Task 1
Task Sheet

Waters of Croatia: From the Emerald Rivers to the Deep Blue Sea

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EOES
European Olympiad of Experimental Science

Introduction to the task:

Croatia is rich in many natural resources and is especially renowned for its crystal-clear sea, beautiful lakes and rivers. These natural features play a prominent role in its economy and cultural heritage, influencing sectors such as agriculture, industry, tourism and biodiversity.

The Adriatic Sea is a very popular tourist destination due to its deep blue color and beautiful beaches. The Croatian coastline is 1880 km long, and has more than 1000 islands, islets, rocks and reefs, making it one of the most indented coasts in Europe and world. The Adriatic Sea has great biodiversity, including some endemic species of algae and fish. Croatia has long held prominent maritime position due to its geographical position. The country is also home to the oldest saltworks in Europe, and possibly even in the world. The Ston saltworks date back to the 14th century and are still active today.

Another important natural resource of Croatia is its freshwater ecosystems, including lakes, rivers, streams and wetlands, which are home to many endemic species and are considered “biodiversity hotspots”. These ecosystems also attract millions of tourists, with popular destinations including the breathtaking waterfalls of Krka National Park and canyons of the Cetina and Zrmanja rivers. Among Croatia’s many beautiful lakes, Plitvice Lakes stand out with their impressive tufa formations and interconnected waterfalls. As the oldest and largest national park in the country, Plitvice Lakes are also a UNESCO World Heritage Site.

In summary, Croatia's natural resources - from its diverse freshwater systems and rich biodiversity to its stunning Adriatic coastline - are essential to the country's economy, cultural identity and deep-rooted historical traditions.

Here are the approximate times you will need to spend on each problem.
Problem 1 – Ready, Set, Go! - 3.5 hours (total marks: 47)
Problem 2 – Salts From Croatian Saltworks - 3.5 hours (total marks: 37)
Problem 3 – Bending Light in Aqueous NaCl Solution - 3.5 hours (total marks: 36)

Note: read the entire Task Sheet first and then start the experiment.

Problem 1. Ready, Set, Go!

Materials and equipment

- Eppendorf tubes with caps (2 mL) – 20
- Plastic Eppendorf tube holder (blue racks) – 1
- Plastic Petri dishes (ø 6.5 cm) – 4
- Graduated plastic droppers (3 mL capacity) – 3
- Curved metal tweezers – 1
- Straight metal tweezers – 1
- Laboratory needle – 1
- Stereomicroscope with built-in light – 1
- Instructions for using the stereomicroscope (in the envelope) – 1
- Macroinvertebrate identification key (in the envelope) – 1

Solutions/liquids in bottles with droppers

- Ethanol solution, 70 % (*v/v*) (Et-OH) – 1 bottle (250 mL)
- Demineralized water (dH_2O) – 1 bottle (500 mL)

Samples

- in transparent plastic beakers with yellow lids, labeled:
  Sample **A** – 1
  Sample **B** – 1

Introduction

Freshwater macroinvertebrates is the term used in freshwater (river, stream, lake) ecology for invertebrates larger than 0.5 mm that live in or on sediments of freshwater bodies. They are excellent bioindicators of freshwater quality. Some of these organisms are streamlined, i.e. they have smooth, narrow bodies that reduce water resistance and thus facilitate movement through the water current, while others are more robust, heavier and/or worm-shaped so that they can remain anchored or burrowed into the soft sediment.

Your task is to sort and identify macroinvertebrates from two different freshwater ecosystems and then use them to calculate benthic density.

Step 1.1. The great macroinvertebrate sorting challenge

On your desks are samples from two different freshwater ecosystems (water bodies) labelled Sample A and Sample B. The organisms are preserved in 70% ethanol (*v/v*).

1. Transfer each sample from the plastic beakers with the yellow lid into several Petri dishes (preferably the deeper part of Petri dishes). **Make sure that you have transferred all the organisms** and that they are distributed so that you can clearly observe each one. Use the tweezers and/or needle to isolate the organisms and move them around as you observe them. You can also isolate them in another Petri dish to improve observation. Add a small amount of ethanol and/or demineralized water from the wash bottles to each Petri dish to **constantly keep the samples moist**.

2. Sort the organisms by taxonomic groups from both samples separately using the stereomicroscope and **Macroinvertebrate identification key.** Use tweezers and laboratory needles to

handle the specimens. ***Warning:*** *Some organisms are fragile and some body parts may be missing (e.g., legs, appendages, gills, …), but specimens in your samples are well preserved, and correct identification is possible. Also note that the samples contain specimens at different life stages/sizes.*

3. Place all individuals of the same taxonomic group into one Eppendorf tube and label each tube with the scientific name of the taxonomic group and sample code (A or B) using the permanent marker. ***Warning:*** *When labeling the samples, ethanol can erase the permanent marker. Add a small amount of ethanol and/or demineralized water from the wash bottles to each Eppendorf tube to* ***constantly keep the samples moist****.*
4. Count the number of organisms in each taxonomic group in both samples and fill in *Table 1.1.1.* on your Answer Sheet. Then, **hold up your red card** and ask the assistant to take a picture of your sorted material. Place Eppendorf tubes with sorted material in the sample containers with yellow lids and put team code stickers on the containers with yellow lids.
5. In *Table 1.1.1.*, calculate the benthic density (number of individuals/m^2) for each taxonomic group, based on the sample area of 0.049 m^2. Round the calculation to two decimal places.
6. In *Table 1.1.1.*, also fill in the respective columns by checking whether your organisms are segmented (i.e., if they have distinct body segments along the longitudinal axis) and/or whether they have legs.

1.1.1. Complete Table 1.1.1. in the Answer Sheet by following the instructions provided above and on the Answer Sheet. (19.25 p)

1.1.2. How do segmentation and legs specifically contribute to organisms' adaptations to fast-flowing aquatic environments? Enter the corresponding letter in the Answer Sheet. (0.5 p)

A. They help the organisms to adhere completely to the substrate.
B. They provide no advantage to the organism's survival in aquatic habitats.
C. They enable these organisms to live as part of the plankton in fast-flowing waters.
D. They help the organisms to anchor themselves in fast-flowing waters, providing stability.

1.1.3. Some organisms are better adapted to faster-flowing waters, while others are better adapted to slower-flowing waters. How does flow velocity in an ecosystem influence macroinvertebrate composition and adaptations? Enter the letter corresponding to the correct answer on the Answer Sheet. (0.5 p)

A. In slow-flowing waters, laterally flattened organisms are more common near the bottom.
B. In fast-flowing waters, streamlined bodies are more common than in slower-moving waters.
C. In slow-flowing waters, only dorsoventrally flattened organisms appear.
D. In fast-flowing waters, organisms with larger bodies are more common, as they can move more efficiently through the current.

