EUROPEAN OLYMPIAD OF EXPERIMENTAL SCIENCE
2024
LUXEMBOURG

TASK2

D'Schueberfouer

Duration: 4 hours

EOES 2024, 11.04.2024

Introduction to the task:

The Schueberfouer stands as a notable cultural event within Luxembourg's summer calendar. Drawing approximately 2 million visitors annually, it spans three weeks from late August to early September, showcasing a myriad of attractions rooted in tradition dating back to the Middle Ages. This year marks the 683rd iteration of this event.

Established in 1340 by Jang de Blannen, the Schueberfouer remains the largest funfair in Luxembourg and the Greater Region. Comprising a diverse array of over 200 attractions, it encompasses large thrill rides tailored for the whole family. Additionally, the event features a variety of culinary offerings including large restaurants, beer tents, sweet shops, as well as entertainment such as lotteries and shooting galleries.

Here are approximate times you will need to spend on each problem:

Problem 1 – Chemistry (Analysis of Lët'z limo) - 2h (including 1h drying (waiting) time)
Problem 2 – Chemistry (Analysis of Luxembourgish mustard) - 2 hours
Problem 3 - Physics (Looping and LEDs) - 3.1. 1h45 + 3.2. 1h45 (the 2 parts 3.1. and 3.2. are not linked and can be fulfilled independently)
Problem 4 - Biology (Osmosis) - 2 hours
Problem 5 - Biology (Analysis of fish scales) – 1.5 hours

TASK2: D'Schueberfouer

As you stroll along the Schueberfouer, the many smells and flavors from all over the world threaten to overwhelm you. So come on, let's take a short break to quench our thirst with the Luxembourgish lemonade Lët'z Limo. Ah, refreshing and tasty! Of course, as young scientists, you are interested in the composition of the lemonade. Let's find out together what its juicy secret is!

Note: Start with Problem 1, as this task requires 60 minutes drying time!

Problem 1 - Analysis of the *Lët'z limo* – Lemon and Lime

Materials

- **Drying Oven** (shared)
- **Scale (precision: 0,01 g)** (also for Problem 2)
- **magnetic stirrer and heating plate** (also for Problem 2)
- **distilled water bottle** (also for Problem 2)
- **stirring bar remover** (also for Problem 2)
- 2 **spatulas**
- **magnetic stirring bar** (preparation of calcium chloride solution)
- **Calcium chloride hexahydrate** inside Falcon tube; approx. 10g (preparation of calcium chloride solution)
- **150mL glass beaker** (preparation of calcium chloride solution)
- **opened bottle of lemonade** (lemonade preparation)
- **100mL graduated cylinder** (lemonade preparation)
- **250mL Erlenmeyer flask** (lemonade preparation)
- **NaOH 3M** inside Falcon tube approx. 10mL (to bring the pH to 8-9)
- **Pasteur pipette** (to bring the pH to 8-9)
- **pH paper** (pH control) (amount not limited)
- **glass thermometer** (to control temperature of lemonade solution)
- **100mL glass beaker** (80°C water wash solution for vacuum filtration of calcium citrate)
- **vacuum flask + suction filter + seal** (for hot vacuum filtration)
- **vacuum tubing + water-jet pump** (for hot vacuum filtration)
- **a pair of cotton gloves** (for hot vacuum filtration)
- **crucible tongs** (for taking hot calcium citrate from the oven)
- periodic table
- timers

The acidity of Lët'z limo—as in many other lemonades—comes from the citric acid that it contains. In **Problem 1**, your goal is to **determine the citric acid content** of the lemonade.

1.1. The chemical formula of citric acid (2P)

Citric acid is an organic compound with a non-trivial formula consisting of six carbon atoms, eight hydrogen atoms and several oxygen atoms per molecule.

☐ **Question 1.1.:** Determine the exact **number of oxygen atoms** contained in a molecule of citric acid, knowing that the molar mass of citric acid is $192.13\frac{g}{mol}$ and that the molecule contains 58.3% oxygen by mass. Detail your calculations on the **ANSWER SHEET Question 1.1**.

Before continuing, please raise the golden card to let a supervisor check your answer. If the result is wrong, you will get no points for this question, but the correct result will be provided.

1.2. Chemical equation for the precipitation of calcium citrate (3P)

To determine the citric acid content in the lemonade, we want to use a simple precipitation reaction using calcium ions: To do this, we add sufficient amount of calcium chloride to the acidic solution, whereby the sparingly soluble salt calcium citrate $Ca_3(C_6H_5O_x)_2$ is formed (x assumes the value determined in question 1.1.). Furthermore, this reaction also forms an acidic solution that is commonly found in any laboratory.

☐ **Question 1.2.:** Write the balanced equation of this reaction on the **ANSWER SHEET Question 1.2.**

Before continuing, please raise the golden card to let a supervisor check your balanced equation. If the result is wrong, you will get no points for this question, but the correct result will be provided.

1.3. Preparation of calcium chloride solution (2P)

To carry out the reaction described above; we need approximately 100 mL of a 0.3 mol/L calcium chloride solution.

☐ **Question 1.3.:** Calculate the **mass of calcium chloride hexahydrate** (assumed to be 100% pure) you need for this. Detail your calculations on the **ANSWER SHEET Question 1.3**.

Before continuing, please raise the golden card to let a supervisor check your result. If the result is wrong, you will get no points for this question, but the correct result will be provided.

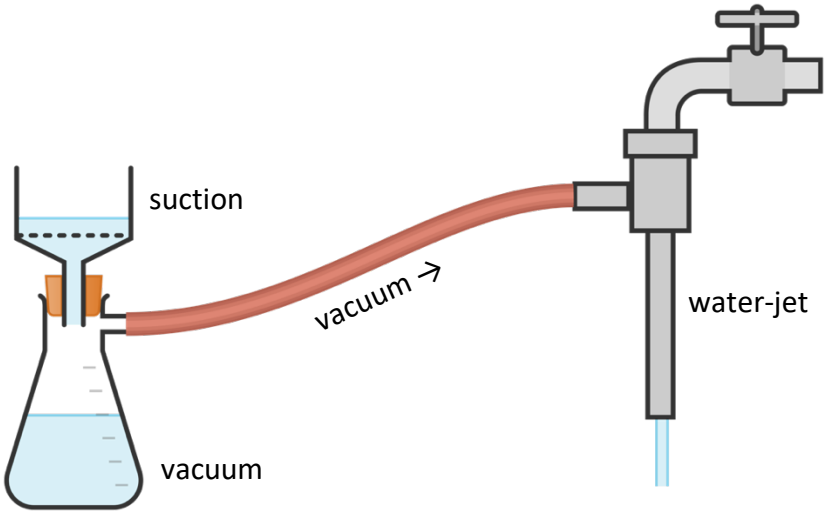
After your calculations are checked by a supervisor, prepare the solution in a 150 mL beaker.

1.4. Precipitation of calcium citrate (9P)

- In a graduated cylinder, pour exactly 100 mL of the lemonade (without shaking the bottle) and transfer it into a 250 mL Erlenmeyer flask. Add all of the previously prepared calcium chloride solution while stirring. Add dropwise the sodium hydroxide solution (3M) until you obtain a **pH value** of **8-9** (takes approx. 100-130 drops). Monitor the pH value using **pH indicator paper** while adding the sodium hydroxide solution!

- Heat the resulting solution to 80°C (**use the provided cotton gloves**) and keep it at 80°C for approximately 5 minutes. The calcium citrate should precipitate completely. In the meantime, weigh the **empty weight** of the available suction filter and heat about 50 mL of distilled water to approx. 80°C in a separate 100 mL beaker. In the next step, this will be used as warm wash water for the precipitate.

- The hot reaction mixture should now be filtered through a suction filter (vacuum filtration, see figure below). **Use the provided cotton gloves** to avoid burning your hands. **Do not touch the hot Erlenmeyer flask without protection!** Gradually rinse the emptied Erlenmeyer flask with the warm wash water to completely transfer the formed calcium citrate into the suction filter.



- Place the suction filter containing the calcium citrate in the drying cabinet at 180°C for **60 minutes** to dry. Then remove the **(!!hot!!)** suction filter using the **crucible tongs** and **allow it to cool** on the work counter before weighing it.

☐ **Question 1.4.:** Now calculate the total citric acid mass in a 330 mL bottle of Lët'z Limo in grams. Detail your calculations on the **ANSWER SHEET Question 1.4**.

1.5. Determination of the number of lemons in one bottle of Lët'z Limo (2P)

To calculate how many lemons are needed for a bottle of Lët'z Limo, we need to make the following theoretical assumptions:

1. Lemon juice has an average concentration of 0.3 mol/L citric acid.

2. One lemon contains 50 mL of lemon juice.

☐ **Question 1.5.:** Calculate the theoretical number of lemons contained in one bottle of Lët'z Limo. Detail your calculations on the **ANSWER SHEET Question 1.5**.

Refreshed by the delicious lemonade, you continue along the Schueberfouer and realize that you are getting hungry. You look around to see what's on offer here and notice that many fairground visitors flavor their food with Luxemburgish mustard. Your spirit of enquiry is awakened: Why is this mustard so popular? What might be the secret of this mustard? In the following experiments, you will get to the bottom of this question.

Problem 2: Analysis of Luxembourgish mustard "Moutarde de Luxembourg"

A glance at the list of ingredients of Luxembourgish mustard reveals its main components: Water, mustard seeds, **vinegar**, salt, sugar and **spices**.

Your **first step** is to determine the **amount of vinegar** as well as the **acidity** of the mustard. As you probably know, vinegar is an aqueous solution of acetic acid (CH_3COOH).

Your **second step** is to identify **one of the spices** in the spice mixture and to subsequently determine the amount of that spice in the mustard recipe.

Materials

- **iPad + pencil**
- **calculator**
- **set square (triangle)**
- **metal spatula**
- **NaOH solid** inside Falcon tube; a few grams
- **pipetting aid**
- **Mustard 90g**; 1 tube
- **100mL glass beaker** (to mix/homogenize 10g mustard from the tube)
- **glass rod** (to mix/homogenize 10g mustard from the tube)
- **2x 150mL glass beaker** (to put exactly 1g of homogenized mustard)
- **magnetic stirring bar** (to mix 1g of homogenized mustard with deionized (DI) water)
- **2x plastic funnel** (gravity filtration of mustard mixture)
- **6x pleated filter paper** (gravity filtration of mustard mixture) (amount not limited)
- **2x 500mL Erlenmeyer flask** (gravity filtration of mustard mixture)
- **burette set** (burette + stand + clamp) (for titration)
- **NaOH 0.1 M** inside Falcon tube ; 30-50mL (titrant)
- **250mL volumetric flask** with stopper (for titrant dilution)
- **25mL glass pipette** (for titrant dilution)
- **150mL glass beaker** (waste during filling of the burette)
- **plastic funnel** (to fill the burette)
- **2x magnetic stirring bar** (for titration)
- **PASCO pH electrode** (for titration)
- **250mL Erlenmeyer flask** (to keep PASCO pH electrode in deionized (DI) water between measurements)
- **Ethanol 96%** inside a bottle; approx. 80mL (for extraction of spices and mustard)
- **25mL glass pipette** (ethanol mustard solution)
- **100mL glass beaker** (ethanol mustard solution)

- **magnetic stirring bar** (ethanol mustard solution)
- **3 spices** (Annatto, Saffron, Turmeric); small quantity (ethanol extraction of spice)
- **4x glass tubes** (for gravity filtration)
- **4x plastic funnel + paper filter** (for gravity filtration)
- **Plastic pipette** (to add approx. 10mL of Ethanol)
- **4x Pasteur pipette** (transfer to cuvette)
- **5x cuvettes** (for spectrometric analysis)
- **PASCO spectrometer** (for analysis of colored molecule in spice/mustard)
- distilled water bottle
- periodic table

2.1. The neutralization reaction between acetic acid and sodium hydroxide (2P)

☐ **Question 2.1.:** Write the balanced chemical reaction (using molecular formulas) between the acetic acid and sodium hydroxide (base) on the **ANSWER SHEET Question 2.1**.

