EUROPEAN OLYMPIAD OF EXPERIMENTAL SCIENCE

2024 LUXEMBOURG

TASK 1

CANCER

Duration: 4 hours

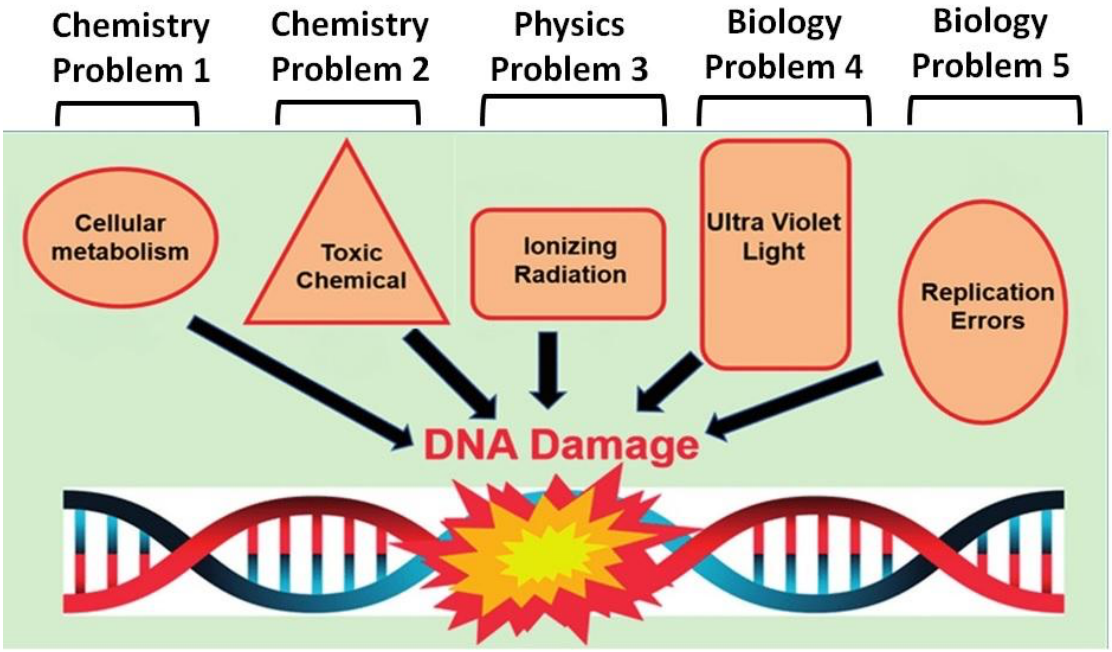
EOES 2024, 09.04.2024

Introduction to the task:

Cancer is a genetic disease which is characterised by an uncontrolled division and growth of abnormal cells. It can start in any of the many tissues in our body and can later spread to other tissues. The deregulation of cellular functions is often based on genetic modifications which can be inherited or acquired over time.

In our daily life, we are exposed to many factors which can affect our genome by damaging the DNA. These are called genotoxic agents. Examples of physical agents are asbestos, ultraviolet (UV) light or ionizing radiation such as radioactive radiation. Such agents can act via different mechanisms but very often, they lead to the formation of highly reactive free radicals which react with DNA. Carcinogenic chemical agents can also act via different mechanisms, but many directly bind to DNA and thereby modify cellular functions. Fortunately, our body possesses mechanisms that can correct DNA damage or remove abnormal cells. However, sometimes, damaged cells can survive and develop into cancer cells over time.

Today, you will help us to fight cancer by investigating some chemical and physical factors which can induce DNA damage and by studying biological effects of UV light.



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

Problem 1 - Chemistry (quantification of Fe^{2+} ions) - 1.5 hours

Problem 2 - Chemistry (carcinogen chaos) - 1.5 hours

Problem 3 - Physics (ionizing radiation) - 3.5 hours

Problem 4 - Biology (effect of UV light on cell growth) - 1.5 hours

Problem 5 - Biology (effect of UV light on genetic material) - 2 hours

TASK1: CANCER

Problem 1 – Quantification of Fe^{2+} ions (27 points)