Step 1.2. Decoding the secret meaning of the Simpson Diversity Index

A diversity index is a quantitative measure that indicates the number of different taxa (e.g., species) in a community. In ecological contexts, a diversity index usually focuses on species, but it can also refer to other categories such as genera, families, or functional (feeding) groups. SDI (Simpson Diversity Index) can be calculated using the following formula:

$$\mathbf{SDI} = 1 - \left\{ \sum_{i=1}^{S} \left(\frac{n_i}{N}\right)^2 \right\} = 1 - \left\{ \left(\frac{n_1}{N}\right)^2 + \left(\frac{n_2}{N}\right)^2 + \cdots + \left(\frac{n_S}{N}\right)^2 \right\}$$

n_i = the number of individuals in species / taxonomic group i
N = total number of individuals of all species / taxonomic groups
n_i/N = pi (proportion of individuals of species / taxonomic group i)
S = species / taxonomic group richness

The SDI value ranges from 0 to 1, where 0 represents no diversity and 1 indicates infinite diversity. In other words, the higher the SDI value, the greater the diversity.

1.2.1. Using the formula above and the data in the table below, calculate the SDI values for Sample C and Sample D, enter the calculated values (rounded to three decimal places) on your Answer Sheet and determine which sample has greater diversity based on your SDI calculations and record this on your Answer Sheet. (3 p)

Sample	Taxonomic group	Number of individuals in the sample
Sample C	**Isopoda**	**6**
	Ephemeroptera	**2**
	Diptera	**45**
	Oligochaeta	**134**
	Bivalvia	**4**
	Gastropoda	**24**
Sample D	**Amphipoda**	**25**
	Ephemeroptera	**13**
	Plecoptera	**7**
	Trichoptera	**4**
	Diptera	**6**

1.2.2. Which of the following factors can decrease SDI of an ecosystem? Enter the letter corresponding to the correct answer on the Answer Sheet. (0.5 p)

A. Enhancing water quality and reducing pollutants.
B. Introducing invasive species that outcompete native species.
C. Increasing the variety of substrate types on the bottom of the ecosystem.
D. The existence of diverse food sources in the ecosystem.

1.2.3. What would you expect to see in an ecosystem with a high SDI value? Enter the letter corresponding to the correct answer on the Answer Sheet. (0.5 p)

A. A stable population of just one species.
B. A few dominant species with low numbers of other species.
C. A rapid change in the composition of species from year to year.
D. A high number of species that are all relatively equally abundant.

Step 1.3. Using taxonomic metrics to reveal the health of aquatic ecosystems

Certain taxonomic groups of macroinvertebrates indicate better water quality and ecological status of freshwater ecosystems. Examples of such groups are mayflies (Ephemeroptera), stoneflies

(Plecoptera) and caddisflies (Trichoptera), all of which are highly sensitive to water pollution. Their higher share in the community can indicate a better ecological status. The proportion of dipterans (Diptera) and oligochaetes (Oligochaeta) gives a different response; their higher share indicates a lower ecological status and poorer water quality, as they are more tolerant of water pollution.

1.3.1. Calculate the proportion of individuals belonging to mayflies (Ephemeroptera), stoneflies (Plecoptera) and caddisflies (Trichoptera) in samples C and D, and express it as a percentage (%), rounded to one decimal place. Enter your answers in Table 1.3.1. on your Answer Sheet. (4 p)

1.3.2. Calculate the proportion of individuals belonging to the taxonomic groups Diptera and Oligochaeta in samples C and D, and express it as a percentage (%), rounded to one decimal place. Enter your answers in Table 1.3.2. on your Answer Sheet. (3 p)

1.3.3. Given the proportions you calculated, which sample is likely to have better water quality? Enter the letter corresponding to the correct answer on the Answer Sheet. (0.5 p)

A. *Sample C is likely to have better water quality because it does not contain any caddisflies.*
B. *Sample D is likely to have better water quality because it has a higher proportion of pollution-tolerant taxa.*
C. *Sample C is likely to have better water quality because it has a higher proportion of taxa sensitive to water pollution.*
D. *Sample D is likely to have better water quality because it has a higher proportion of taxa sensitive to water pollution.*

Step 1.4. Using ecological metrics to reveal the health of aquatic ecosystems

In this task, you will determine macroinvertebrates' **feeding types** in samples C and D.

1.4.1. Using the macroinvertebrate identification key provided, check for all possible feeding types of the taxonomic groups found in your Samples C and D. Enter your answers on your Answer Sheet in Table 1.4.1. (2.75 p)

1.4.2. Shredders feed on coarse particulate organic matter. Organisms belonging to shredders have (enter the corresponding letter on the Answer Sheet) (0.5 p)*:*

A. *Poisonous glands to kill prey.*
B. *Strong mandibles to bite food particles.*
C. *Sucking mouthparts to ingest liquid food.*
D. *Large gills to filter particles from the water.*

1.4.3. If you find a plenty of predators in your sample, what might this suggest about ***the ecosystem****? Enter the corresponding letter on the Answer Sheet.* (0.5 p)

A. *It is overrun with algae and detritus.*
B. *It is under threat from invasive species.*
C. *It has poor water quality and low biodiversity.*
D. *It has a well-balanced food web with a healthy diversity of organisms.*

1.4.4. Which of the following could indicate a polluted ecosystem when considering the feeding types of the macroinvertebrates? Enter the corresponding letter in the Answer Sheet. (0.5 p)

A. *A high proportion of detritivores like oligochaetes.*
B. *A balanced distribution of all feeding types across the sample.*

C. *A high proportion of scrapers and predators like some stoneflies.*
D. *A high proportion of filter-feeders and shredders like some caddisflies.*

Step 1.5. Drift into action: Calculating macroinvertebrate drift density and propensity

In macroinvertebrate studies, the phenomenon known as **drift** refers to the downstream movement of organisms in the water column. The composition and appearance of drift can be influenced by various abiotic and biotic factors, categorizing it as either:

1. **Active drift**: Initiated by biotic factors, where organisms move downstream in search of food, new habitats, or due to life cycle changes.
2. **Passive drift**: Resulting from changes in physico-chemical parameters, such as flow velocity or nutrient and pollutant concentrations.