Before continuing, please raise the golden card to let a supervisor check your balanced equation. If the result is wrong, you will get no points for this question, but the correct result will be provided.

Step 1: Titration of acetic acid

These are the necessary steps:

1. Take approximately 10 g of mustard and mix it thoroughly in a 100 mL beaker using a glass rod. Thereof transfer exactly 1.00 g of mustard into a 150 mL beaker, then add approximately 100 mL of distilled water before stirring it for approximately 3 minutes on the magnetic stirrer at room temperature.
2. Filtrate the mixture by means of a pleated filter in a glass funnel on top of a 500 mL Erlenmeyer (gravity filtration). Rinse the residue several times with distilled water and keep the filtrate.
3. Use the filtrate to carry out a titration with a sodium hydroxide solution. This titration consists in dripping a solution of a strong base (using a burette) into a weak acid solution. Follow the procedure (1 mL steps until 40 mL are reached) according to the measurement steps written in the SparkVue-App. An acid-base reaction takes place. Keep the mustard solution in the same 500 mL Erlenmeyer flask to carry out the titration.

   You can simply place the PASCO pH electrode in the Erlenmeyer flask without fixation. Position it in such a way that the dripping titrant solution does not fall onto the electrode casing directly. Use a magnetic stirrer and make sure it does not bump into the electrode. You can keep the electrode in the solution at all times.

4. The necessary sodium hydroxide solution with a concentration of 0.01 M needs to be produced beforehand by diluting the provided sodium hydroxide solution with a concentration of 0.1 M. Assume that the concentration is exactly 0.100 M. You may only use the provided material for this task.

Note: Please refer to the provided video in the Photo-App. Please ask the instructor on any remaining questions concerning the technical equipment.

5. The equivalence point is determined with the help of a pH meter. Use the PASCO® pH meter connected to an iPad to measure the pH during the titration. A jump in the pH curve can be observed when the equivalence point is reached. **When not in use, keep the pH meter immersed in distilled water in a 250mL Erlenmeyer.**
6. Repeat the steps 1 to 5 a second time and use the average value to answer Question 2.2:
    - Take the 1.00g of mustard from the same sample as for the first titration
    - Fill up the same burette as for the first titration
    - Use the same magnetic stirring bar (rinse it using the distilled water bottle)

2.2. Amount of acetic acid (10 points)

☐ **Question 2.2.:** Calculate the molar amount of acetic acid in exactly 1,00 g of Luxembourgish mustard (5P). Do not delete the graph of the titration – it will be also graded (5P). Detail your calculations on the **ANSWER SHEET Question 2.2**.
Please mark your graph with your country and group number!

2.3. Mass percent of acetic acid in mustard (2P)

☐ **Question 2.3.:** Calculate the mass percent of acetic acid in Luxembourgish mustard. Detail your calculations on the **ANSWER SHEET Question 2.3**.

2.4. Vinegar in mustard (2P)

☐ **Question 2.4.:** Determine the volume of vinegar (ρ = 1.005 g/mL, contains 5% of acetic acid by mass), which is necessary for preparing 90 g of mustard (one tube of mustard). Detail your calculations on the **ANSWER SHEET Question 2.4**.

Step 2

The spice recipe of most products is usually a well-kept secret. Let's identify one of the spices in Luxembourgish mustard, henceforth called "spice A".

Spice A contains a certain percentage of a colored molecule, henceforth called "molecule B".

1. In a 100 mL beaker, mix exactly 1.00 g of mustard with 25 mL ethanol (use a pipette) and stir for 3 min at room temperature.
2. Put a plastic funnel and filter paper on top of a glass test tube and carry out a filtration of the mixture to collect a yellow filtrate.

Note: Please refer to the video of the PASCO® spectrometer in the Photo-App. Please ask the instructor on any remaining questions concerning the technical equipment.

3. Using the PASCO® spectrometer, collect an absorption spectrum and a fluorescence (@405nm) spectrum.

2.5. Peak absorption (2P)

☐ **Question 2.5.:** What is the peak absorption wavelength (in nm) of molecule B? Write your answer on the **ANSWER SHEET Question 2.5**.

2.6. Peak fluorescence (2P)

☐ **Question 2.6.:** What is the peak fluorescence wavelength (in nm) of molecule B? Write your answer on the **ANSWER SHEET Question 2.6**.

2.7. Molar concentration of molecule B (2P)

☐ **Question 2.7.:** Using Lambert-Beer law, what is the molar concentration of molecule B in the filtrate? Detail your calculations on the **ANSWER SHEET Question 2.7**.

Lambert-Beer law

$$A = \varepsilon \cdot c \cdot d$$

A= absorbance (= log(I_0/I))
ε = extinction coefficient ($L \cdot mol^{-1} \cdot cm^{-1}$)
d = distance (= length of cuvette = 1 cm)

Use $\varepsilon = 55000\ L \cdot mol^{-1} \cdot cm^{-1}$

2.8. Mass percentage of spice A (3P)

☐ **Question 2.8.:** Assuming that spice A contains 2 mass percent of molecule B. What mass percentage of the commercial spice A does the company use in their mustard? The molar mass of molecule B is 368,38 g/mol. Detail your calculations on the **ANSWER SHEET Question 2.8**.

Now let's find out which spice the mustard contains. On your desk you find 3 yellow-orange spices (Saffron, Turmeric (Curcuma) and Annatto). These spices are typically used for food coloring and flavoring. Only one of these is present in the mustard recipe.
Use the small pipettes to add approximately 10 mL of ethanol to the corresponding glass test tube. Put a plastic funnel and filter paper on top of 3 glass test tubes and carry out a filtration.

2.9. Spice A (4P)

☐ **Question 2.9.:** Using the spectrometer, determine which spice A is contained in this mustard. **Only one of the three mentioned spices is present**. Analyze the statements in the table of the **ANSWER SHEET Question 2.9.** and decide whether these are **true** or **false**. Tick (✓) the correct answer.

2.10. Alternative chemical way to spice A (3P)

☐ **Question 2.10.:** Using only materials available on your desk, find another chemical way to quickly guess that mustard, among the three commercial spices available at your desk, contains only spice A. Analyze the statements in the table of the **ANSWER SHEET Question 2.10.** and decide whether these are **true** or **false**. Tick (✓) the correct answer.

Note: pOH + pH = 14

Problem 3: Physics – Looping & LEDs

Problem 3.1. Looping (24 P)

Introduction

The vibrant lights and joyous screams at the Schueberfouer announced its presence long before you arrived. The iconic Ferris wheel stood tall against the twilight sky, beckoning you in. As you wandered through the bustling crowds, a roller coaster with multiple, thrilling loops caught your eye (Figure 3.1).

Figure 3.1 Rollercoaster at the Schueberfouer

As a physicist, a question sparked in your mind: What makes the cart loop solely under gravity?

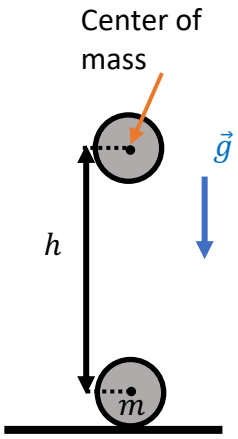
Back in your lab, a wave of nostalgia hit you as you retrieved your childhood Hot Wheels looping set (Figure 3.2). Setting it up, just like the picture in your mind, you were ready to unravel the scientific mystery behind the roller coasters.

Figure 3.2 Hot Wheels Looping Set

Theory

To investigate the physics of a rollercoaster, you recognize that the principle of conservation of mechanical energy offers a useful approach. To apply it, we need to neglect energy loss due to friction.
The mechanical energy E_{mech} of a system is the sum of its potential energy E_{pot} and kinetic energy E_{kin}.

$$E_{\text{mech}} = E_{\text{pot}} + E_{\text{kin}}$$

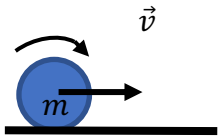


An object of mass m located at a height h has a gravitational potential energy of

$$E_{\text{pot}} = m \cdot g \cdot h$$

With $g = 9{,}81\,\frac{\text{m}}{\text{s}^2}$ the gravitational acceleration.

The kinetic energy of a **rotating without slipping homogeneous sphere** of mass m and velocity v is the sum of the linear (translational) and rotational kinetic energy:



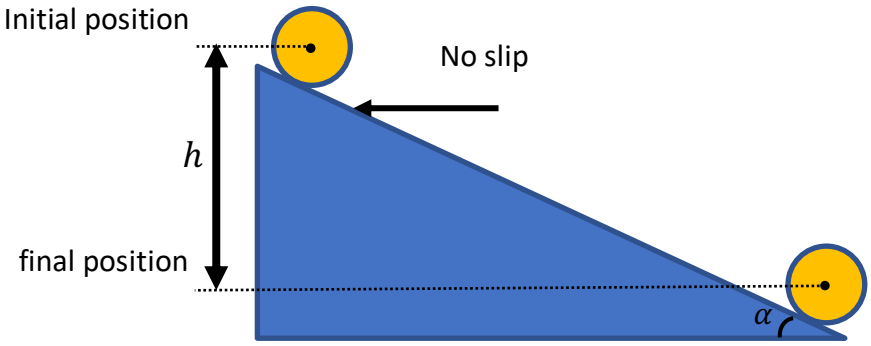
$$E_{\text{kin}} = E_{\text{kin,translation}} + E_{\text{kin,rotation}}$$
$$E_{\text{kin}} = \frac{1}{2}mv^2 + \frac{1}{5}mv^2$$

The law of the conservation of mechanical energy theorem states, that without losses due to friction, the mechanical energy is conserved. This means that the mechanical energy of an object in an initial state $E_{\text{mech,initial}}$ equals the mechanical energy $E_{\text{mech,final}}$ in a later state:

$$E_{\text{mech,initial}} = E_{\text{mech,final}}$$

Exercises

By applying the law of conservation of mechanical energy, we can determine the theoretical velocity of a homogeneous sphere (marble) of mass m, **rolling** down an inclined plane of angle α after releasing it from rest from a height h as it is shown in the following figure.



➔ **Question 3.1.:** Use the law of conservation of mechanical energy to derive a mathematical expression for the velocity v in dependence of h of the marble rolling down the inclined plane from an initial to the final position. Detail your calculation on the **ANSWER SHEET Question 3.1.** **(1 point)**

This result is critical for question 3.3. Raise your golden card for a supervisor to verify your answer. If incorrect, you get 0/1 point for question 3.1., but you will receive the correct result.

➔ **Question 3.2.:** Consider a marble sliding (only sliding, no rotation) down an inclined plane and a marble rolling (no sliding, only rotation) down the same plane. If both start from rest at the same height, which object will reach a greater final velocity at the bottom? Tick the respective box on the **ANSWER SHEET Question 3.2**. **(0.5 point)**

We now introduce the equations of motion for a uniformly accelerated object in one dimension. Assuming constant acceleration, its motion is described by the following equations:

$$a = const. \neq 0$$
$$v(t) = a \cdot t + v_0$$
$$d(t) = \frac{1}{2} \cdot a \cdot t^2 + v_0 \cdot t$$

With a the acceleration, v the time dependent velocity, t the time, v_0 the initial velocity and $d(t)$, the travelled distance. In our case, since the marble starts from rest, the initial velocity v_0 is zero. Therefore, the equations of motion can be simplified to:

$$a = const. \neq 0$$
$$v(t) = a \cdot t \quad (1)$$
$$d(t) = \frac{1}{2} \cdot a \cdot t^2 \quad (2)$$

- **Question 3.3.:** In question 3.1, you derived a mathematical expression for the velocity v of a marble rolling down an inclined plane. Building on this result and utilizing the relationship between velocity, acceleration (equation (1)), distance traveled (equation (2)), height h and angle of inclination α **derive** the following expression for the acceleration:

$$a = \frac{5}{7} g \sin \alpha.$$

Hint: use $\frac{height}{distance} = \frac{h}{d} = \sin \alpha$

We recommend starting with your result for the velocity from question 3.1. Write your calculations on the **ANSWER SHEET Question 3.3**. **(2 points)**

Experiment - Part 1 (75 minutes)

Materials

- Marble (20 g)
- Hot wheels track builder unlimited
- 2 Light barriers connected to a timer
- 6 Cables
- Graph paper
- Ruler
- Wooden inclined plane

To determine the gravitational acceleration g experimentally, set up two light barriers: one at position 1 and one at 2 along the inclined plane (figure 3.3). Measure the time t it takes the marble to travel the distance d between the barriers. The steps as well as the questions will be explained below. The relation between the distance d, time t and acceleration a is given by $d(t) = \frac{1}{2} a t^2 = \frac{1}{2} \left(\frac{5}{7} g \sin \alpha \right) t^2$.

