: *Evaluating skills.doc* from the ProBase website.

Student sheet: Determination of nitrate in water with VIS spectrophotometry

Scope
This method is used for the determination of nitrate in water.

Principle
With this method, xylenol is nitrated to nitroxylenol. The nitroxylenol will be distilled and captured in a solution of sodium hydroxide. The absorbance of the yellow solution will be measured at a wavelength of 430 nm. The intensity of the colour of nitroxylenol is proportional to the concentration of nitrate.

Apparatus
Equipment - instruments
- calibrated analytical balance, accuracy = 0.1 mg
- UV-VIS spectrophotometer.

Equipment - glassware
- 100 mL volumetric flasks (class A)
- 0.5 L volumetric flasks (class A)
- calibrated volumetric pipettes (2, 10, 20, 25 mL)
- 300 mL conical flask
- distillation assembly
- measuring cylinders

Materials
- 1-hydroxy-3,4-dimethylbenzene (xylenol)
- sodium nitrate
- sulfuric acid 98%
- sodium hydroxide

Reagent solutions
- Standard sodium nitrate solution 0.1 mol $NaNO_3$ /L

Weigh 4.25 g of reagent-grade $NaNO_3$ into a 0.5 L volumetric flask. Dissolve in water, fill it to the mark and mix well.

- Xylenol solution

Weigh 0.25 g of reagent-grade 1-hydroxy-3,4-dimethylbenzene into 10 mL sodium hydroxide solution. Dissolving time is about 60 minutes.

- Sulfuric acid solution $c(H_2SO_4)$ = 13 mol/L

Carefully mix 720 mL concentrated sulfuric acid with 280 mL water.

- Sodium hydroxide solution c(NaOH) = 0.2 mol/L

Preparation
Apparatus
The wavelength of the spectrophotometer can be checked by using a tungsten lamp at 656.1 nm.

Sample
Pipette 10 mL water sample into a 300 mL conical flask. Add 2 mL xylenol reagent and 40 mL sulfuric acid. Close the flask with a stopper, shake well and leave for 30 minutes at 20 oC. Add 100 mL water, and distil the nitroxylenol in a 100 mL calibrated flask in 10 mL NaOH solution. Finish the distillation when about 50 mL has been distilled. Rinse the condenser with 15 mL NaOH and water. Fill the flask to the mark with water. Measure the absorbance after 1 hour at a wavelength of 430 nm.
Measure the values for blank solutions of all the reagents without nitrate.
No special handling is necessary for the waste.

The calibration curve
Transfer 10 and 20 mL of the standard sodium nitrate solution to 100 mL volumetric flasks, fill to the mark with distilled water and mix well. Transfer 10 mL solution in a conical flask; add 2 mL xylenol reagent and 40 mL sulfuric acid. Repeat the procedure (sample preparation) from "Close the flask".

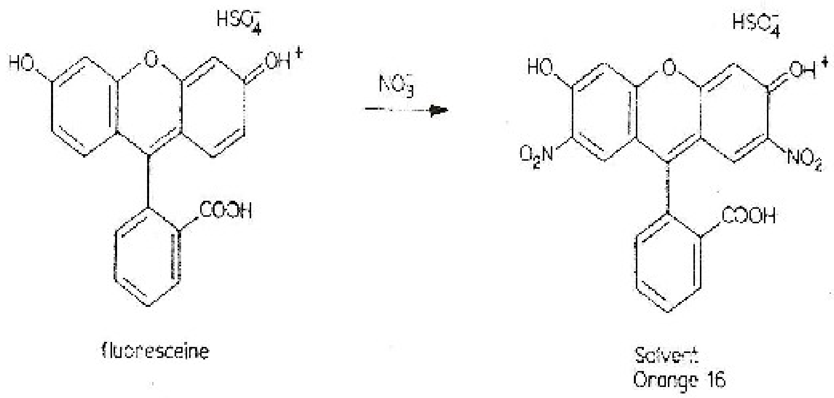
Calculation
Plot absorbance against concentration (mg/L). The concentration of the sample can be determined by interpolation.

Expression of results
The results will be given in mg/L.

Student sheet: Determination of nitrate in water with fluorescence photometry

Scope
This method is used for the determination of nitrate in water.

Principle
Fluorescein is a fluorescent molecule with an excitation wavelength of 435 nm and an emission wavelength of 485 nm. When nitrate is added to a solution containing fluorescein (in concentrated sulfuric acid), the reaction leads to the formation of Solvent Orange 16 which is a not fluorescent molecule. (See the diagram below.)