Drift sampling is conducted using drift samplers, which consist of a 1.5 m long drift net with a 214 µm mesh diameter attached to a cylindrical plastic tube measuring 50 cm x 7.5 cm, with an opening area of 44.2 cm^2. During the sampling, three drift samplers are deployed to collect replicate samples (**Figures 1.5.1. and 1.5.2.**). After the designated sampling time, the drift nets are removed from the water, separated from the tubes, and their contents, including macroinvertebrates, are preserved in 70 % (*v/v*) ethanol.

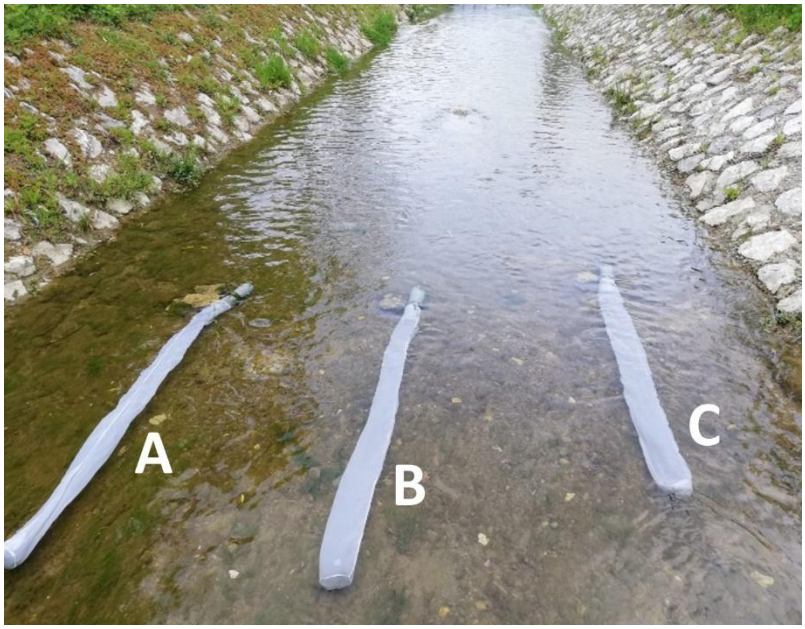


Figure 1.5.1. Drift sampling across stream width (A – Drift net 1, B – Drift net 2, C – Drift net 3)

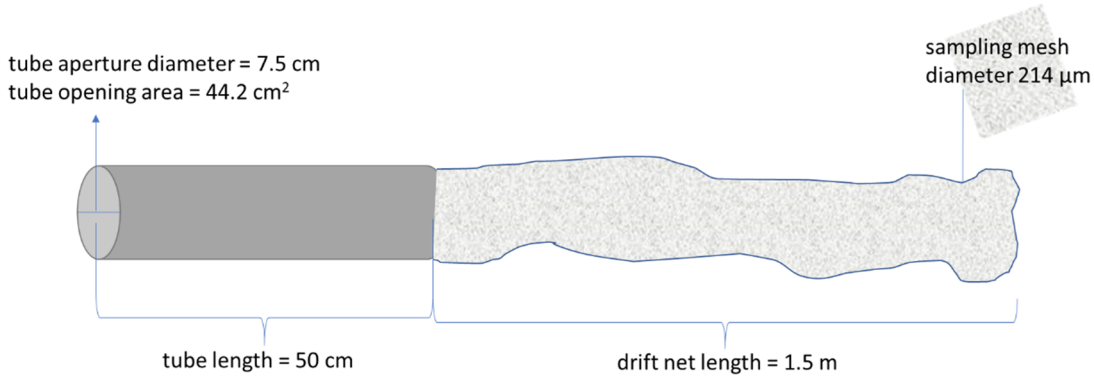


Figure 1.5.2. Scheme of a drift net with the corresponding dimensions

1.5.1. Here are the data obtained during and after the collection of the drift samples using the drift nets 1, 2 and 3, all of the same dimensions: each drift net has a length of 1.5 m, a sampling mesh diameter of 214 µm, with a cylindrical tube length of 50 cm, an aperture diameter of 7.5 cm, and an opening area of 44.2 cm²:

Drift net 1	Drift net 2	Drift net 3
Sampling duration: 13:20 to 14:05 **Water flow velocity**: 0.5 m/s **Macroinvertebrate counts**: ● Ephemeroptera: 22 ● Amphipoda: 36 ● Diptera: 12	**Sampling duration**: 13:20 to 14:07 **Water flow velocity**: 0.7 m/s **Macroinvertebrate counts**: ● Ephemeroptera: 28 ● Amphipoda: 38 ● Diptera: 10	**Sampling duration**: 13:20 to 14:10 **Water flow velocity**: 0.4 m/s **Macroinvertebrate counts**: ● Ephemeroptera: 15 ● Amphipoda: 22 ● Diptera: 17

Calculate the volume of water passing through each drift sampler during the sampling period and drift density (number of organisms/m³) for each taxonomic group in each drift sample and the average drift density for each group considering all three replicate drift samples. Enter the results (rounded to two decimal places) in Table 1.5.1. on your Answer Sheet. (8 p)

1.5.2. Calculate the drift propensity value for each taxonomic group (Ephemeroptera, Amphipoda, Diptera) using the following data (1.5 p)*:*

	Benthic density (Number of individuals/m²)	Drift density (Number of individuals/m³)
Ephemeroptera	429	10
Amphipoda	3531	24
Diptera	122	12

Use the following formula to estimate the drift propensity:

Drift propensity = Drift density / Benthic density

1.5.3. A higher drift propensity value indicates that the organism is more likely to drift downstream. Enter the results (rounded to three decimal places) in Table 1.5.2. on the Answer Sheet and draw conclusions about which groups are more likely to drift downstream. Enter your answers in Table 1.5.3. on the Answer Sheet. (1.5 p)

Problem 2. Salts from Croatian Saltworks

Introduction

Iodine is an important nutrient needed for the proper functioning of thyroid. Not enough iodine in the diet can cause various health problems, such as enlarged thyroid gland and hypothyroidism.
Even though iodine can be found in trace amounts in dairy products, eggs and seafood, fortifying table salt with iodine increases iodine intake for human consumption. Iodine in nature can be found in various forms, such as iodine (I_2), or iodide (I^-) and iodate (IO_3^-) salts.
Salt for human consumption can be obtained by evaporating seawater or by mining mineral deposits. Croatia is known for its three saltworks in Ston, Nin and Pag. Only two of these saltworks fortify their table salts with iodine. Your task is to determine which saltworks add iodine to their salts, in what form(s) iodine is present in those salts, and in what concentration.

