SUMMARY OF STANDARDS FOR

WATER ANALYSIS

ON COURSE WAT-E2120
SPRING 2018

- electrical conductivity
- turbidity
- color
- COD_{Mn}
- UV-absorbance
- NPOC
- bacteria
- total iron
- alkalinity
- ATP

Edited by Aino

Determination of electrical conductivity

Ability to carry electrical current is called conductivity of a solution. It varies both with the number and type of ions the solution contains. Most dissolved inorganic substances in water supplies are in the ionized form and so contribute to the specific conductance. It is expressed in millisiemens per meter (1S/m = 10^4 µS/cm = 10^3 mS/m).

Procedure:

Here it is possible to use same sample than in pH-measurement.
Measure temperature of the sample and adjust the reading to the conductivity meter. Also the temperature value from pH-measurement can be used. Rinse the conductivity probe with RO-water and immerse into the sample and measure the conductivity. Rinse the probe with RO-water after measuring.

The results are reported in terms of millisiemens per meter (mS/m).

Literature: SFS-EN 27888, dated 1994. Water quality. Determination of electrical conductivity

Determination of turbidity

The term turbidity means reduction of transparency of a liquid caused by the presence of undissolved matter. The turbidity may be caused by wide variety of suspended materials, which range in size from colloidal to coarse dispersions, depending upon the degree of turbulence.

Turbidity is reported in terms of Formazine Nephelometric Units (FNU).

Procedure:

Check before measuring that in the equipment's display
-Range Mode is AUTO
-SIGNAL AVG and SAMPLE have green light

Handle the turbidity tubes only in the top of the tube in spiral part.

Mix sample well so that you cause as little air bubbles as possible and pour into the cell. Wipe off the drops with soft paper and finger marks with the piece of cloth. Close the tube. Put the cell in the turbidity meter and read the turbidity value from the instrument scale. After measuring rinse the tubes first with warm tap water and after that with RO-water.

Report the results as follows:

- If the turbidity is less than 0,99 FNU, to nearest 0,01 FNU
- If the turbidity is between 1,0 FNU and 9.9 FNU, to the nearest 0,1 FNU
- If the turbidity is between 10 FNU and 40 FNU, to the nearest 1 FNU

Literature: SFS-EN ISO 7027, dated 2000. Water quality. Determination of turbidity

Determination of color

Natural waters are mostly colored yellowish brown by particular components of iron, clay particles and by humic matter. Also algae and other impurities can cause color to water. True color of water means color due to only to dissolved substances, determined after filtration of the water sample through a membrane filter of pore size 0,45 µm. (SFS-EN ISO 7887, dated 2011, page 2)

Reagents:

Color calibration solution, 100 mg/l Pt

Procedure:

Filter the sample through 0,45 µm membrane filter.
Measure samples absorbance using spectrophotometer at wavelength 410 nm and 4 cm optical glass (OG) cell. Measure also the absorbance of calibration solution. Rinse cell carefully after every measurement.

Calculation:

Specific absorption for the calibration solution a $(mm^{-1}(mg/l\ Pt)^{-1}$

$a = A_{410}/(100*d)$

A_{410} absorbance of the color calibration solution
100 the color of the calibration solution in mg/l Pt
d the optical path length, in millimeters, of the optical cell

True color of sample mg/l Pt $= A_{410S}/ad$

A_{410S} absorbance of the sample

Report the value to the nearest mg/l Pt.

Litterature: SFS-EN ISO 7887, dated 2012. Water quality. Examination and determination of colour

Determination of chemical oxygen demand (COD_{Mn}-value or $KMnO_4$-number)

The chemical oxygen demand test is widely used as a means of measuring organic matter in the waters. This method (oxidation with permanganate) is used mainly in Finland. Permanganate value is that value which indicates that amount of potassium permanganate in milligram per litre, which one litre of water consumes in the conditions mentioned in the standard 3036.

Reagents:

1. Sulphuric acid, 4,0 mol/l
2. Potassium permanganate solution, 0,002 mol/l
3. Potassium iodide solution, 0,1 mol/l
4. Starch – indicator
5. Sodium thiosulfate solution (Na2S2O3), 0,01 mol/l

Procedure:

Make two identical tubes per sample. Shake the sample well and pipette 10 ml sample or its dilution to both of two tubes. Make two blank sample tubes by pipetting 10 ml RO-water in to the tubes and handle them equally with the other samples. Add 0,5 ml 4 M sulphuric acid and 2,0 ml potassium permanganate solution. Put the tubes in the boiling water bath for 20 minutes. Cool the tubes.