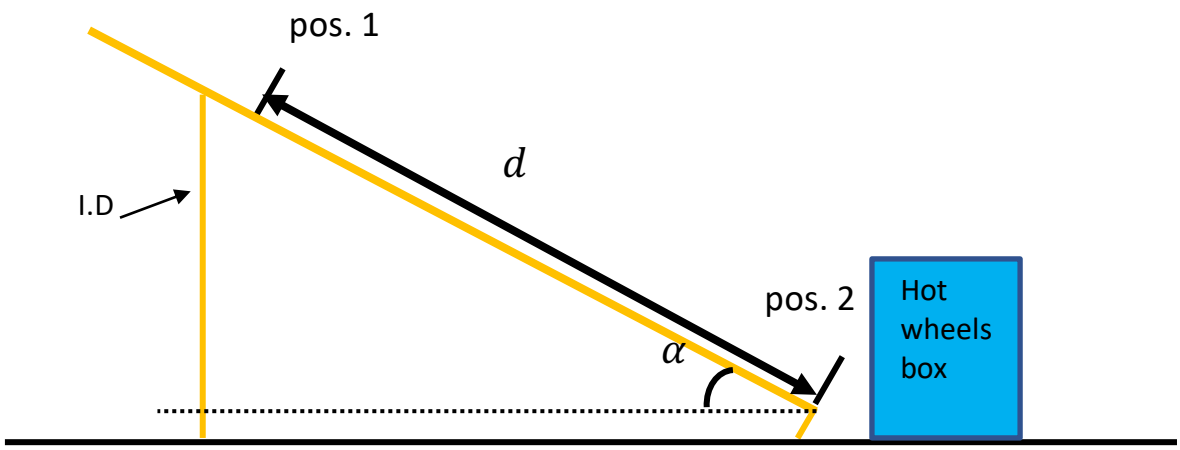


Figure 3.3 Setup to measure the gravitational acceleration

To Do:

1) You need to do six measurements with a marble of 20 g.
2) For each measurement, choose a distance *d* between 20 and 100 cm and measure the time it takes the marble to cover this distance by using the light barrier.

MAKE SURE TO READ THE FOLLOWING INSTRUCTIONS:

1) Place the Hot Wheels Box at the end of the track (opening towards the track) to catch the marble.
2) To keep the distance between the ball and the light barrier constant for each measurement, a plasticized piece of paper is used as a barrier. Hold it between the ball and the light barrier for each measurement and then pull it away abruptly (see Figure 3.4).

Figure 3.4 Marble release

3) The formula $d(t) = \frac{1}{2}at^2 = \frac{1}{2}\left(\frac{5}{7}g\sin\alpha\right)t^2$ assumes that the initial velocity of the marble is zero. This is however not correct as the marble travels a distance of $0.5\ \mathrm{cm}$ within $0{,}06\ \mathrm{s}$ from the point of release to the center of the forked barrier. For each measurement this additional distance and time needs to be added to the measured distance and time.
4) For the first experiment, the equipment is ready. Verify the timer setting matches Figure 3.5. To begin a new measurement, press the reset button.

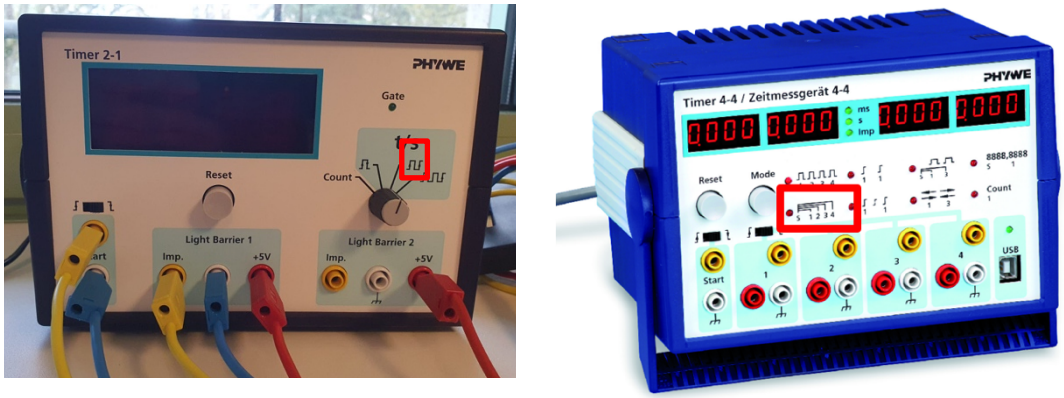


Figure 3.5 Timer 2-1 (left) or Timer 4-4 (right)

QUESTIONS

➔ **Question 3.4.:** Write the Identification number (I.D.) of the inclined plane on the **ANSWER SHEET Question 3.4.** (see Figure 3.3).

Note: make sure the string of the inclined plane is tight throughout the measurements.

➔ **Question 3.5.:** Determine the angle of inclination α of your setup and write the angle in degrees on the **ANSWER SHEET Question 3.5.** (see Figure 3.3). Note down your measured results to $0.1°$. This result is critical for **Question 3.10.** Raise your golden card for a supervisor to verify your answer. If the absolute error of α is between 0.5° and 1°, 0.5 points will be deducted. If the absolute error of α is > 1°, 0/1 point will be given for this question. In both cases you will receive the correct result from a supervisor. (**1 point**)

Hint: use $\sin\alpha = \frac{height}{distance} = \frac{h}{d}$ to calculate α

➔ **Table 3.6.:** (**4.5 points**)

- Write your six distance (d) and time (t) measurement pairs (write down your measurements to $0.001\ \mathrm{m}$ & $\pm\ 0.001\ \mathrm{s}$) in **table 3.6. on the ANSWER SHEET**.
- Calculate the corrected values for distance $d' = d + 0{,}005\ \mathrm{m}$ and time $t' = t + 0{,}060\ \mathrm{s}$ and finally calculate t'^2. Write your results in the **table 3.6. on the ANSWER SHEET**.

➔ **Graph 3.7.:** (**2.5 points**)

- Represent the six different corrected distances *d'* and the corresponding corrected times squared t'^2 in a $d'(t'^2)$ diagram on graph paper, using S.I units and clearly labeling the axes. After completing the diagram, label the graph with the corresponding sticker!
- Draw a regression line (best fit on the eye) through your data points.

➔ **Question 3.8.:** Should the regression line theoretically intersect the origin? Circle the correct answer on the **ANSWER SHEET Question 3.8**. (**0.5 points**)

➔ **Question 3.9.:** Calculate the slope of the regression line. Subsequently, utilize this slope value to calculate the acceleration of the marble. Write your calculations on the **ANSWER SHEET Question 3.9.** and express them in SI Units. All calculations involving numerical values must include units. (**1.5 points**)

➔ **Question 3.10.:** Determine the gravitational acceleration of the Earth g from the acceleration calculated in **Question 3.9**. Show your calculations on the **ANSWER SHEET Question 3.10.** and indicate your result in SI units. All calculations involving numerical values must include units. (**1 point)**

➔ **Question 3.11.:** Calculate the absolute and relative deviation of your result with respect to the theoretical value of $g = 9{,}81\ \frac{m}{s^2}$. Show your calculations on the **ANSWER SHEET Question 3.11.** and indicate your result in SI units. All calculations involving numerical values must include units. (**1 point**)

Experiment - Part 2 (45 minutes)

Materials

- Marble (20 g)
- Hot Wheels track builder unlimited
- Additional parts to change the radius of the loop
- Graph paper
- Ruler
- Mounts and clamps for stabilizing the loop
- Wooden inclined plane

It can be shown that the theoretical minimum height h for a marble of radius r_{marble} passing a loop of radius r is given by:

$$h_{\min} = 2.7(r - r_{marble}) \quad (3)$$

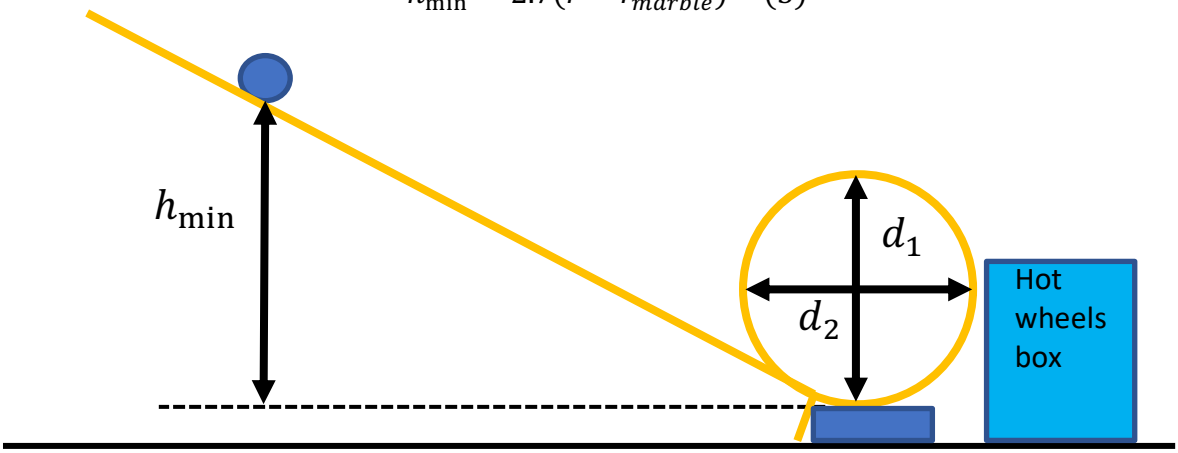


Figure 3.6 Hot wheels looping

To do:

Before continuing, remove the light barriers from the stand and replace them with the clamps.

In the following experiment you need to build different loops using different combinations of the **orange** Hot Wheels tracks of 30, 19 and 12 cm (see Figure 3.7). For five different loop radii, determine the minimum release height at which the marble completely traverses the loop without detaching from the track. To **minimize energy loss, ensure the loop is as circular as possible and well-stabilized using the metal mounts and clamps. This will help reduce deformities in the loop.**

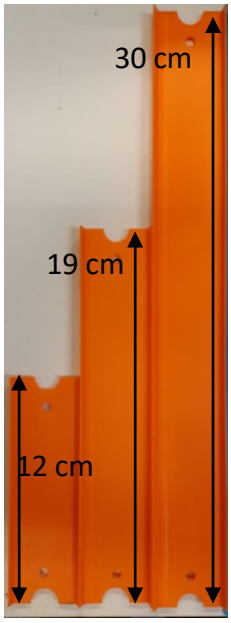


Figure 3.7 Hot Wheels Tracks

MAKE SURE TO READ THE FOLLOWING INSTRUCTIONS:

- Place the Hot Wheels Box at the end of the track (opening towards the track) to catch the marbles.
- **For the loop only use the orange tracks and handle them gently to avoid deforming them too much.**
- **The maximum loop consists of three pieces of 30 cm.**

For **five** different loops of radius *r*:

➔ **Question 3.12.:** Measure the radius of the marble and note down your measurements to 0.001 m on the **ANSWER SHEET Question 3.12.**

➔ **Table 3.13.:**

- Measure the diameter of the loop vertically d_1 and horizontally d_2 (note down your measurements to 0.001 m). Take the mean value as diameter d, calculate the mean radius r. Subtract the radius of the marble from the mean radius of the loop and write the values in **table 3.13**. on the **ANSWER SHEET.** (**2.5 points**)
- Determine experimentally the minimum height $h_{\min}$ (note down your measurements to 0.001 m) for which the marble **completely** passes each of the five loops and write the values in **table 3.13**. on the ANSWER SHEET. (**2.5 points**)

$h_{\min}$ is defined as the vertical displacement of the bottom of the marble relative to the loop's lowest point.