Step 2.1. Chemical reactions for determination of iodine species

Materials and equipment

- 6 mL test tube – 14
- Test tube holder
- 25 mL glass beaker – 3
- Glass dropper – 3
- Waste beaker and bottle
- Demineralized water (dH_2O)

Solutions in 20 mL bottles with droppers (M = mol dm^{-3})

- KI, c(KI) = 0.5 M
- I_2 in KI, $c(I_2)$ = 0.05 M
- KIO_3, $c(KIO_3)$ = 0.05 M
- HNO_3, $c(HNO_3)$ = 1.9 M
- $FeCl_3$, $c(FeCl_3)$ = 0.18 M
- Starch, w = 0.2 % (w/v)
- $AgNO_3$, $c(AgNO_3)$ = 0.1 M
- NH_3, $c(NH_3)$ = 4.0 M
- NaCl, c(NaCl) = 0.3 M

Sample solutions

- in bottles marked as Salt **1**, Salt **2** and Salt **3 (mass of salt and final volume of solution is given on the label)**

Your first task is to examine the specific reactions of iodine (I_2), iodate ion (IO_3^-), iodide ion (I^-) and chloride ion (Cl^-) in aqueous solution. Choose five test tubes (T1–T5) from the test tube holder. Add specific reagents into each test tube as follows in Table 2.1. Mix gently after each addition. Observe and note the changes in Table 2.1.1. in the Answer Sheet.

Table 2.1. Procedures for specific reactions of iodine (I_2), iodate ion (IO_3^-), iodide ion (I^-) and chloride ion (Cl^-) in aqueous solution

Test tube		Procedure
T1	Detection of iodine	Into T1 add 1–2 drops of I_2 and 1–2 drops of starch solution.
T2	Detection of iodate	Into T2 add 1–2 drops of KIO_3, followed by 1–2 drops of HNO_3, then 1–2 drops of starch solution, and finally 1–2 drops of KI. $IO_3^-\ (aq)\ +\ 5I^-(aq) + \ 6H^+(aq)\ \rightarrow\ 3I_2\ (aq) + \ 3H_2O\ (l)$
T3	Detection of iodide	Into T3 add 1–2 drops of KI, followed by 1–2 drops of starch solution, and then 1–2 drops of $FeCl_3$. $2Fe^{3+}\ (aq) + 2I^-\ (aq) \rightarrow\ 2Fe^{2+}\ (aq) + \ I_2\ (aq)$
T4	Detection of iodide	a) Into T4 add 1–2 drops of KI, and then 1–2 drops of $AgNO_3$.
		b) Then add 4–5 drops of NH_3 solution to the same test tube (T4).
T5	Detection of chloride	a) Into T5 add 1–2 drops of NaCl solution, followed by 1–2 drops of $AgNO_3$.
		b) Then add 4–5 drops of NH_3 solution to the same test tube (T5).

2.1.1. Fill the Table 2.1.1. (Answer Sheet) with your results by marking the observed change with an X. (3.5 p)

2.1.2. Write the balanced chemical reactions in test tubes T4a and T5a (write states of matter) into Table 2.1.2. (Answer Sheet). (1 p)

Step 2.2. Testing salt solutions

Your task is to determine the form in which iodine is present in the table salt samples marked as Salt **1**, Salt **2** and Salt **3**.
Pour approximately 5 mL of each of these salt solutions into three small glass beakers. Choose nine test tubes from test tube holder (T6–T14). Into the first three test tubes (T6–T8) add 1–2 drops of solution of Salt **1**. Into the second three test tubes (T9–T11) add 1–2 drops of Salt **2** solution. Into the last three test tubes (T12–T14) add 1–2 drops of Salt **3** solution.
Determine the form in which iodine is present in Salt **1**, **2** and **3** by adding specific reagents for determination of iodine (I_2), iodate ion (IO_3^-), and iodide ion (I^-) as described in Table 2.1., reactions in T1–T3:

- T6, T9 and T12 – perform experiment for detection of iodine (T1)
- T7, T10 and T13 – perform experiments for detection of iodate (T2)
- T8, T11 and T14 – perform experiments for iodide (T3)

(**NOTE:** I^- over time oxidizes to I_2 which gives a false positive reaction even if IO_3^- is not present. Therefore, only note the changes observed **up to 1 minute** after the addition of reagents).

2.2.1. Fill in Table 2.2.1. on the Answer Sheet by marking the observed change with an X. (1.5 p)

2.2.2. Fill in Table 2.2.2. on the Answer Sheet by marking the observed change with an X. (1.5 p)

2.2.3. Fill in Table 2.2.3. on the Answer Sheet by marking the observed change with an X. (1.5 p)

2.2.4. In Table 2.2.4. mark with an X the correct form of iodine that is present in table salt samples or mark no iodine if it is not present. (1.5 p)

Step 2.3. Measuring iodine in table salts

Materials and equipment

- 50 mL pipette – 2
- Pipette bulb
- 25 mL burette and stand
- 5 mL pipette – 3
- 300 mL Erlenmeyer flask – 3
- Glass funnel
- Red card
- Waste beaker and bottle
- Demineralized water (dH_2O)

Solutions in 100 mL bottles (M = mol dm^{-3})

- KI, *c*(KI) = 0.005 M
- H_2SO_4, *c*(H_2SO_4) = 1.5 M
- Starch, *w*(starch) = 0.2 % (*w*/*v*)
- NaClO, *c*(NaClO) = 0.02 M
- HCOOH, *c*(HCOOH) = 0.5 M
- $Na_2S_2O_3$, *c*($Na_2S_2O_3$) ≈ 0.001 M standardized solution (**exact concentration is given on the label**)

Sample solutions

- in bottles marked as Salt **1**, Salt **2** and Salt **3 (mass of salt and final volume of solution is given on the label)**

Your next task is to analyze **only** salt sample(s) that are fortified by iodine in the form of I_2, I^- or IO_3^- and to determine the amount of iodine in them.
The iodine content in salt samples can be determined by iodometric titration. The process is similar for all three forms of iodine, with the primary difference occurring in the initial step, where all iodine anionic forms are converted to I_2:

A) Iodine is present in the form of I_2, and it can be directly titrated with thiosulfate in the presence of starch.

$$I_2\ (aq) + 2S_2O_3^{2-}\ (aq) \rightarrow 2I^-\ (aq) + S_4O_6^{2-}\ (aq)$$

B) Iodine is present in the form of IO_3^- so the first step is reduction of iodate to iodine.

$$IO_3^-\ (aq) + 5I^-(aq) + 6H^+(aq) \rightarrow 3I_2\ (aq) + 3H_2O\ (l)$$

C) Iodine is present in the form of I^- so the first step is oxidation of iodide, removal of excess oxidant and addition of potassium iodide to form free iodine.

$$I^-\ (aq) + 3ClO^-\ (aq) \rightarrow IO_3^-\ (aq) + 3Cl^-\ (aq)$$
$$IO_3^-\ (aq) + 5I^-(aq) + 6H^+(aq) \rightarrow 3I_2\ (aq) + 3H_2O\ (l)$$

Subsequently, the iodine can be titrated with a standard sodium thiosulfate solution in the presence of starch.