Apparatus
Equipment - instruments
- calibrated analytical balance, accuracy = 0.1 mg
- fluorimeter with a variable wavelength

Equipment - glassware
- 50 mL volumetric flasks (class A)
- 1000 mL volumetric flask (class A)
- calibrated volumetric pipettes (0.2, 0.4, 0.6 mL)
- calibrated volumetric pipettes (1.0, 5.0 mL)
- measuring cylinders

Materials
- fluorescein sodium
- potassium nitrate
- sulfuric acid 98%

Reagent solutions
- Standard potassium nitrate solution 0.2 mmol NO_3^-/L

Weigh 0.2020 g of reagent-grade KNO_3 into a 1 L volumetric flask. Dissolve in water, fill it to the mark and mix well.

- Disodium fluorescein 5.10^{-4} mol/L

Dissolve 0.0940 g of disodium fluorescein in 500 mL sulfuric acid. Always use a freshly prepared solution.

Preparation

Apparatus

Set up the spectrophotometer in accordance with the user manual.

Sample

Pipette 1-5 mL of the water sample (containing maximum 0.005 mg nitrate) into a 50 mL graduated flask. Fill the flask with a freshly prepared disodium fluorescein solution. Measure the fluorescence after 45 minutes (and no longer than 47.5 minutes) at an excitation wavelength of 435 nm and an emission wavelength of 485 nm.

Calibration curve

Transfer 0.2, 0.4, and 0.6 mL of the standard potassium nitrate solution to 50 mL volumetric flasks and fill them to the mark with freshly prepared disodium fluorescein solution.
Adjust the fluorescence with concentrated sulfuric acid on 0 (zero) and the solution with the highest concentration on 100. Measure the fluorescence of all the calibration solutions with a fluorimeter.
Calculate the nitrate content in ppm.

Calculation

Plot intensity against concentration (mg/L). The concentration of the sample can be determined by interpolation.

Expression of results

The results will be given in mg/L.

Example

Concentration nitrate mg/L	**Intensity**
0.00	100
0.04	66.1
0.08	33.2
0.12	1.30

Regression curve, using least squares, gives:

Function	Y = -824.25 x +99.52
Correlation coefficient	0.99991

Sample

		VI (95%)	
Y value	X (calculated)	X (min)	X (max)
17.1	0.10000	0.09584	0.10429
17.4	0.09964	0.09548	0.10392
Mean	0.09982	0.09566	0.10410

Teacher’s document

Overview
Students find out the best method for an investigation taking into account their laboratory costs, precision and accuracy. To be effective, they need to have appropriate scientific skills and knowledge, be able to work together as a team and to communicate effectively.

Students learn to:
- tackle scientific problems by applying their practical skills and their knowledge and understanding of spectrophotometry (UV-VIS) and fluorescence photometry
- observe, measure and analyse data from spectrophotometry and to evaluate these
- work with others and manage relationships with people
- communicate by writing a report on water pollution
- manage a budget.

Developing students' skills
One third of the students will carry out a photometric analysis of nitrate with xylenol, one third a fluorescence analysis of nitrate and one third a phosphate analysis. They should receive feedback after each one.
Appropriate questions should be asked as prompts for them to think about.

Assessment of skills will depend on the required assessment objectives for the course being followed by the students. The completed report on the ‘best’ method for the determination of nitrate and a conclusion about the water quality could be used as assessment evidence.

Organising and managing the class
The students should have experience of spectrophotometry (UV-VIS and fluorescence).
It is suggested that students work in groups
- minimum group size: 3
- minimum number of groups: 3

If there are more than three groups, some can do the same procedure.

Every student should take part in the planning, the practical activity and writing the report. Make sure students have sufficient time to share and discuss their results before starting to write the reports for the head of the laboratory (teacher) and the camp site owner.

Session plan for teachers
A suggested approach:
- Teacher’s introduction to activity and checking prior knowledge (30 minutes)
- Arranged into groups, students discuss and allocate the work (15 minutes)
- Carry out the practical work (about 305 minutes)
- Groups come together to share and discuss results (about 25 minutes)
- Complete reports (about 40 minutes)

Budget management example
Students will find budget management easier if they use a programme such as *Microsoft Excel*.

They should compare the theoretical with the actual costs.