Individual materials and equipment

- **1 × Spectrophotometer** (different models, operation instructions on site)
- **1 × plastic rack with three 50 mL tubes** with solutions A and B (labelled "A" and "B")), and *o*-phenanthroline solution (0.5 % in ethanol) (labelled "OP")
- **1 × 100 mL glass bottle** with acetate buffer (CH_3COOH/CH_3COONH_4) solution, pH = 5 (labelled "buffer")
- **1 × 250 mL volumetric flask** containing 100 mL of an acidified solution to yield 250 mL of sample F1 (labelled "F1")
- **1 × 100 mL volumetric flask** for the preparation of diluted solution F2
- **1 × 50 mL volumetric flask** for the preparation of diluted solution F3
- **2 × 100 mL beaker** (for solution F3 and H_2O)
- **1 × 10 mL graduated pipette** (for F1)
- **5 × 5 mL graduated pipette** (for A, B, H_2O, F2 and *o*-phenanthroline solution)
- **1 × 25 mL volumetric pipette** (for buffer solution)
- **1 × 5 mL volumetric pipette** (for buffer solution)
- **1 × 1 mL volumetric pipette** (for the *o*-phenanthroline solution)
- **1 × pipetting aid** (Brand™)
- **1 × wooden test tube rack** with **8 test tubes**
- **8 pieces of Parafilm™** to close the test tubes.
- **1 × plastic cuvette rack** with **9 macro cuvettes** (b = 1 cm, see *Figure 1.1*)
- **1 × spray bottle** with demineralized H_2O (also for Problem 2)
- **1 × 1L glass bottle** with demineralized H_2O (also for Problem 2)
- **Graph paper (mm paper)**
- **calculator**
- **set square** (triangle)
- **permanent marker for the glassware** (also for Problem 2)

Introduction:

Iron metabolism is implicated in multiple cancer types and elevated concentrations of Fe^{2+} ions are found in cancer cells if compared to normal cells. For example, it is thought that Fe^{2+} ions contribute to the carcinogenic mechanism by which asbestos can induce a type of cancer called mesothelioma. In the form of the Fe^{2+} ion, iron can induce the formation of reactive oxygen species (ROS) via a reaction called Fenton reaction. ROS are mostly reactive radicals which can induce DNA damage by oxidising or breaking DNA strands. Fe^{2+} ions can also trigger other carcinogenic mechanisms. However, scientists are currently also researching how the elevated ion concentrations could be used in cancer treatment, as Fe^{2+} is also able to induce a special kind of cell death called ferroptosis. Triggering ferroptosis in abnormal cells with higher Fe^{2+} levels could thus be used to destroy these cells.

In this first problem, you are investigating the concentration of Fe^{2+} ions in aqueous solution.

Interaction between matter and light

Many experimental methods for qualitative and quantitative investigation of the structure of matter are based on the interaction of it with electromagnetic radiation.

In the electromagnetic wave range from the near-ultraviolet (UV, approx. 300 nm) to the near-infrared (IR, approx. 850 nm), the interaction of radiation with molecules or ions in solution can cause transitions between quantified energy levels through absorption. This interaction depends, among other things, on the structure of the chemical entity concerned.

The term absorptiometry is used to describe a general method based on the measurement of the proportion of light energy absorbed at a particular wavelength (or at all wavelengths in a range) by a transparent solution. Absorptiometry has come to play an important role in quantitative chemical and biological analysis. Techniques using light absorption are colorimetry and spectrophotometry.

To describe the absorbing properties of a chemical species, we use (as a fingerprint) its absorption spectrum, i.e. a graph of a function of the attenuation of a beam of radiation as a function of wavelength, frequency or wavenumber. Two terms are commonly used to measure this attenuation: **transmittance** (***T***) and **absorbance** (***A***). **Figure 1.1** provides a schematic representation of the fundamental parameters in the case of light absorption. A parallel beam of light passes through a layer of solution of **thickness *b*** and **concentration *c*** of an absorbing species. As a result of interactions between photons and absorbing particles, the **intensity** of the beam is attenuated from I_0 to I.

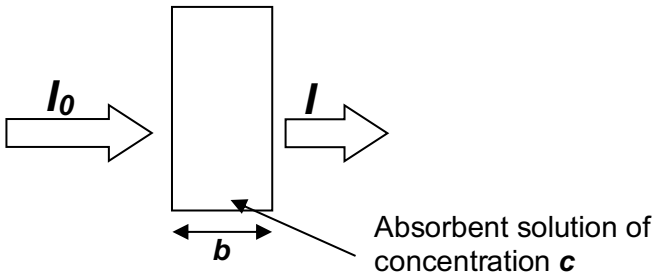


Figure 1.1: Attenuation of a beam by an absorbent solution

The **transmittance** ***T*** of the medium is defined as the fraction of incident radiation that is transmitted by the medium:

$$T = I/I_0 \qquad (1)$$

The transmittance *T* can be converted into the **absorbance** ***A***. The exact conversion formula is irrelevant for the solution of this problem.