➔ **Graph 3.14.:** Represent $h_{\min}$ versus $(r - r_{\text{marble}})$ graphically and calculate the slope of the linear regression on graph paper. Use S.I. units and clearly label the axes. After completing the diagram, label the graph with the corresponding sticker! (**3 points**)

➔ **Question 3.15.:** Does the regression line theoretically intersect the origin? Circle the correct answer on the **ANSWER SHEET Question 3.15**. (**0.5 points**)

You must not be surprised if your result does not match the theoretical value of 2.7. Your measurements will be compared with our experimental data.

Problem 3.2. LEDs (26P)

Introduction:

A visit to the *Schueberfouer* is most enjoyable in the evening when the stalls and rides, adorned with thousands of colorful lights, create a special atmosphere.
In this task, you will delve into LEDs and experimentally determine one of the great constants of nature: the Planck constant.
The Planck constant, introduced by the German physicist Max Planck in 1900 as part of his groundbreaking work on blackbody radiation, plays a fundamental role in determining the color (wavelength) of light emitted by LEDs.

Aim:
Determination of Planck's constant using different LEDs

1. *Measurement of the I-V characteristic of an LED (45* min*)*

Material

- Breadboard with integrated power supply (5V **DC**)
- Electrical resistor ($R_2 = 470\ \Omega$)
- Potentiometer (variable resistor) R_1
- Red LED
- 2 Multimeters
- Wires (4)
- Alligator clips (4)

The smaller of the two multimeters must be used as a ammeter and the larger one as an voltmeter.

Experimental setup

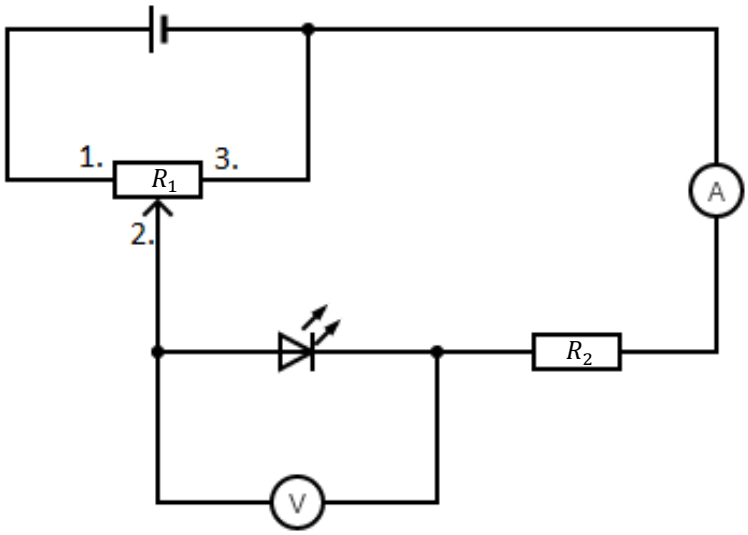
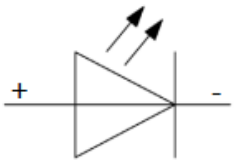
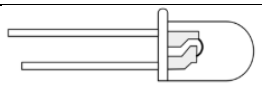
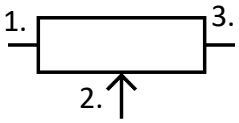
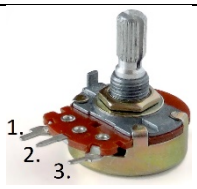


Figure 3.8: Electrical circuit

Hints:

	Circuit symbol	Connection of the pins
Resistor		Can be placed in any orientation
LED		 Positive pole: long pin Minus pole: short pin
Potentiometer		

Experimental procedure

➔ **Question 3.16.: Construct the electrical circuit (figure 3.8), then present it to a supervisor for verification (=> show your golden card; signature of a supervisor on the ANSWER SHEET Question 3.16.)**. Make sure that the power supply is switched off during the construction of the circuit (the green LED on the power supply does not light up). If you encounter difficulties, you can consult a supervisor for guidance, but you will lose 1 point for each hint. After 3 hints you will get a working circuit. (**3 points**)

The voltage at the LED can be changed by turning the wheel on the potentiometer. Slowly adjust the potentiometer knob to gradually increase the voltage V across the LED. Observe the LED. At a specific voltage, called the forward voltage, V_f, it will begin to emit light.

➔ **Question 3.17.:** Record the forward voltage V_f (write down your measurements to 0.01 V) on your **ANSWER SHEET Question 3.17**. (**1 point**)

For a more accurate forward voltage determination, you will now measure the $I - V$ characteristic of the LED.
The ideal $I - V$ characteristic of an LED is shown in figure 3.9.

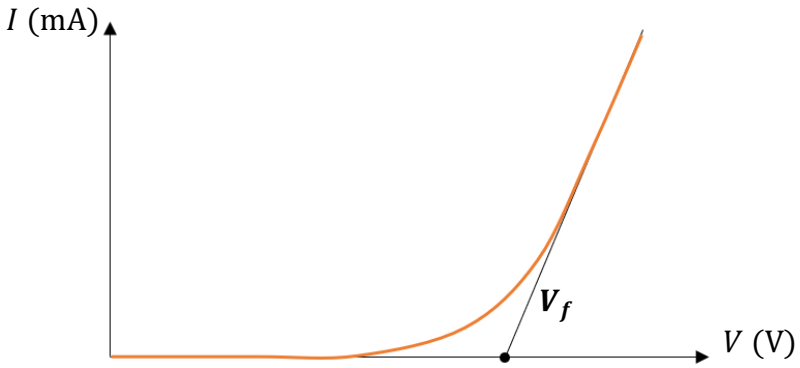


Figure 3.9: *I-V* characteristic of an LED

To record the *I-V* characteristic, ensure that:

- the ammeter and voltmeter are set to the **DC mode**.
- you identify the areas in which more measurements are required.
- you measure the current up to an magnitude of current of 4.00 mA.

➔ **Table 3.18.:** Record the *I-V* characteristic of the red LED in **table 3.18. on the ANSWER SHEET.** Take at least 15 measurement points (write down your measurements to 0.01 V & 0.01 mA). (**3.5 points)**

➔ **Graph 3.19.:**

- Plot the *I-V* characteristic, *I* versus *V,* on the provided graph paper (using at least 15 data points), ensuring that the graph and both axes are clearly labeled. After completing the diagram, label the graph with the corresponding sticker! (**2.5 points)**
- To determine the forward voltage V_f of the red LED, draw a trendline (best fit on the eye) through the linear region of the *I-V* characteristic. The point where this line intersects with the voltage axis closely approximates the forward voltage V_f. (see figure 3.9.) Note the value (write down your measurements to 0.01 V) on the **ANSWER SHEET at Graph 3.19**. (**1 point**)

2. *Measurement of the wavelength of the LEDs (30 minutes)*

Theory

When an LED's light shines through a diffraction grating, a series of narrow, closely spaced slits, it creates spherical waves of light from each opening. These waves interact with each other behind the grating, either amplifying (constructive interference) or canceling (destructive interference) each other.
This interaction creates an interference pattern (figure 3.10) dependent on the light's wavelength λ and the grating's slit spacing g. This pattern consists of a series of bright spots, called interference maxima.

Figure 3.10: Interference pattern of laser light

In the middle is the central interference maximum ($n = 0$), on its left and right side are the interference maxima of higher order ($n > 0$).

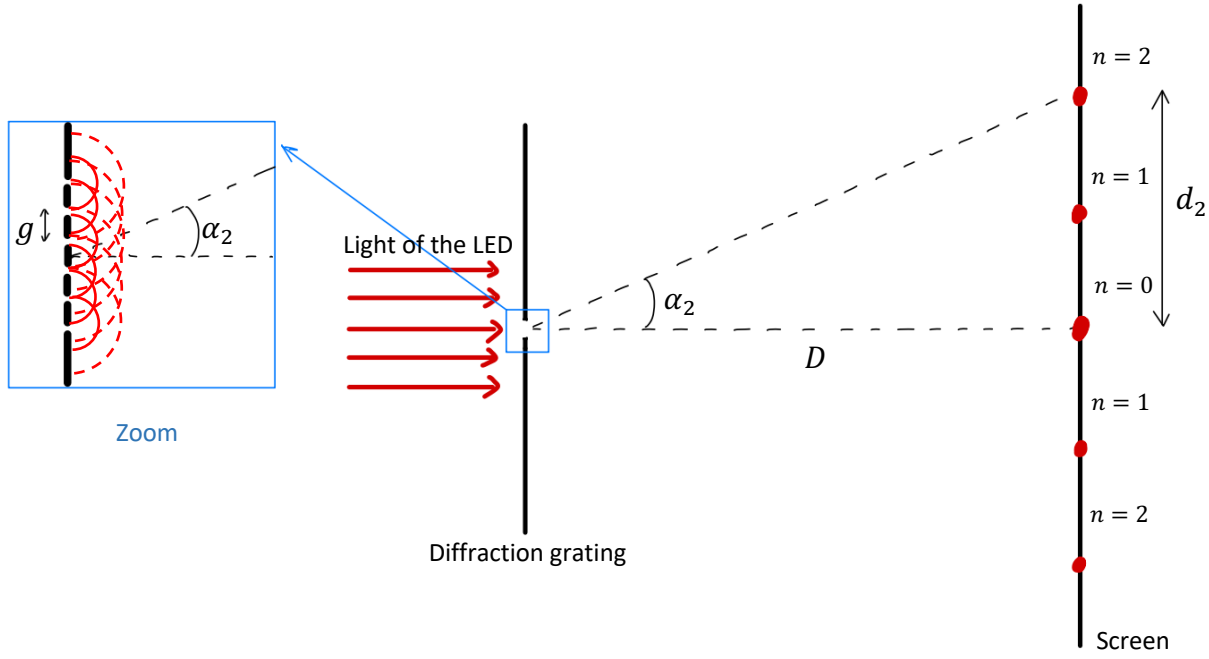


Figure 3.11: Diffraction experiment of laser light

The position of the different interference maxima can be calculated using the following formulae:

$$\tan \alpha_n = \frac{d_n}{D} \quad (1) \qquad \sin \alpha_n = \frac{n \cdot \lambda}{g} \quad (2)$$