Your first task is to choose the samples you will analyze and then select the procedure for the form of iodine present in the table salt samples, based on the results from 2.2.4.

2.3.1. Determine the oxidation states of iodine in iodine molecule, iodate ion and iodide ion. Write the answer in Table 2.3.1. on the Answer Sheet. (1.5 p)

2.3.2. In the reaction of iodine and thiosulfate ion, determine what species is being reduced and what species is being oxidized. Indicate your answer by X in appropriate cell in Table 2.3.2. on the Answer Sheet. (1 p)

2.3.3. What is the role of starch solution in the titration of iodine with the standard solution of sodium thiosulfate? Enter the corresponding letter in the Answer Sheet. (0.5 p)

a) starch is used as indicator in the detection of endpoint of reaction involving iodine solution.

b) starch is used to stabilize the solution of iodine.

c) starch is used to achieve acid medium needed for the reduction of iodate ions into iodine.

2.3.4. In Table 2.3.4. mark with an X which salt samples have you chosen based on the previous results. (1 p)

2.3.5. In Table 2.3.5. ***write which of the following procedure you have chosen, and raise the red card so the lab assistant can check your answer. Only after that can you start with the experiment. The lab assistant will check answers for tasks 2.2.4, 2.3.4. and 2.3.5. and sign them if the answers are correct. If the answers are incorrect, you will not get points for these tasks, but the lab assistant will provide you with correct answers.*** (1.5 p)

Based on the results of Step 2.2. or correct answers provided by lab assistant select one of the following procedures for quantitation of iodine in the table salts:

A) <u>If the iodine is present in the form of I_2 procedure is as follows</u>:
Use a pipette to transfer 50 mL of salt solution into the Erlenmeyer flask.
Titrate solution of salt samples with sodium thiosulfate solution until salt solution changes color from yellow to a very pale yellow.
Add 1 mL of starch solution and continue titrating until blue color completely disappears.

B) <u>If the iodine is present in the form of IO_3^- procedure is as follows</u>:
Use a pipette to transfer 50 mL of salt solution into the Erlenmeyer flask.
Add 5 mL of potassium iodide solution and 2 mL of sulfuric acid solution.
Titrate solution of salt samples with sodium thiosulfate solution until salt solution changes color from yellow to a very pale yellow.
Add 1 mL of starch solution and continue titrating until blue color completely disappears.

C) <u>If the iodine is present in the form of I^- procedure is as follows</u>:
Use a pipette to transfer 50 mL of salt solution into the Erlenmeyer flask.
Add 2.5 mL of sodium hypochlorite solution and stir well.
Add 5 mL of formic acid solution and swirl to remove excess oxidant.
Add 2.5 mL of potassium iodide solution and 2 mL of sulfuric acid solution.
Titrate with the sodium thiosulfate solution until salt solution changes color from yellow to a very pale yellow.
Add 1 mL of starch solution and continue titrating until blue color completely disappears.

Perform the chosen procedure **AT LEAST** three times for each sample. Write the volumes of $Na_2S_2O_3$ used for each titration on the Answer Sheet (Problems 2.3.6–2.3.8).
Note: Use the 50 mL pipettes for pipetting samples, and 5 mL pipettes for pipetting reagents solutions.

(Total marks for 2.3.6 + 2.3.7 + 2.3.8: 12 p)

*2.3.6. If you analyzed sample Salt **1**, in Table 2.3.6. on the Answer sheet write the volumes of $Na_2S_2O_3$ used for titration, calculate the mean volume and circle the volumes used in your calculation. If you didn't analyze sample Salt **1** write "**/**" in the cells of Table 2.3.6.*

*2.3.7. If you analyzed sample Salt **2**, in Table 2.3.7. on the Answer sheet write the volumes of $Na_2S_2O_3$ used for titration, calculate the mean volume and circle the volumes used in your calculation . If you didn't analyze sample Salt **2** write "**/**" in the cells of Table 2.3.7.*

*2.3.8. If you analyzed sample Salt **3**, in Table 2.3.8. on the Answer sheet write the volumes of $Na_2S_2O_3$ used for for titration, , calculate the mean volume and circle the volumes used in your calculation. . If you didn't analyze sample Salt **3** write "**/**" in the cells of Table 2.3.8.*

2.3.9. Calculate the molar mass (in g mol^{-1} units) of sodium thiosulfate ($Na_2S_2O_3$), iodine molecule (I_2), potassium iodate (KIO_3) and potassium iodide (KI). Write the answer in the Table 2.3.9 (Answer sheet). (2 p)

*2.3.10. In the table 2.3.10. on the Answer sheet calculate the amount (in moles) of sodium thiosulfate used for titration of samples. Indicate the sample you are performing calculation for (sample Salt **1**, Salt **2** or Salt **3**).* (1 p)

*2.3.11. In the table 2.3.11. on the Answer sheet calculate the amount (in moles) of iodine species (I^-, I_2 or IO_3^-, according to the form of iodine which is present in the given sample) present in the initial sample solution (250 mL). Indicate the sample you are performing calculation for (sample Salt **1**, Salt **2** or Salt **3**).* (2 p)

*2.3.12. In the table 2.3.12. on the Answer sheet calculate the mass of iodine species (KI, I_2 or KIO_3, according to the form of iodine which is present in the given sample) in the initial sample solution (250 mL). Indicate the sample you are performing calculation for (sample Salt **1**, Salt **2** or Salt **3**).* (1 p)

*2.3.13. In the table 2.3.13. on the Answer sheet calculate the fraction (as mg per kg of salt) of iodine species (KI, I_2 or KIO_3, according to the form of iodine which is present in the given sample) in the sample(s), rounded to 1 decimal place . Indicate the sample you are performing calculation for (sample Salt **1**, Salt **2** or Salt **3**).* (1 p)

*2.3.14. Based on the results, mark on the map the saltworks from which the salt samples (Salt **1**, Salt **2** and Salt **3**) came, if you know that the saltwork in Ston does not add iodine into their salt, and that saltwork in Pag adds more iodine into their salt than the saltwork in Nin.* (2 p)

Figure 2.1. Map of Croatia with marked capital city (Zagreb) and cities where the saltworks are located.