Just before titrating add 1 ml potassium iodide and few drops of starch to the tube. Mix content of the tube with magnetic stirrer and titrate with 0,01 M sodium thiosulfate until blue color disappears.

Calculation:

$$CODMn = (V2 - V1) \cdot c1 \cdot 800 \cdot f$$

CODMn = chemical oxygen demand, mg/l
V1 = the volume of sodium thiosulfate consumed by the sample, ml
V2 = the volume of sodium thiosulfate consumed by the blank sample
c1 = concentration of sodium thiosulfate solution, mol/l
factor 800 = half of the molecular weight of oxygen changed to milligrams and divided by the volume of the sample (16 / 2 · 1000 / 10)
f = dilution factor

From CODMn (mg/l) you get permanganate reading (mg/l MnO4) by multiplying the CODMn (mg/l) by factor 3,95.

Factor 3,95 comes from the formula 158/(16*2,5) where
158 − mole mass of KMnO4
16 − mole mass of oxygen (O)
2,5 − 1 mole permanganate is equivalent to 2,5 moles of oxygen (O)

Literature: SFS 3036, dated 1981. Veden kemiallisen happen kulutuksen (COD_{Mn}-arvon tai $KMnO_4$-luvun) määritys (only in Finnish)

UV-absorbing organic constituents

Some organic compounds commonly found in water and wastewater strongly absorb ultraviolet (UV) radiation. Strong correlations may exist between UV-absorption and organic carbon content, color and precursors of trihalomethanes. UV-absorbing organic constituents in a sample absorb UV light in proportion to their concentrations. (Standard Methods for the examination of water and wastewater, 21st edition 2005, pages 5-72 – 5-73).

Procedure:

Filtrate sample through 0.45 µm syringe filter. UV-absorption is measured at 254 nm using 1 cm or 4 cm quartz cuvette.

After measuring, wash the cuvette well with tap water and rinse with RO-water.

Literature: Standard Methods for the examination of water wastewater, (21st edition, 2005), page 5-71

Determination of total organic carbon (NPOC)

In addition to organic carbon the water sample may contain carbon dioxide or ions of carbonic acid. Prior to the TOC determination, it is essential that this inorganic carbon is removed by purging the acidified sample with a gas which is free from CO_2 and organic compounds. Alternatively, both total carbon (TC) and total inorganic carbon (TIC) may be determined and the organic carbon content (TOC) may be calculated by subtracting the total inorganic carbon from the TC.

Shimadzu TOC-V-analyzer we use method, where inorganic carbon is first removed and then TOC is measured. Then we call result NPOC (nonpurgeable organic carbon). Results are in tables. They are reported in milligram per liter using two expressive numbers.

Literature: SFS-EN 1484 dated 1997, Water analysis. Guidelines for the determination of total organic carbon (TOC) and dissolved organic carbon (DOC) + Shimadzu TOC-5000A – manufactures instruction

Bacteria

SFS-EN ISO 6222, dated 1999. Water quality enumeration of culturable micro-organisms. Colony count by inoculation in a nutrient agar culture medium

SFS 4088, dated 2001. Lämpökestoisten koliformisten bakterien lukumäärän määritys kalvosuodatusmenetelmällä (only in Finnish) and Standard Methods for the examination of water and wastewater, 21st edition 2005, pages 9-66 − 9-68

SFS-EN ISO 8199, dated 2008. Water quality. General guidance on the enumeration of micro-organisms by culture.

Determination of total iron

Major part of iron in oxygenous natural water is combined with humic matter in complex form, colloid deposit or combined with suspended solids. Measuring the amount of total iron ferrous iron is oxidized to ferric state (Fe^{2+}). Oxidation takes place in an autoclave under high pressure at temperature 120 °C.

Reagents:

1. Potassium peroxide sulfate, powder
2. 4 M sulfuric acid
3. Hydroxylammoniumcloride-solution
4. TPTZ-solution
5. Sodium acetate solution

Procedure:

Weight 0,25g ± 0,05g potassium peroxide sulfate into each oxidation bottle. Pipette 25 ml sample or its dilution and add 250 µl 4 M sulfuric acid. Make also blank sample by pipetting 25 ml RO-water and add the reagents like in to the samples. Close the bottles tightly. Put samples in the autoclave for 30 minutes. Let them cool. (Autoclaving takes about 2 hours.)