Material

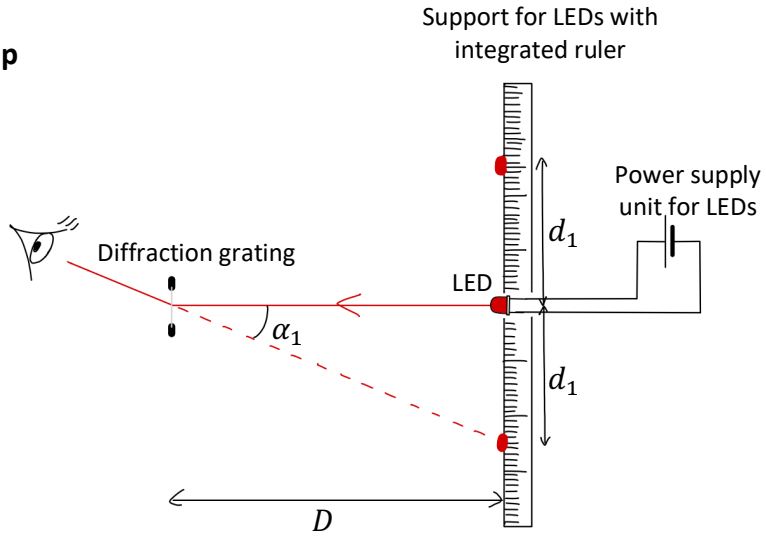
- Power supply unit for LEDs (labeled **Nr. 1**)
- 4 different LEDs (red, green, yellow, blue)
- Diffraction grating (500 slits/mm) (labeled **Nr. 2**)
- Support for the diffraction grating (labeled **Nr. 3**)
- Support for LEDs with integrated ruler (labeled **Nr. 4**)
- 1 ruler

➔ **Question 3.20.**: Calculate the distance g between the centers of two adjacent slits of the diffraction grating which has 500 slits/mm. (All calculations involving numerical values must include units). Detail your calculations on the **ANSWER SHEET Question 3.20**. (**1 point**)

➔ **Question 3.21.**: For violet light of wavelength $\lambda = 380\ \mathrm{nm}$, calculate the angle at which the 1st order interference maximum $(n = 1)$ can be observed in this diffraction experiment. (All calculations involving numerical values must include units). Detail your calculations on the **ANSWER SHEET Question 3.21**. (**1 point**)

➔ **Question 3.22.**: What is theoretically the maximum number of the observable interference maxima, when the grating is illuminated with violet light of wavelength $\lambda = 380\ \mathrm{nm}$? Include your calculations to support your answer. Detail your calculations on the **ANSWER SHEET Question 3.22**. (All calculations involving numerical values must include units). (**1 point**)

Experimental setup



Measurements

For the four different LEDs:

- Connect the LED to the power supply unit.
- Put the LED through the hole in the center of the support for LEDs.
- Position the diffraction grating at a distance of $D = 40.0\ \mathrm{cm}$ from the LED.
- Look through the diffraction grating (in the direction of the LED) and measure the distance $2 \cdot d_1$ between the two 1st order interference maxima. You will see an elliptic light spot on the ruler. Take the approximate middle of the spot for your measurements. Sometimes you may also see more than one colour. Consider only the part corresponding to the colour of the LED.
  (Hint: If the interference maxima are not immediately visible, try viewing the grating slightly off-center.)

➔ **Table 3.23.:** Record your measurements (write down your measurements to $0.001\ \mathrm{m}$) on the **ANSWER SHEET in table 3.23**. (**3 points**)

Evaluation

➔ **Question 3.24.:** Use the measured values (from **table 3.23**) to calculate for each LED the angle α_1 at which you were able to observe the 1st interference maximum. Provide a **detailed calculation only for the red LED**, demonstrating the steps involved. Record the calculated values on the **ANSWER SHEET Question 3.24**. (All calculations involving numerical values must include units). (**1.5 points**)

➔ **Question 3.25.**: Use the calculated values (from **Question 3.24**) to calculate the wavelength λ of each LED. Provide a **detailed calculation only for the red LED**, demonstrating the steps involved. Record the calculated values on the **ANSWER SHEET Question 3.25**. (All calculations involving numerical values must include units). (**1.5 points**)

3. Determination of Planck's constant (30 minutes*)*

Theory

Light-emitting diodes, or LEDs use a process called electroluminescence to generate light. This process involves the movement of electrons within a semiconductor material, resulting in the release of light particles called photons.
However, as observed in our first experiment, a LED only illuminates when the applied voltage exceeds a specific value, known as the forward voltage V_f. This voltage is necessary to push the electrons across the semiconductor layers.
During this process, the electrons gain energy, which they release finally in form of a photon.

- The energy E_g released by an electron while emitting a photon depends on the forward voltage of the LED and is calculated as follows:

$$E_\mathrm{g} = e \cdot V_\mathrm{f}$$

with $e = 1.602 \cdot 10^{-19} C$ – the elementary charge; V_f – forward voltage of the LED in V.

- The energy of a photon is given by the formula:

$$E_\mathrm{p} = h \cdot \nu$$

with h is Planck's constant; ν is the frequency of the light in s^{-1}.

- Knowing the wavelength λ of light and using the following formula allows you to calculate its frequency ν:

$$\nu = \frac{c}{\lambda}$$

with $c = 2.998 \cdot 10^{8} \frac{\mathrm{m}}{\mathrm{s}}$ – speed of light; λ is the wavelength of the light in m.

- The energy released by an electron E_g equals the energy E_p of the emitted photon.

$$E_\mathrm{g} = E_\mathrm{p}$$

➔ **Question 3.26.**: Derive a formula that allows you to determine the forward voltage V_f of a LED as a function of the frequency ν of the emitted light. Write your answer on the **ANSWER SHEET Question 3.26**. (**0.5 points**)

Evaluation

➔ **Question 3.27.:** Calculate the frequency ν of the light emitted by the 4 different LEDs and write the values in the **ANSWER SHEET Question 3.27**. Use the values from Question 3.25. (**1 point**)

➔ **Graph 3.28.:** Plot the $V_\mathrm{f} - \nu$ characteristic, V_f versus ν, on the provided graph paper. Label your graph clearly. Fit your data points with an appropriate regression curve on the graph (best fit on the eye)! After completing the diagram, label the graph with the corresponding sticker. (**2.5 points**)

Use the following values for the forward voltage V_f of the green, yellow and blue LEDs.

	V_f (V)
Red LED	Value from **Question 3.19.**
Blue LED	2.44
Green LED	1.99
Yellow LED	1.91

➔ **Question 3.29.:** Calculate the slope of the regression curve. Subsequently, utilize this slope value and the formula derived under **Question 3.26**. to calculate Planck's constant. Detail your calculations on the **ANSWER SHEET Question 3.29**. (**2 points**)

Remark: Slight deviations between your experimental value and Planck's constant are expected, as the forward voltage of the LED is also temperature-dependent, which this used simplified formula doesn't consider.

Problem 4: Biology (Osmosis) (27P.)

The classical dish at the “Schueberfouer”: “*gebaakene Fësch*”. Your task today will consist in analysing and comparing blood smears of fish and beef and to determine the evolutionary position of the group called “fish”.

To make sure that the prepared dish is “gebaakene Fësch” and the chef didn’t use a cheaper ingredient, your first task is to determine how one could distinguish by microscopy the differences between fish and beef. Therefore you will produce fish as well as beef blood preparations and examine them microscopically for cell properties.

Material needed:

- gloves
- trout
- Dissection tray
- scalpel
- scissors
- 0,3 mL syringe
- Micropipettes (P10 & P1000)
- pipette tips in red box (for P10 micropipette)
- pipette tips in blue box (for P1000 micropipette)
- tweezers
- Hemacolor I (Fixing solution) **(S1)**
- Hemacolor II (Coloration solution) **(S2)**
- Hemacolor III (Dye solution) **(S3)**
- Microscope

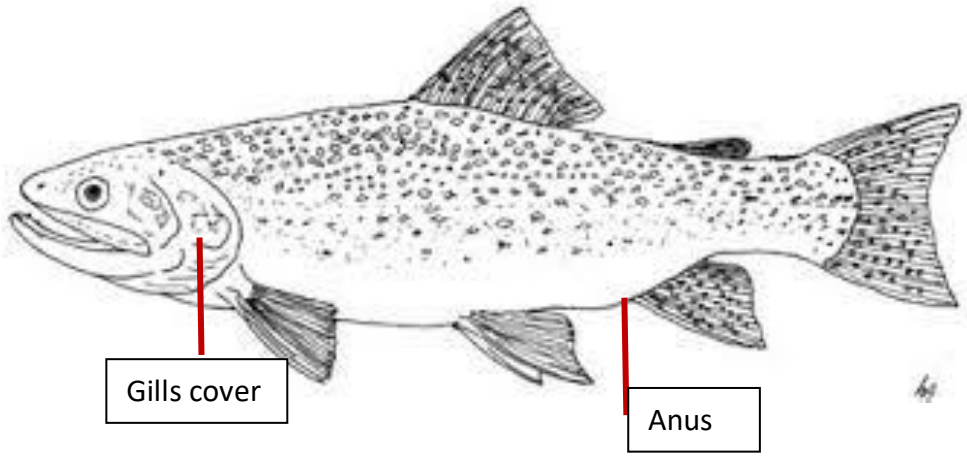
- 6 microscope slides
- 6 coverslips
- Red onion
- Solution A **(A)**
- Solution B **(B)**
- Solution C **(C)**
- 4 Pasteur pipette
- 10 mL beef blood (= solution **S4**)
- 2 test tubes
- pencil, colour pencil (red)
- 2 holder tubes
- 1 microtube (eppendorf) H_2O
- Parafilm
- Petri Dish

Problem 4.1. Blood smear

You are to make a blood smear of two animals.

A) Fish

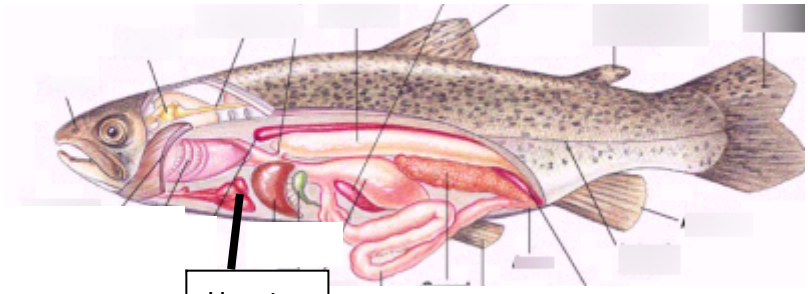
A trout is lying in front of you. To take the blood from the heart of the trout, you have to uncover the heart. The heart lies diagonally under the gill cover, close to the edge of the body floor.



fao.org/3/i2125e/i2125e.pdf

1. Cut along the bottom of the body using the scissors, starting at the anus and working towards the gill cover. Make sure you cut the skin and the tissue underneath, but not too deep to avoid damaging the internal organs.
2. Cut to about the level of the middle of the gill cover. Now cut upwards using a scalpel or the scissors, towards the gill cover. You may need to cut the gill covers with scissors.
3. Use tweezers to lift the skin and muscle at the cut. Carefully cut along the inside of the body with the scalpel to separate the muscle flesh from the organs.

In the front, lower area, between the gill cover and the ventral fin is the heart of the trout.



https://quizlet.com/244315817/trout-dissection-diagram/

☐ **Question 4.1.:** Once you think you uncovered the heart, call a supervisor to check and point the heart with your scissors. The supervisor will put a STAMP on your **ANSWER SHEET Question 4.1**.

4. Carefully remove the cap and then carefully insert the 0,3 mL syringe into the heart. The heart is very small, so be careful not to puncture it.
5. Now aspirate the blood. Apply one to two drops of blood to the microscope slide, 1 cm from the edge, and smear with an obliquely placed cover glass, without pressure. (Fig.4.1)
6. Place 1 small Petri dish into 1 larger Petri dish (Fig. 4.2) (The Petri dishes are only used not to stain any surfaces) Prepare this 3 times (for the solution S1, S2 and S3)
7. Put your specimen in the small Petri dish.
8. Cover the specimen in Hemacolor I solution **(S1)** with a Pasteur pipette, then take out the specimen using tweezers off the solution and redip it 5 times into the solution. Keep the Pasteur pipette for the coloration of the beef blood.
9. Use tweezers to transfer your specimen to the second Petri dish.