Problem 3: Bending Light in Aqueous NaCl Solution

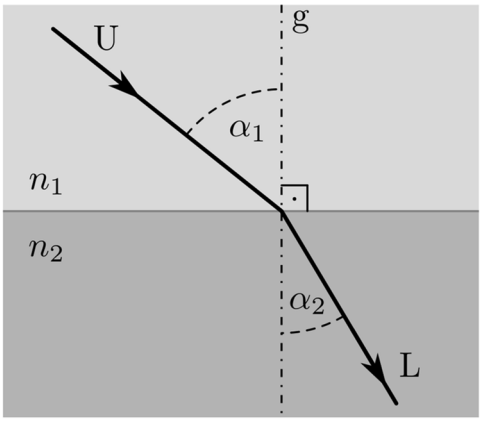
Materials and equipment

- A plexiglass tub, with external dimensions 28 cm x 14.5 cm x 8 cm
- Table salt
- A kitchen scale
- Graphing papers – 3 (you can ask the assistant for additional papers if needed)
- 100 pins (used to track the path of the light rays, and to fix graphing papers)
- A ruler
- Styrofoam pieces – 2
- Weights – 2
- Bottle of demineralized water
- Two plastic spoons
- A paper/plastic cup for salt weighing
- A computer

3.1. Introduction

In this problem you will measure the refractive index of light in demineralized water and in a 20 % *mass fraction* aqueous table salt (NaCl) solution.

In a (partially) transparent homogeneous optical medium (e.g. air, vacuum, water, glass …) light propagates in a straight line. When light passes from one medium to another, it refracts as described by Snell's law, and as shown on Figure 3.1. The constants n_1 and n_2 are called the indices of refraction of corresponding media and are numbers without units.



Snell's law

$$n_1 \sin\alpha_1 = n_2 \sin\alpha_2$$

Figure 3.1. The propagation of a light ray passing from one medium to another. ' U ' - incoming ray, ' L ' - refracted ray, ' g ' – a line normal to the plane border of the media, α_1 (α_2) - angles of the incoming and the refracted ray from the line 'g'. n_1 and n_2 - indices of refraction of the media.

The approximate value of the refractive indices of some materials is given in Table 3.1. In this task, we use the approximate value for air $n_{\text{air}} = 1.000$.

Table 3.1: Several approximate values of refractive indices n for visible light.

Substance	**Refractive index n**
vacuum	1.000
air	1.0003
glass	1.551
plexiglass	1.495

Substance	**Refractive index n**
glycerin	1.473
ethanol	1.362
ice	1.310
sapphire	1.770

3.2. Light ray propagation

3.1.1. In Figure 3.2 a) and b) the incoming beam (gray dashed line) reaches the boundary between two media (full black line), in this case between sapphire and ethanol. (Two protractors are also drawn on the Figure, one on each side of a border between two media.) Based on the data from Table 3.1 and Snell's law, draw the refracted rays for both cases on the Answer Sheet. (The accuracy of the drawing may not be worse than ±1 degree.) Show your calculation. (2 p + 2 p)

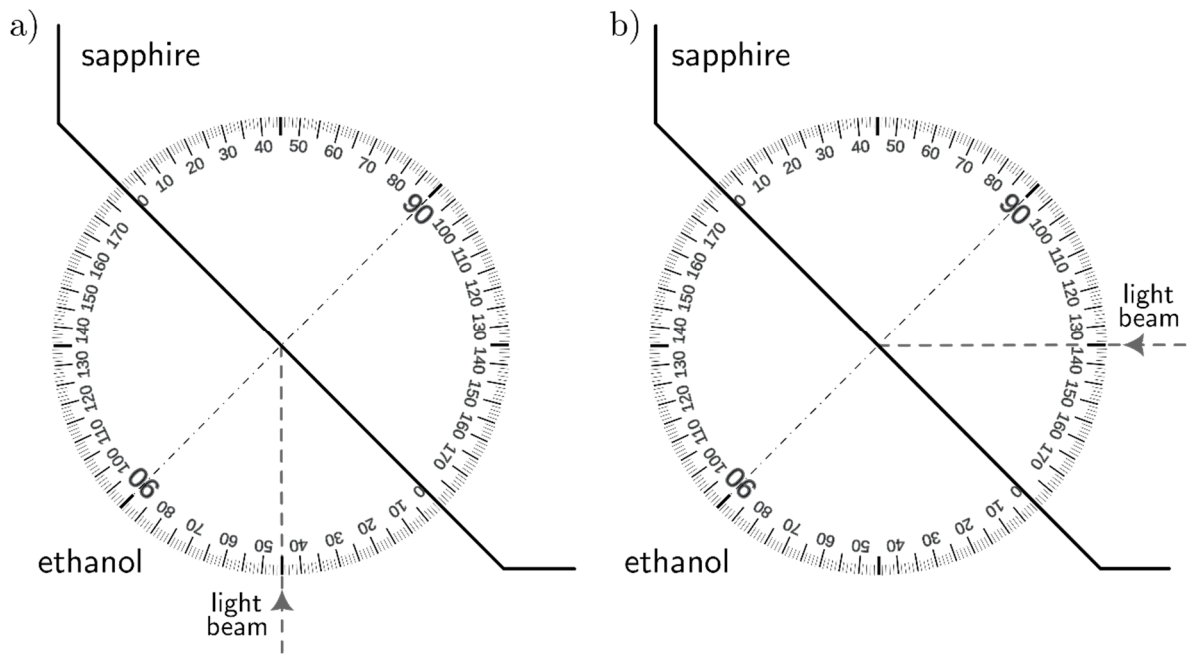
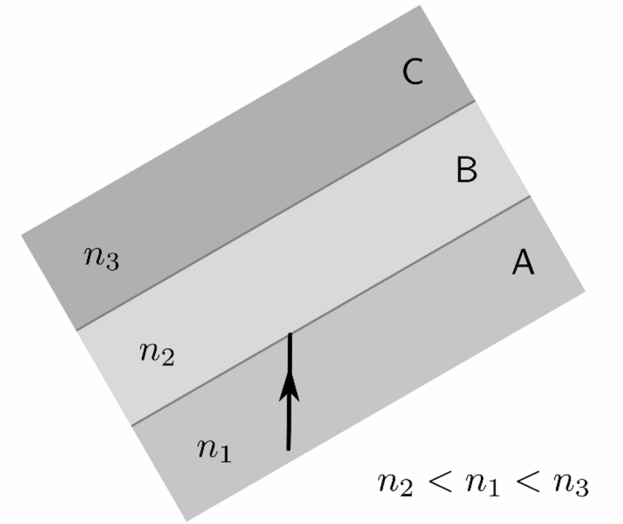


Figure 3.2. Full black line: the boundary between ethanol and sapphire. Gray dashed line: the incoming light beam (the arrow indicates the direction of the propagation).