The spreadsheet could be set up as in this example:

Chemicals	**Price** € /kg	**Quantity used** kg	**Cost*** €
disodium fluorescein	140.00	1.0×10^{-4}	0.014
potassium nitrate	25.00	0.202×10^{-4}	0.005
sulfuric acid	10.00	0.92	9.20
Hire	**Price** € /hour	**Time used** hours	**Costs** €
apparatus	50.00	3	150
building (including water, electricity, heating)	200.0	3	600
analysis time	50.00	4	200
Total costs			**959.22**

*cost = price x quantity used

Run the spreadsheet twice; once using estimated quantities/time and then again using the actual values.

Technical notes

- **Equipment and materials**

Materials and their CAS numbers

Name	**Grade**	**CAS number**
1-hydroxy-3,4-dimethylbenzene	AR	95-65-8
sodium nitrate	AR	7757-79-1
sulfuric acid 98%	AR	7664-93-9
sodium hydroxide	AR	1310-73-2
fluorescein sodium	AR	518-47-8
potassium nitrate	AR	7757-79-1

Instruments
- fluorescence photometer
- UV-VIS spectrophotometer
- calibrated analytical balance

Glassware
- distillation assembly
- 1 L volumetric flask (Class A)
- 2 x 500 mL volumetric flask (Class A)
- 100 mL volumetric flasks (Class A)
- 5 x 50 mL volumetric flask (Class A)
- 0.2, 0.4, 0.6, 1, 1.5, 2, 5, 10, 20, 25 mL pipettes (Class A)
- 300 mL conical flask
- measuring cylinders

- **Preparation**

Sample
- water from a lake which has a slightly green colour

Filter the water before use.
- sodium nitrate

Dry the sample at 140 °C for 1 hour

Fluorescence and VIS photometry
Students who determine the nitrate content with fluorescence photometry must use p.a. quality sulfuric acid for good results.

The nitrate and/or phosphate sample solution (water from a lake) should give a reading in the middle of the calibration curve.

It takes about 60 minutes to dissolve the xylenol into the sodium hydroxide solution.

Note: The technical notes of the determination of phosphate are included in the text of the procedure to be downloaded from the StandardBase website (www.standardbase.com).

TESTING PRIOR KNOWLEDGE

Choose the correct answer.

1. Which statement is correct?
   a. Excitation wavelength is always lower than the emission wavelength
   b. Excitation wavelength is always higher than the emission wavelength
   c. Answers a and b could both be right: it depends on the analyte
   d. Excitation wavelength is equal to the emission wavelength

2. Emission proceeds if an electron goes:
   a. from first singulet to the ground singulet state
   b. from first triplet to the ground singulet state
   c. from first triplet to the ground triplet state
   d. from first triplet to the second ground state

3. What is the cost of potassium nitrate (15.00 €/kg) for a standard solution of potassium nitrate (c = 0.2 mmol/L)?
   a. 0.003 €
   b. 0.03 €
   c. 0.3 €
   d. 0.0003 €

4. What will be the profit margin (in % of the personal cost) if the fee for an analysis is 350 €?
man-hour cost: 50 €
overhead costs: 20 % of the total costs
time for sampling: 1.5 hours
time for analysis: 2 hours
time to write report: 1 hour
   a. 55.5
   b. 129.6
   c. 22.9
   d. 5.55

Note: answers are given in red. These need to be removed before the test is used.

TESTING PRIOR KNOWLEDGE

Choose the correct answer.

1. Which statement is correct?
   a. Excitation wavelength is always lower than the emission wavelength
   b. Excitation wavelength is always higher than the emission wavelength
   c. Answers a and b could both be right: it depends on the analyte
   d. Excitation wavelength is equal to the emission wavelength

2. Emission proceeds if an electron goes:
   a. from first singulet to the ground singulet state
   b. from first triplet to the ground singulet state
   c. from first triplet to the ground triplet state
   d. from first triplet to the second ground state

3. What is the cost of potassium nitrate (15.00 €/kg) for a standard solution of potassium nitrate (*c* = 0.2 mmol/L)?
   a. 0.003 €
   b. 0.03 €
   c. 0.3 €
   d. 0.0003 €

4. What will be the profit margin (in % of the personal cost) if the fee for an analysis is 350 €?
man-hour cost: 50 €
overhead costs: 20 % of the total costs
time for sampling: 1.5 hours
time for analysis: 2 hours
time to write report: 1 hour
   a. 55.5
   b. 129.6
   c. 22.9
   d. 5.55