10. Cover the specimen in Hemacolor II solution **(S2)** with a new Pasteur pipette, then take out the specimen using tweezers off the solution and redip it 4 times into the solution. Keep the Pasteur pipette for the coloration of the beef blood.
11. Use tweezers to transfer your specimen to the third Petri dish.
12. Cover the specimen in Hemacolor III solution **(S3)** with a new Pasteur pipette, then take out the specimen using tweezers off the solution and redip it 4 times into the solution. Keep the Pasteur pipette for the coloration of the beef blood.
13. Lift your specimen with tweezers up, rinse with distilled water, drip off, **not rub off!**, put it on filter paper, let it dry.

The slide you just prepared will be referred to as **preparation 1,**

Keep the Petri dishes, you will have to reuse them after Question 4.6.

Please close the beakers S1, S2 and S3 with Parafilm.

Please arrange the scalpel and the used syringe as shown in the following picture. Do not attempt to put the protective cap back on the needle and put the tools back into the holder tubes after use.

This preparation will be needed at question (4.10)

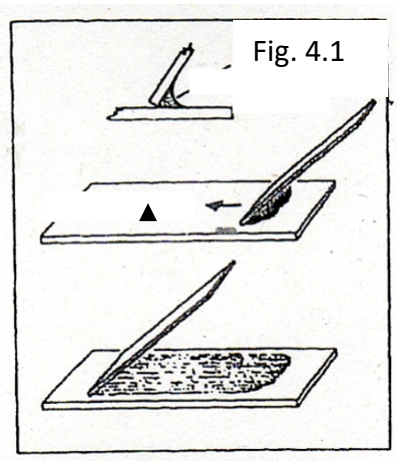
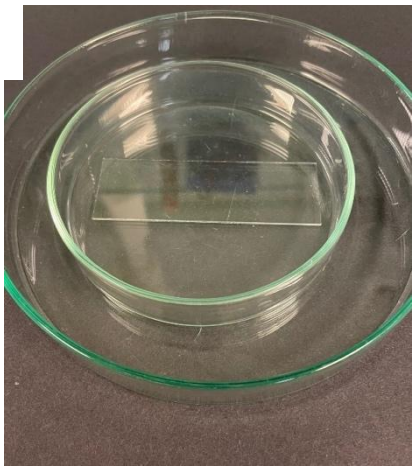


Fig. 4.1

Fig. 4.2



B) Blood smear of beef blood

In front of you is beef blood **(S4)**. Before you can make the smear, you have to dilute the blood 100 times with one of the solutions A, B or C in front of you.

Only one solution is the right one you can use.

To find out which of the three solutions (A, B or C) you can use, carry out the following experiment.

Make 3 preparations of the red epidermis of a red onion scale (Fig. 4.3). Remove the skin from a fresh onion and peel off one onion scale. Use a scalpel to score small squares of approx. 0.5 cm edge length into the red outer side and carefully remove a thin piece with tweezers (Fig. 4.3). Make sure to remove only the upper skin.

Use a new Pasteur pipette for each solution (A, B or C).

Place one specimen in a drop of solution A. Cover your specimen with the coverslip.

Place one specimen in solution B. Cover your specimen with the coverslip.

Place one specimen in solution C. Cover your specimen with the coverslip.

Mark your preparations with a permanent marker.

Observe the 3 preparations under the microscope.

Fig. 4.3

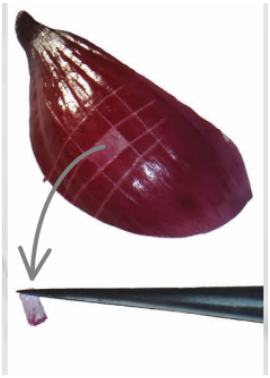


Fig. 4.3:https://www.schule-bw.de/faecher-und-schularten/mathematisch-naturwissenschaftliche-faecher/biologie/unterrichtsmaterialien/7-10/zelle/mikro/pdf/AB_zwiebel.pdf

For the following experiments, always use new Pasteur pipettes!

- **Question 4.2.:** Which solution is the hypertonic solution? (1P.)

Observe and make a sketch of a purple red onion cell in the hypertonic solution. Annotate the different cell components using the letter corresponding for the correct scientific annotations provided on the **ANSWER SHEET Question 4.2! (3.5P.)**

All drawings have to be made by pencil, the annotation lines need to be drawn with a ruler and must be parallel. It is prohibited to take a picture of your microscopic observations. You need to draw what you actually see under the microscope.

- **Question 4.3 Which cell structure is <u>mainly</u> affected by the hypertonic solution? Circle the right answer. (1P.)**

Letter	**Label**	**Letter**	**Label**
A	Chloroplast	E	Vacuole
B	Cell wall	F	Cell membrane
C	Cytoplasm	G	Mitochondria
D	Nucleus	H	Golgi body
I	Lysosome	J	Centrosome

- **Question 4.4 a) What phenomenon takes place in the red onion cell when it is put in a hypertonic solution. Circle the right answer(s). (2P.)**

Letter	**Label**
A	A movement of cytoplasmic organelles is generated
B	The sodium and chloride ions diffuse into the cell structure.
C	The water from the affected cell structure diffuses outside of the cell.
D	Nucleus is destroyed.
E	The water from the cytoplasm diffuses outside the cell.

- **Question 4.4 b) Which cell structure allows the phenomenon of osmosis? Circle the right answer(s). (2P.)**

Letter	Label
A	plasmodesma
B	gap junctions
C	aquaporin
D	canal proteines
E	cell membrane
F	chloroplast

☐ **Question 4.5.:** Which of the 3 solutions will you use for dilution? Write your answer on the **ANSWER SHEET Question 4.5.** and go and see a supervisor at the shared bench for a STAMP. Show your answer, do not talk!

☐ **Question 4.6.:** Now dilute the blood 100 X, to get a volume of 2 ml.

What volume of solution do you use? What volume of blood do you take? Write your answer on the **ANSWER SHEET Question 4.6**.

Put one small drop (10µl) of the diluted beef blood on the microscope slide, 1 cm from the edge, and smear with an oblique cover slip, without pressure. (same procedure as on see Fig.4.1).

Follow the next steps (same procedure as on Fig. 4.2)

1. Put your specimen in the small Petri dish with Hemacolor I solution **(S1)**
2. Take your specimen with tweezers and dip it 5 times briefly into the solution **(S1)**.
3. Use tweezers to transfer your specimen to the second Petri dish with Hemacolor II solution **(S2)**.
4. Take your specimen with tweezers and dip it 4 times briefly into the solution **(S2)**.
5. Use tweezers to transfer your specimen to the third Petri dish with Hemacolor III solution **(S3)**.
6. Take your specimen with tweezers and dip it 4 times briefly into the solution **(S3)**.
7. Lift your specimen with tweezers up, rinse with distilled water, drip off on filter paper, let it dry.

The slide you just prepared will be referred to as **preparation 2.**

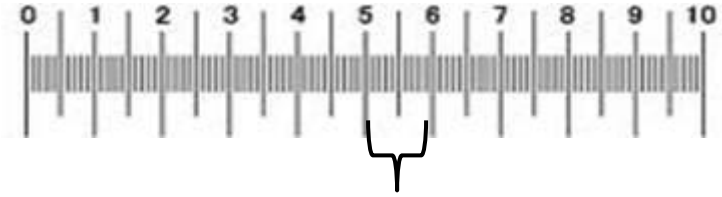
This preparation will be needed at question (4.12)

☐ **Question 4.7.:** One of the three solutions is a hypertonic solution.

Dilute the beef blood 100X with this hypertonic solution to get a volume of 2 ml.

Observe under the microscope. Make a biological drawing of a red blood cell. Label the different cell components using the letter corresponding for the correct scientific annotations provided on the **ANSWER SHEET Question 4.7.**!

In the ocular is a graduated scale with 10 large units, respectively 100 small units (Fig. 4.4).



1 large unit = 10 small units

Fig. 4.4

The table below indicates the real size of a small unit.

Magnitude	**Meaning of a small unit**
100X	10 µm
200X	5 µm
400X	2,5 µm

☐ **Question 4.8.:** Determine the diameter of the red blood cell observed under 4.7 by calculating the average of 3 measured diameter of different red blood cells from the preparation 4.7. Write your answers on the **ANSWER SHEET Question 4.8**.

☐ **Question 4.9.: Theoretical question:** The beef blood is put in 3 different test tubes with the 3 solutions A, B and C. After centrifugation by 200 x g for 8 min at 4°C, what would these test tubes look like. Draw and label the expected results in the test tubes on the **ANSWER SHEET Question 4.9**.

☐ **Question 4.10.:** Observe **preparation 1** under the microscope. Make a drawing of a red blood cell. Label the different cell components using the letter corresponding for the correct scientific annotations provided on the **ANSWER SHEET Question 4.10**!

☐ **Question 4.11.:** Measure the diameter of 3 red erythrocytes. Determine the average value of the diameter of an erythrocyte! Write your answers on the **ANSWER SHEET Question 4.11**.

☐ **Question 4.12.:** Observe **preparation 2** under the microscope. Make a drawing of a red blood cell. Label the different cell components using the letter corresponding for the correct scientific annotations provided on the **ANSWER SHEET Question 4.12**!

☐ **Question 4.13.:** Measure the diameter of 3 red erythrocytes. Determine the average value for an erythrocyte! Write your answers on the **ANSWER SHEET Question 4.13**.

☐ **Question 4.14.:** The isotonic solution has a concentration of 9g/L NaCl. Knowing the molecular masses of Na = 22.99 g/mol and Cl = 35.45 g/mol, determine how many moles of sodium (Na) and chlorine (Cl) ions are required to prepare 100 mL of a 9g/L isotonic NaCl (sodium chloride) solution? Detail your calculations on the **ANSWER SHEET Question 4.14**.

Problem 5: Biology (Evolution) (23P.)

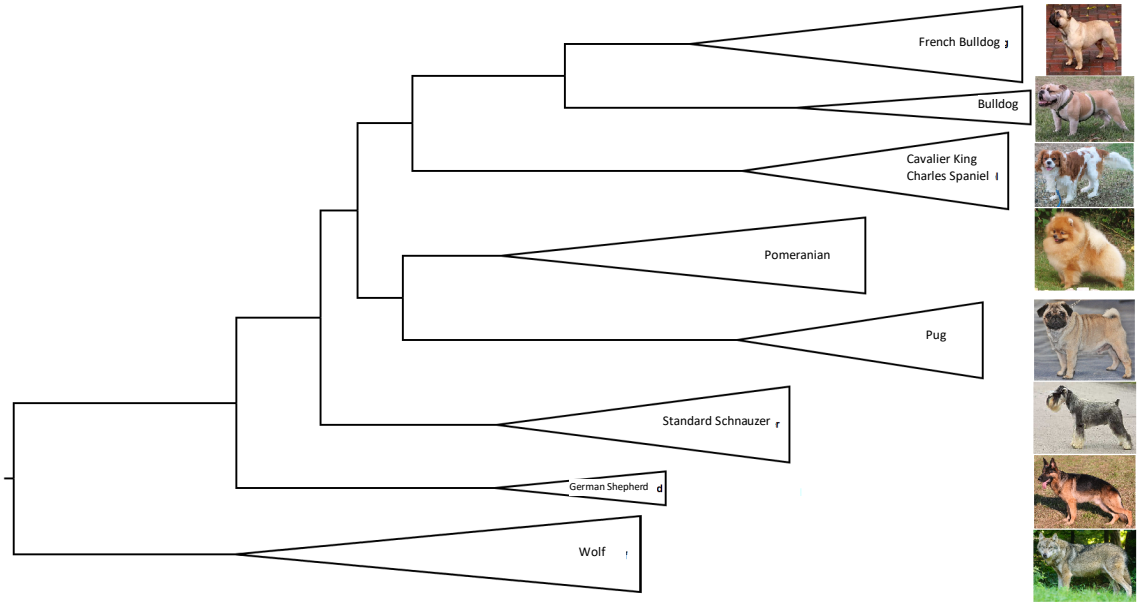
The classical dish at the “Schueberfouer”: “*gebaakene Fësch*”. As it tastes good and the food is tradition, nobody cares about the correct denomination! But, as a person interested in biology, you could ask yourself:
Is the word “fish” a term correctly used when you’re a biologist?