Figure 3.3. The black line with an arrow represents the direction of the incoming light ray in medium A (with refractive index n_1).



3.1.2. In Figure 3.3, a ray of light is successively refracted at several boundaries of different media. For the incoming ray in medium A, as indicated in the figure, qualitatively sketch the approximate path of the light ray in media B and C on the Answer Sheet. Pay special attention to the relative direction of the rays in media A and C. (3 p)

3.3. Measurement of the refractive index

3.3.1. Introduction

To measure the refractive index of demineralized water (and later also the aqueous table salt solution), we will use the set-up sketched in Figure 3.4. which is based on tracking a light beam, i.e. its refraction at the air-water interface, by measuring the entry angle of the light beam and the distance d.

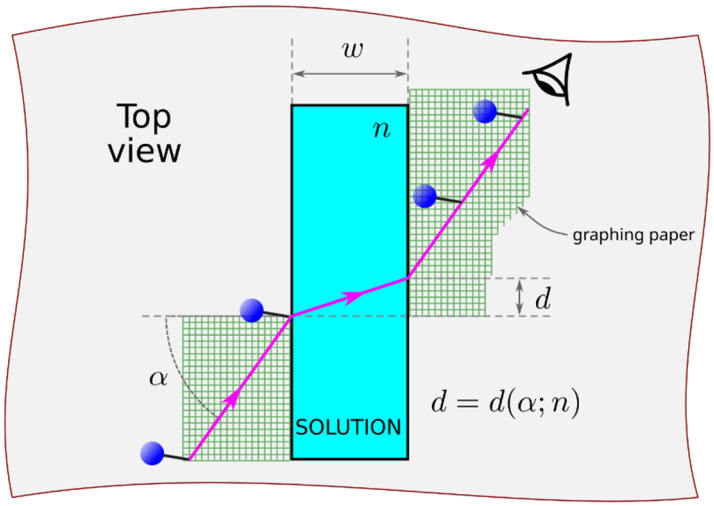


Figure 3.4. A sketch of the setup used to measure the refractive index *n* of a liquid.

We see that the distance d between the perpendicular lines to the points where the light ray enters and exits the water depends on the angle of incidence α. From Snell's law we can obtain the relation

$$d(\alpha; n) = w \frac{\sin \alpha}{\sqrt{n^2 - \sin^2 \alpha}} \tag{3.1}$$

This is the main relation we will use to determine the value of the refractive index of the liquid by using the measured data for various angles α and distances d. Measuring distance d for various angles α we can determine refractive index n.

Given that the values of the refractive indices of demineralized water and an aqueous salt solution are very similar, it is of particular interest to perform the measurements as accurately as possible.

3.3.2 Experimental setup

To measure the value of the refractive index, we will use the setup shown in Figure 3.5.

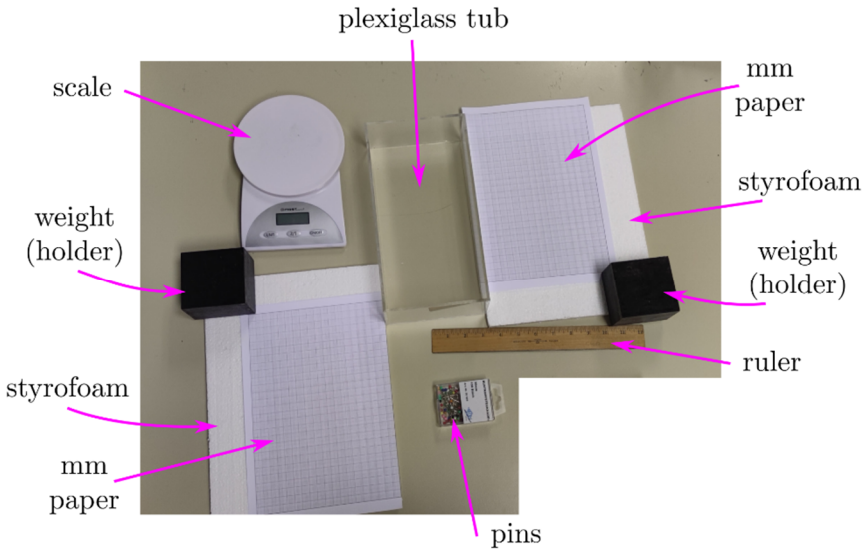


Figure 3.5. Photograph of the experimental setup. The incoming light beam enters the setup from the left side of the photograph and the observation of the beam is made from the right side of the photograph.

On the Answer Sheet is a partially completed Table in which you can enter your measured and calculated values. The explanation of the columns in the Table is:

Column title	Meaning
α (deg)	The angle of incident light rays.
x exact (cm)	Exact (calculated) values of the distances from the origin, which depend on angle α.
y exact (cm)	
x (cm)	The values of the x and y coordinates which will approximately correspond to the angle α, and which use a half-integer value in centimeters (such suitable values are, for example, 2.0 cm or 4.5 cm, while inappropriate values are, for example, 2.3 cm or 4.6 cm).
y (cm)	
d (cm) 0%	Experimentally determined distance values d for the demineralized water (0%) and for aqueous table salt solution (20% mass fraction).
d (cm) 20%	

3.3.2.1 Determination of the angle α

As the values of the refractive indices of water and the aqueous salt solution are slightly different, we will set angles α by choosing convenient positions/coordinates on the graphing paper. We will start with integer angles and try to choose the coordinates on the graphing paper that approximately correspond to them. This will enable us to determine the angles more precisely (e.g. instead of choosing the coordinate 1.487 cm which would correspond to an integer angle, it is more accurate to choose the coordinate 1.5 cm). The partially filled Table will help us for this purpose (see an example in Table 3.3).

α (deg)	x exact (cm)	y exact (cm)	x(cm)	y(cm)	d(cm) 0%	d(cm) 20%
0	17	0	17	0	0	0
5	17	1.487	17	1.5	...	...
10	17	2.998	17	3.0	...	...
15	...	...	...	...		
20	...	...	...	...		
...	...	...	...	...		
70						
75						

Table 3.3: An example of a Table used to enter the measured and the calculated values.

In the first column are predetermined angles α for which we want to determine the distances d. We set the beam path by sticking two pins into the graphing paper placed on the Styrofoam. One pin is placed in the corner of the graphing paper next to the tub containing the liquid (see Figure 3.6. a). This pin is given the coordinates $(x, y) = (0,0)$. The other pin is placed on the graphing paper on given (x, y) coordinates. We can trace light direction by looking from the side, when pins overlap in your view.