Add	2 ml hydroxylammoniumcloride-solution
	2 ml TPTZ -solution
	2 ml sodium acetate solution

Mix well after each addition.
Measure the absorbance of each sample using the spectrophotometer at 593 nm after 5 min and before 2 hours. Use 1 cm optical glass (OG) cell.

Subtract the absorbance of blank sample from the absorbance of the sample. Use the calibration curve (i.e. regression line) to get the concentration of the total iron. Finally notice the dilution factor, if samples have been diluted.

Literature: SFS 3028, dated 1976. Veden raudan määritys. Fotometrinen menetelmä (only in Finnish)

Determination of alkalinity

The alkalinity of water is a measure of its capacity to neutralize acid. Hydroxides, carbonates and bicarbonates cause the major portion of the alkalinity in neutral waters. The sample is titrated with the standard acid solution to pH 4.5.

Reagent:

Hydrochloric acid HCl, 20 mmol/l or 100 mmol/l

Procedure:

Measure 50 ml well shaken sample with 50 milliliter's volumetric glass into the narrow and high beaker, add magnet and immerse the pH-probe carefully in to the beaker. Stir at a rate at which a vortex is just not perceptible. Measure the pH value of the sample. Titrate the sample with 20 mmol/l hydrochloric acid until pH reaches 4.5 and stays stabile at least 30 seconds. Note the volume of acid consumed.

Concentration of used hydrochloric acid: ________ mmol/l

Calculation:

$$X = (a \cdot c) / V$$

X = alkalinity, mmol/l
a = the volume of hydrochloric acid solution consumed to reach pH 4.5, ml
c = the actual concentration of the hydrochloric acid solution used, mmol/l
V = the volume of the sample, ml

The results are reported in terms of millimoles (H^+) per litre (mmol/l).

Literature: SFS-EN ISO 9963-1 (dated1996)

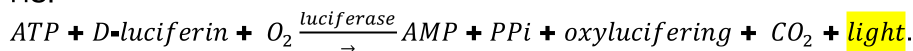
Determination of Total ATP

Edited by Panu Laurell, 2018

ATP test is a process of rapidly measuring actively growing microorganisms through detection of adenosine triphosphate (ATP). All living cells contain ATP where it plays the role of energy currency between different cellular processes. The intracellular concentration of ATP is carefully regulated to similar levels in all types of cells and as such it gives a direct measure of biological concentration and health. ATP is quantified by measuring the light produced through its reaction with the naturally occurring firefly enzyme luciferase using a luminometer. The amount of light produced is directly proportional to the amount of ATP present in the sample. Most bacterial cells contain approx. 2x 10-18 mol ATP per cell, while most eukaryotic cells, as a result of their larger size, contain 10-15 mol ATP or more.

Assay principles

Before the assay, ATP is released from the cell using Extractant B/S. ATP is assayed using ATP reagent HS.



$$ATP + D\text{-}luciferin + O_2 \xrightarrow{luciferase} AMP + PPi + oxylucifering + CO_2 + light.$$

The intensity of the light is proportional to the amount of ATP and is measured in a luminometer. The light emission is measured before and after addition of a known amount of ATP standard. This makes it possible to calculate the amount of ATP in unknown samples expressing the result in pmol (10-12 mol).

Reagents for assay

1. ATP Reagent HS. Lyophilised reagent containing luciferase and luciferin. The luciferase activity in the reconstituted reagent consumes ATP at a rate of approx. 6%/min.
2. Diluent B 10 mL. Buffer used to reconstitute ATP Reagent HS.
3. Extractant B/S 10 mL.
4. ATP standard 5 mL (10-7 mol/L ATP).

Assay procedure

Use plastic cuvette provided from the lab. For pipetting, use sterile pipet tips.

1. Mix ATP Reagent HS with Diluent B to reconstitute ATP Teagent HS.
2. Add 50 µL Extractant B/S to a cuvette.
3. Add 50 µL sample to the cuvette.
4. Add 400 µL reconstituted ATP Reagent HS and **measure light emission, I_{smp}.**
5. Add 10 µL ATP Standard, i. e. 1 pmol ATP, and **measure light emission, $I_{smp+std}$.**

Calculations

Calculate amount of ATP (pmol) in the sample by the equation:

$$ATP_{smp} = \frac{l_{smp}}{l_{smp+std} - l_{smp}}.$$

Reference: ATP Biomass Kit HS instructions by Bio Therma