In the next hours, you’re about to perform some experiments that will help you to a correct classification of the animals you formerly knew as “fish”.

A) Evolution

The term “fish” stands for all aquatic, craniate gill-bearing animal that lacks limbs with digits. But in fact, some of the animals included in this “fish” group evolved differently.
In biology, often the phylogenetics is used to correctly classify living organism. In fact, this is the study of the evolutionary history and relationships among or within groups of organisms. The relationships between the organisms are determined for example by DNA sequences, amino acid sequences or morphology.
As a result of this analysis, the organisms are represented in a phylogenetic tree.

Below, you can see an example of such a phylogenetic classification, by the example of some dog breeds. This sort of representation is called a dendrogram or cladogram.



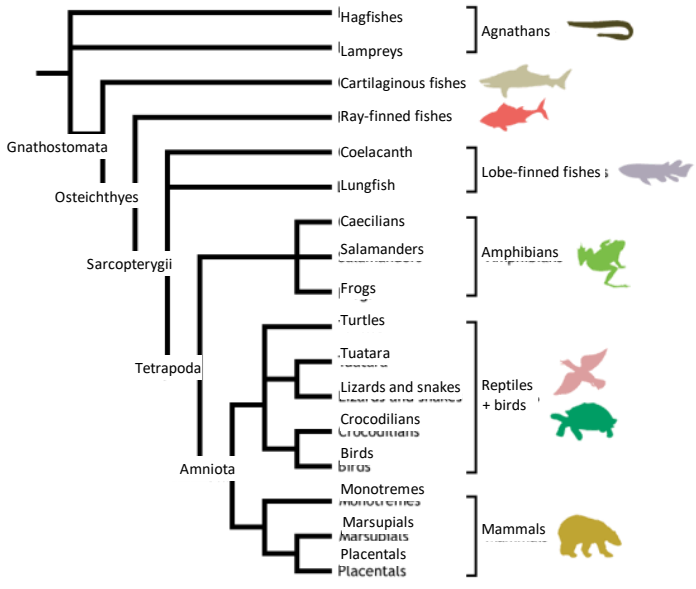
https://en.wikipedia.org/wiki/Phylogenetic_tree

A cladogram shows relations among organisms, it does show how ancestors are related to descendants. The cladogram uses lines, that branch off in different directions, ending in a clade.

A clade is a group of organisms with a last common ancestor.

The branching off points represent a hypothetical last common ancestor. This hypothetical organism would exhibit all the traits shared among the terminal taxa above it. It also would provide clues about the order of evolution of various features, adaptation, and other evolutionary facts about ancestors.

The more traits the organism share, the more related they are to each other.



https://www.ucl.ac.uk/museums-static/obl4he/vertebratediversity/

Above, you can see a simplified phylogenetic cladogram of the major vertebrate clades.

☐ **Question 5.1**: Analyze the statements in **Question 5.1.** on the **ANSWER SHEET** and decide whether these are **true** or **false**. Tick (✓) the correct answers.

a. Crocodilians are more related to birds than they are to lizards.
b. Frogs and turtles share a common trait, the amnios.
c. All the organisms commonly known as fish share the same clade.
d. Lungfish are more related to mammals than they are to ray-finned fishes.
e. Hagfishes and Lampreys share the trait of being jawless.
f. Turtles and birds share one common hypothetical ancestor.
g. Based on the phylogenetic cladogram above, salamanders share more common traits with lungfish than with lizards.

B) <u>Practical work</u>

Your practical work will consist in examining the scales of different species of fish.

Before starting, it is important to understand what the scales are.

☐ **Question 5.2**: Analyze the statements in **Question 5.2.** on the **ANSWER SHEET** and decide whether these are **true** or **false**. Tick (✓) the correct answers.

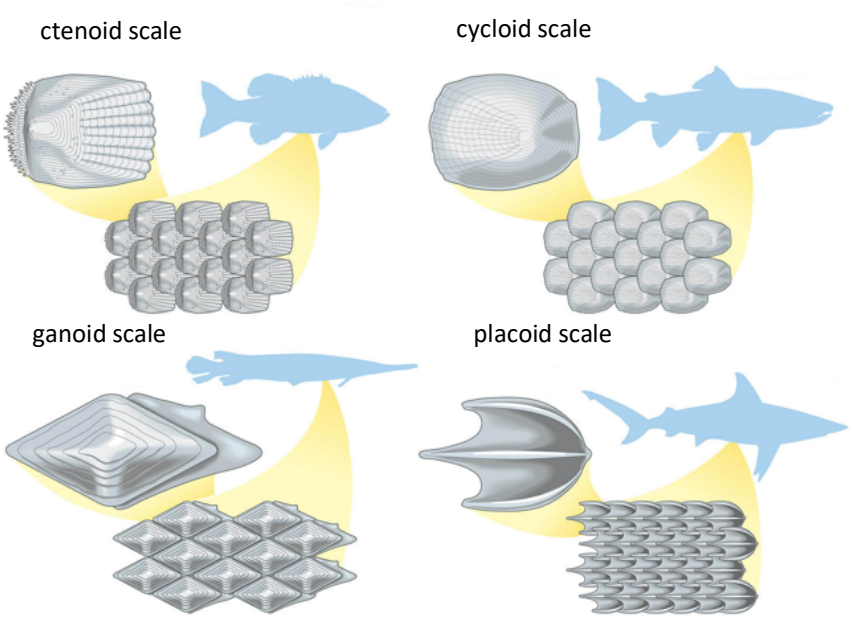
a. A fish scale is a small, rigid plate that grows out of the skin.
b. The scales of different fish species are very similar in material to the scales found in reptiles.
c. The scales are meant to protect the fish's body from injuries.
d. The scales can provide an advantage in camouflage.
e. Fish scales are produced from the mesoderm of dermis.
f. One species of fish can present different types/shapes of scales, according to the part of the body that is considered.
g. The same genes involved in tooth and hair development in mammals are also involved in scale development.
h. The morphology of a scale can help to identify the species of fish.

Now you start your practical work!

You find in front of yourself the skin of a salmon (marked “A”), the skin of a ray (marked “B”), and the skin of a sea bass (marked “C”).

Your task will consist in classifying the three species into different groups, considering the form and aspect of their scales.

Figure: Schematic view of 4 different types of fishscales that can be found in fish



https://www.aquaportail.com/dictionnaire/definition/3061/ecailles-placoides

You will have to prepare/mount a microscopic sample of the scales of each sample of fish skin in front of you.

B.1) Preparation of the microscopic sample of the ray scale (skin B)

Materials:

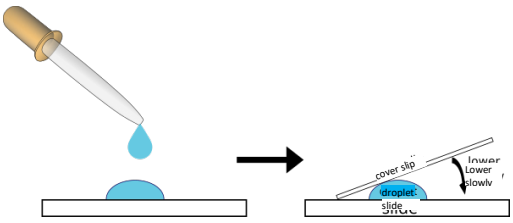
- Fish skin with fish scales (in Petridish labeled “B”)
- Binocular
- Dissection tray (same as used for problem 4)
- Scissors
- Test tube
- Tweezers (same as used for problem 4)

- 1 candle
- 1 beaker (labeled trash)
- 1 test tube
- Solution of 10% KOH labeled “10% KOH”
- 1 watch glass
- Solution of Alizarine Red (for staining) labeled “Alizarine Red”
- Diluted Glycerin (1:1) labeled “Diluted Glycerin”
- Distilled water
- Microscope slide
- Cover glass
- Paintbrush
- Permanent marker
- Safety goggles

Preparation:

1. Cut a small piece of the skin (of the fish) with the scissors (approximately 2 cm x 2 cm). You have to take a part where the skin feels rough when you touch it.
2. Remove (if necessary), with the forceps, all the muscles attached to the interior face of the skin.
3. Put the skin in a test tube and add approximately 4 mL of the 10% KOH solution. (the whole sample must be covered in the solution)
4. **YOU HAVE TO PUT ON THE SAFETY GOGGLES AT THIS POINT!** Boil the skin in the test tube over the flame of the candle until the skin becomes brown.
   The boiling in the KOH solution will detach the scales from the skin.
   Note: Only heat the upper part of the solution; if the KOH solution heats too quickly, it will splash out of the test tube!

5. Let the residue (brown) settle at the bottom of the tube.
6. Remove the KOH solution slowly into a beaker (beaker labeled “**trash”**).
7. Add water to the tube to wash the scales; remove the water into the beaker (“trash”).
   Repeat 5 times.
   Note: Be careful not to remove the scales at the bottom of the tube.
8. After the washing, pour the scales onto a watch glass. You may use the paintbrush to do so.

9. Stain the scales with 2 drops of Alizarin Red solution for **5 minutes**. If the Alizarin Red solution shows a sediment in the bottle, you have to shake it very gently.
10. With the paintbrush, take the scales out of the solution and place them on a microscope slide.
11. Add 2 drops of diluted glycerin on the scales on your slide.
12. Mount carefully with a cover glass. Pay attention to place the cover glass carefully. Lower the cover glass and avoid forming bubbles.
13. 

14. Observe under the binocular!
15. When you find the correct magnitude and the scale is observable, call a supervisor to verify your preparation. If the magnification and the coloration are good, it will be validated on the **ANSWER SHEET Question 5.3.** with a stamp.

☐ **Question 5.3.:**

- On the ANSWER SHEET, you'll find the microscopic pictures of 3 different scales. Choose the one you observe (by ticking the right box) and label it using the pictures in the **APPENDIX after QUESTION 5.6.** and the letters in the table on the **ANSWER SHEET at Question 5.3.!**
- By comparing with the schematic scales (*Figure: Schematic view of 4 different types of fishscales that can be found in fish)*, you can determine what kind of scale you find on the skin of rays. Write your answer (**just the corresponding letter**) on the **ANSWER SHEET Question 5.3**. ("**Type of scale**" in the table).

B.2 Preparation of the scales of the salmon (skin A) and the sea bass (skin C)

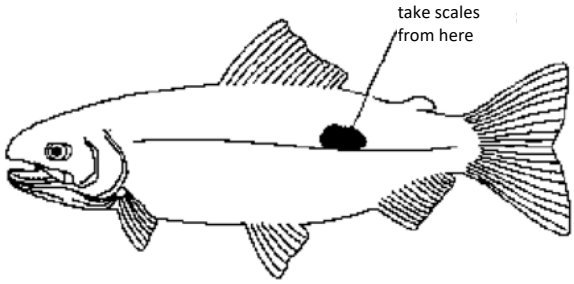
Material (needed for the preparation of the scales of 1 species!)

- Fish skin with fish scales (in Petri dish labeled "A" and "C")
- Binocular
- Microscope
- Dissection tray
- Forceps
- Solution of 10% KOH labeled "10% KOH"

- 2 watch glasses (you use the same for skin A and skin C)
- Solution of Alizarine Red (for staining) labeled “Alizarine Red”
- Diluted glycerin (1:1) labeled “Glycerin”
- Distilled water
- Paintbrush for manipulating the scales
- Microscope slide
- Cover glass
- Permanent marker

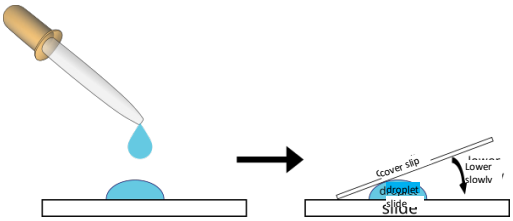
Preparation

1. With the forceps, remove some scales (3-4) from the skin of the fish. The skin that is provided to you comes from the marked area.