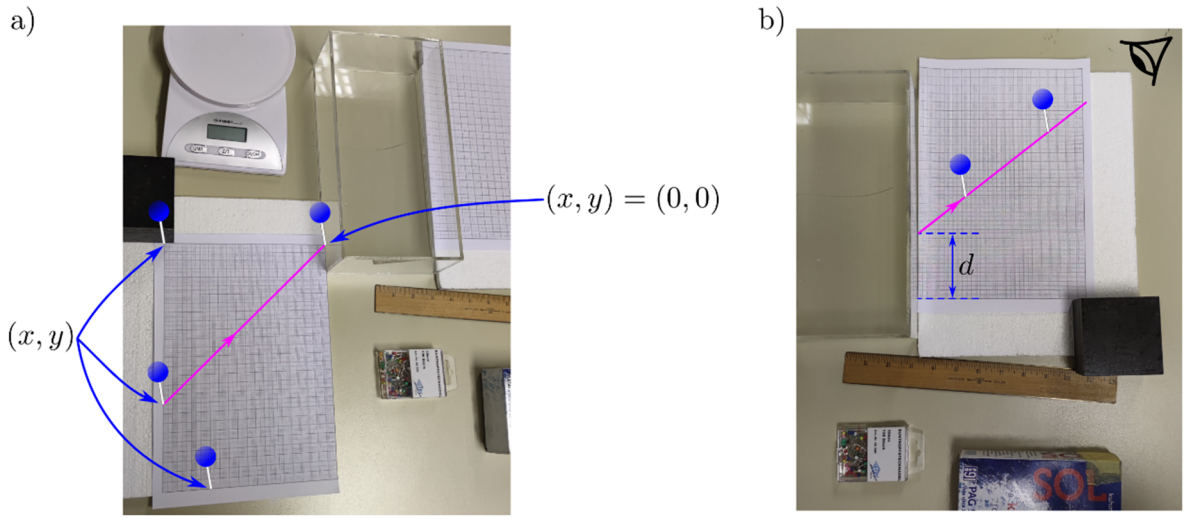


Figure 3.6. a) Sketch of the position of the pins for the incoming light ray. b) Sketch of the position of pins for the outgoing light ray.

3.2.1. To define the angle α as accurately as possible, the pins should be as far as possible from each other. Given the dimensions of the graphing paper, fill in the columns 'x exact (cm)' and 'y exact (cm)' in the Table 3.3 on the Answer Sheet. (For non-integer values you may specify only three significant digits.) Make sure that all the coordinates can be placed on the given graphing paper! (1 p)

3.2.2. Since 'exact' coordinates have a decimal part, placing the pin in the specified coordinates would require an accuracy that is smaller than a millimeter. That's why we replace the 'exact' coordinates with coordinates that are close to them, expressed in halves of centimeters. Fill in the 'x(cm)' and 'y(cm)' columns in Table 3.3 on the Answer Sheet accordingly. Make sure that all coordinates can be placed on the given graphing paper! (3 p)

The coordinates 'x (cm)' and 'y (cm)' are the coordinates for which we can now accurately determine the new angle α, and where you can place your pins.

3.3.2.2 Determination of the distance d

You can follow the path of the imagined light beam exiting the tub by looking from the side and placing *two* pins on the second graphing paper (see Figure 3.6. b). Pay attention to the placement and the alignment of the graphing paper to make sure you determine the distances d correctly. To maximize the accuracy of the measurement for an individual light beam, do not place the two pins too close to each other. **Plan your experimental setup as to not move graphing papers while tracing various rays, as it would incur additional errors in measurements.**

3.2.3. Fill less than half of the tub with demineralized water and place the pins on the graphing paper as described. After placing (all) the pins on the second graphing paper, use a ruler to draw lines connecting the pins for each of the rays, and extend them to the edge of the graphing paper. This will allow you to determine the distance d for each of the rays and enter it in Table 3.3 in the column 'd(cm) 0%' on the Answer Sheet (see Figure 3.6. b). (9 p)

3.2.4. Add the appropriate amount of table salt to the tub so that you get a 20 % mass fraction aqueous solution. (The '20 % mass fraction' means: for 24 g of demineralized water add 6 g of salt.) Write the mass and height of demineralized water, and mass of added salt on the Answer Sheet. Make sure all the salt is dissolved in water. (1 p)

3.2.5. To determine the distances d follow the procedure in 3.2.3., filling up the column 'd(cm) 20%'. (9 p)

3.3.3 Determination of the refractive indices

The expression for the dependence of the distance d on angle α (equation (3.1)) is complicated. However, we can reduce it to (the index i indicates a particular measurement):

$$\sin\alpha_i\sqrt{w^2 + d_i^2} = nd_i \qquad (3.2)$$

(Here, angles α_i correspond to new angles with half-integer coordinates, and w is outer width of the tub.) By introducing the variables

$$X_i = d_i$$
$$Y_i = \sin\alpha_i\sqrt{w^2 + d_i^2} \qquad (3.3)$$

the above relation can be written as

$$Y_i = nX_i \tag{3.4}$$

which is an equation of a line through the origin. Using this expression, we can easily find the value of the refractive index n as the slope of the line $Y = Y(X)$.

To find the slope of the line in expression (3.4), we will use a computer and the *MS Excel* program (use filename as: **COUNTRY**-TEAM-**A**.xlsx), which helps us do two things: calculate the pairs of values (X_i, Y_i) using the measured data and then determine the slope (and its error) of the line $Y = Y(X)$, which is equal to the refractive index n. To do this, enter the obtained measurements from the columns 'x(cm)', ' y(cm)', ' d(cm) 0% ' and 'd(cm) 20%' in the Excel table on the computer. After that, form two columns in which you determine the angles α (both in radians and in degrees). Finally, form two additional columns in which you calculate the pairs of values (X_i, Y_i) according to expressions (3.3), and perform a linear fit using that data. Do not force the intercept of the fitted line to zero. (If you have problems when fitting, raise the red card for help.)

3.3.1. Using a linear fit, determine the slope of $Y = Y(X)$, and hence the refractive indices for demineralized water and the aqueous table salt solution. Write the obtained values (together with their errors with two significant digits) on the Answer Sheet. (4 p)

*3.3.2. In the experiment and the calculation, we ignored the influence of the plexiglass tub walls. Based on the value of the refractive index of plexiglass from Table 3.1, estimate whether the true values of the refractive indices of the liquids are **higher**, **the same,** or **lower** than the ones measured in the experiment. Check the answer (☒) on the Answer Sheet.* (2 p)