2. Put the scales removed in a watch glass and add 4 mL of KOH solution.
3. Leave the scales in the KOH solution for 5 minutes.
4. Put the scales onto another watch glass and add distilled water to wash the scales using a paintbrush.
5. Drain out the water of the watch glass into a beaker (trash).
6. Repeat the washing five times.
7. Drain the water out of the watch glass and stain the scales with Alizarin Red solution by adding 2 drops to the watch glass. If the Alizarin Red solution shows a precipitate in the bottle, you have to gently move it in a circular motion.
8. Let the colorant work for 5 minutes.
9. Using the paintbrush, take the scales out of the watch glass and place them onto a microscope slide.

10. Add 2 drops of dilute glycerin on the scale on your slide.
11. Mount carefully with a cover glass. Pay attention to place the cover glass carefully to avoid bubbles.



12. Observe under the binocular and under the microscope!
13. When you find the correct magnification and the scale is observable, come with your preparation and your **ANSWER SHEET Question 5.4**. to the shared bench, so that a supervisor can validate your preparation and take a picture. You'll have to tell the supervisor what magnification to use.

☐ **Question 5.4.:**

- A picture of your preparation is taken by one of the supervisors and you continue by making a precise drawing (on the **ANSWER SHEET Question 5.4.**) of the scale of the salmon observed. You'll have to label your drawing by using the letters from the **ANSWER SHEET Question 5.4**!
- For your drawing (scale of the salmon) the magnification you used for the observation has to be marked.
- By comparing with the schematic scales (*Document: Schematic view of 4 different types of fishscales that can be found in fish)*, you can determine what kind of scale you find on the skin of salmon. Write your answer (**just the corresponding letter**) on the **ANSWER SHEET Question 5.4**. ("**Type of scale**" in the table).

☐ **Question 5.5.: Repeat the same procedure you did for preparing the scales of the salmon for the sea bass skin!**

Procedure

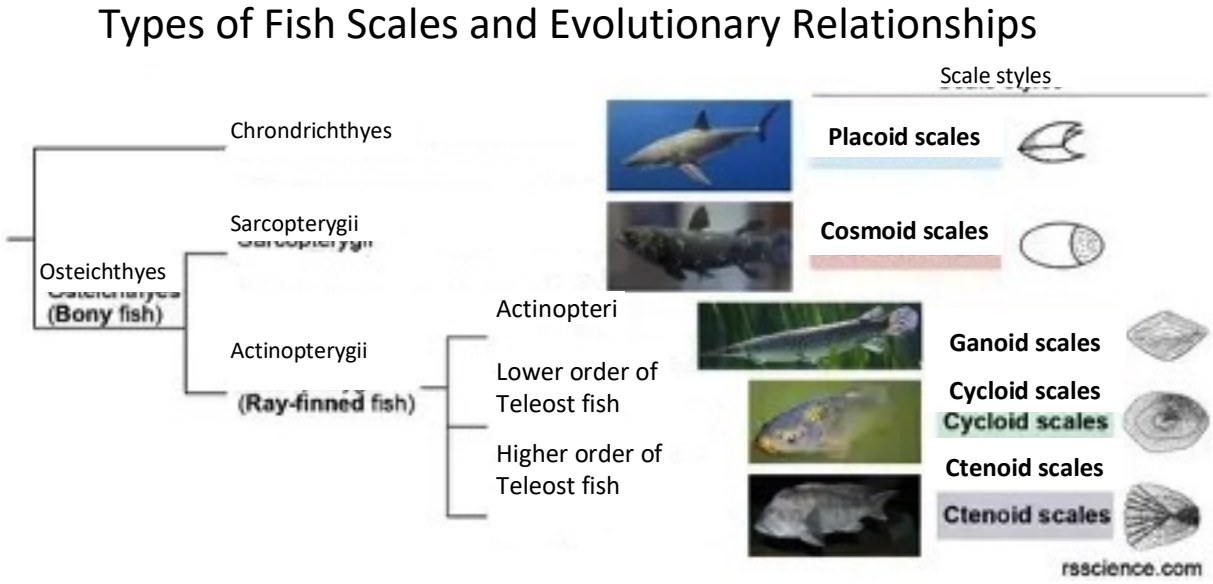
- Observe under the binocular or microscope!
- When you find the correct magnification and the scale is observable, call a supervisor to verify your preparation. If the magnification and the coloration are good, it will be validated on your **ANSWER SHEET Question 5.5.** with a stamp.
- On the **ANSWER SHEET Question 5.5.**, you'll find 3 different scales. Choose the one you observe (by ticking the right circle) and label it using the pictures in the **APPENDIX after QUESTION 5.6.** and letters in the table on the **ANSWER SHEET Question 5.5.**!

- By comparing with the schematic scales (*Document: Schematic view of 4 different types of fishscales that can be found in fish)*, you can determine what kind of scale you find on the skin of the sea bass. Write your answer (**just the corresponding letter**) on the **ANSWER SHEET Question 5.5.**. ("**Type of scale**" in the table).

Keep your preparations! Mark them so that you know, to which species they belong.

Classify the three species of fish to the different classes, using this simplified phylogenetic representation!

☐ **Question 5.6.: Classify the three species of fish observed, based on their scales!**

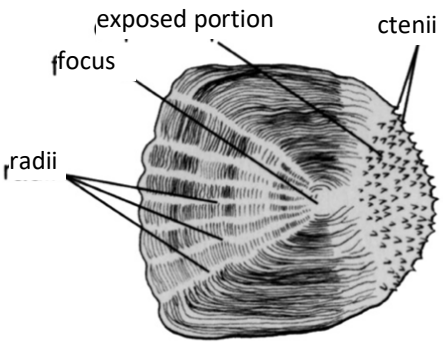


https://rsscience.com/fish-biology-and-fish-scales-under-the-microscope/

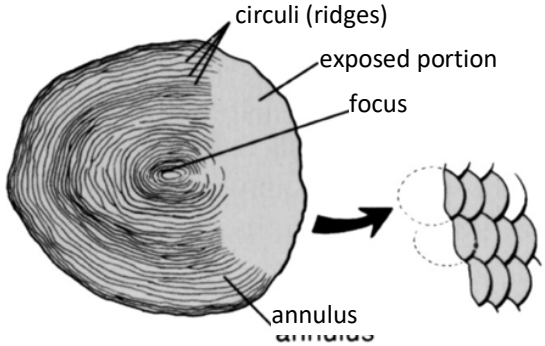
APPENDIX

Pictures for labelling

Ctenoid

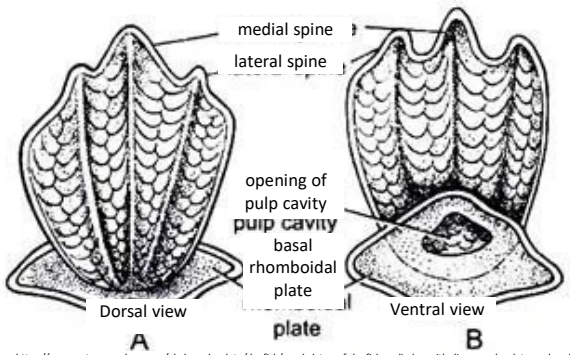


Cycloid



https://quizlet.com/93445240/vert-lab-quiz-2-fishes-flash-cards/

Placoid



https://www.notesonzoology.com/phylum-chordata/dogfish/exoskeleton-of-dogfish-scoliodon-with-diagram-chordata-zoology/7537

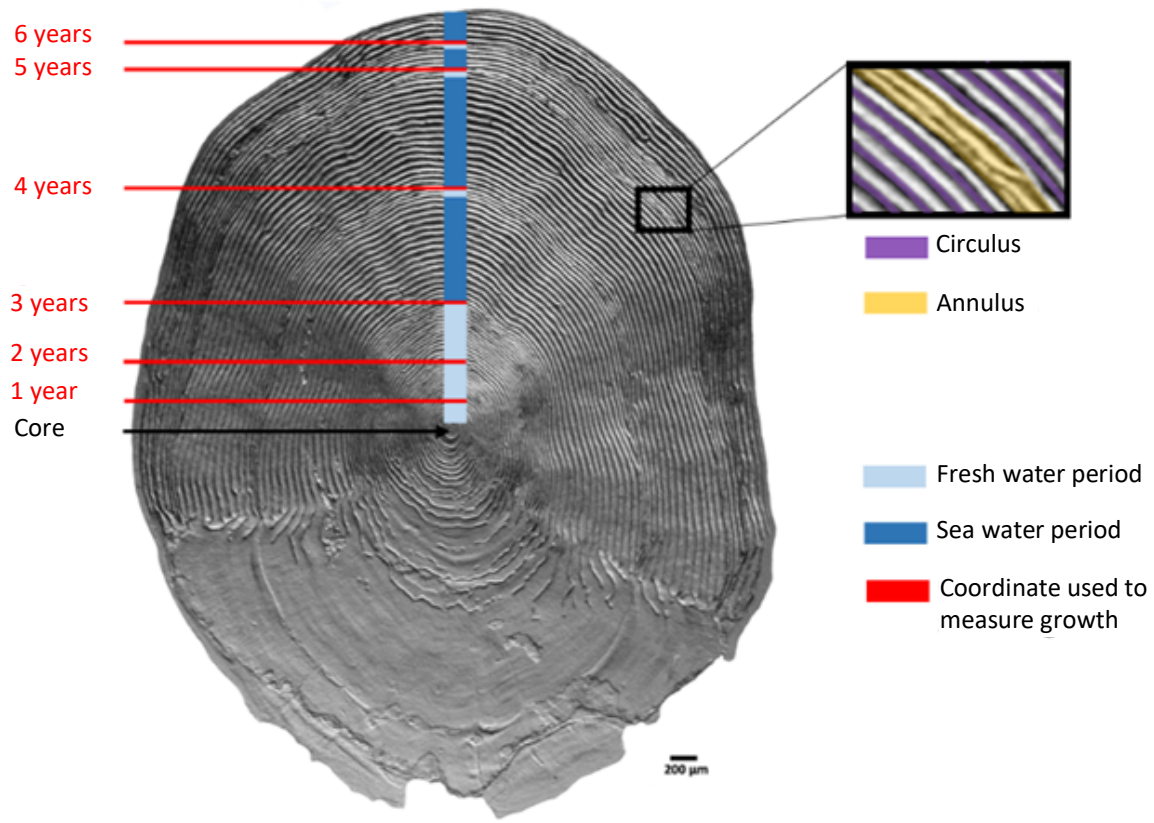
C. Age determination of one species of fish

The different types and morphology of the fish scales can not only be used to classify the different species of fish, but it is also possible to estimate the age by analyzing the ctenoid or the cycloid scales.

Most fish have the same number of scales throughout their entire lives and the scales continue to grow with the fish. Fish do most of their growth throughout the warmer months when food is plentiful, so their scales grow quickly, creating rings that are spaced-out; however, they grow slower when temperatures drop in the winter, so rings developed at this time are close together. These dark, winter rings are called the “annuli” and it is by counting the yearly annuli

that you can estimate their age. Sometimes, growth rings can also differentiate when a fish moves from freshwater to saltwater, such as the scale of this sea-run trout.

Beneath, you find an example of a cycloid scale used for determining the age of the fish.



https://www.facebook.com/photo/?fbid=5703192819693358 (trout unlimited Canada)

☐ **Question 5.7. Your task is to determine the age of the fish you identified, having cycloid scales!**

Add all the necessary explanations to the drawing you realized before only of this specific scale (cycloid scale). Write the determined age on the **ANSWER SHEET Question 5.7**.

You can also realize a new drawing of the mentioned scale if you prefer. (**ANSWER SHEET Question 5.7.**)

End of problem 5:

Check the following bullet points, to make sure that you fulfilled everything:

- You have realized three preparations of the scales of the three species of fish.
- A picture has been taken by a teacher of the preparation you made of the scale of the salmon.
- You realized one drawing of the microscopic/binocular mount of the fish scales you made for the salmon scale, using a microscope or a binocular.
- You classified the three species of fish
- You determined the age of the fish with the cycloid scale.