Lambert-Beer's law

The **absorbance** ***A*** of monochromatic radiation is directly proportional to the absorption path length ("optical path length") ***b*** in the medium and the molar concentration ***c*** of the absorbing species (see **Figure 1.1**). The relationship is:

$$A = \varepsilon \cdot b \cdot c \qquad (2)$$

c: concentration in mol•L^{-1}

b: absorption path length in cm

ε: molar extinction coefficient in L•cm^{-1}•mol^{-1}

The Lambert-Beer law also works using mass concentration (β) and the mass extinction coefficient ε_m !

Some examples of related values of A and T:

If $A = 0$; $T = 100$ %: no absorption

If $A = 1$; $T = 10$ %: 90 % absorption of I_0

If $A = 2$; $T = 1$ %: 99 % absorption of I_0

You are supposed to discard values $A > 2$ for your calculations !

Practical aspects of the colorimetric technique and application in this task

Before a chemical substance can be colorimetrically assayed, it must in most cases be transformed into a coloured substance, using a reaction that must be highly specific, rapid, stoichiometric and give rise to a stable colour (long enough for reliable analysis!).

Your task will be the determination of an unknown Fe^{2+}-ion concentration in a given solution. The characteristic colour of this metal cation is much too weak to be used colorimetrically. So, the ions are quantitatively converted into a red complex with phenanthroline:

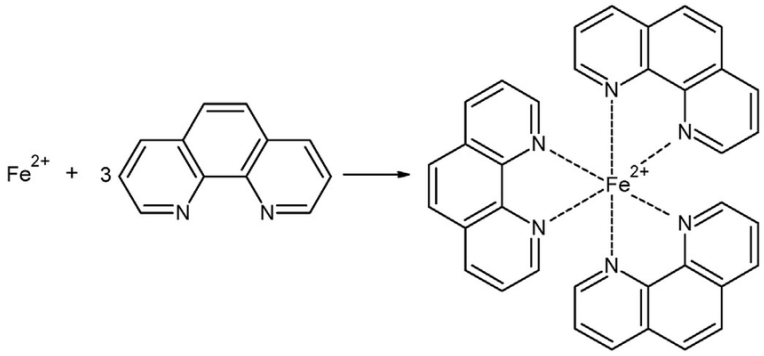


Figure 1.2: Formation of the red iron(II)-phenanthroline complex

(W. Kiciński in *Carbon* 168(1), Licence CC BY 4.0)

At a wavelength of 508 nm (visible green light) our chemical system exhibits maximum absorption. Due to technical reasons, we will use a wavelength of 492 nm in this task. The observed colour of the complex is red, the complementary colour of the absorbed green light.

As the Lambert-Beer law shows, concentration determination can be carried out by measuring ***A*** while knowing ε and ***b***. In practice, a calibration curve is established with known concentrations. This is what you will do during the first step of this task.

Procedure

Gloves, goggles and a lab coat are mandatory during this task! Gloves must be changed if contaminated.

Step 1: Preparation of the calibration samples.

Using solutions A ($\beta(Fe^{2+})$ = 100 mg/L) and B ($\beta(Fe^{2+})$ = 5.00 mg/L), prepare **10.0 mL each** of 8 solutions of given final Fe^{2+} concentrations $\beta(Fe^{2+})$ according to the following table (to be completed in the ANSWER SHEET: **Table 1.1.1.**): *(the method is explained below table 1.1.1.)*

$\beta(Fe^{2+})$ (mg/L)	V(A) (mL)	V(B) (mL)	$V(H_2O)$ (mL)	V(buffer) (mL)	V(*o*-phenanthroline) (mL)
12.0	?		?	5.00	1.00
10.0	?		?	5.00	1.00
5.00	?		?	5.00	1.00
1.50		?	?	5.00	1.00
1.00		?	?	5.00	1.00
0.500		?	?	5.00	1.00
0.250		?	?	5.00	1.00
0		?	?	5.00	1.00

☐ Detail your calculations **only for the first line** in the ANSWER SHEET below **table 1.1.1.**!

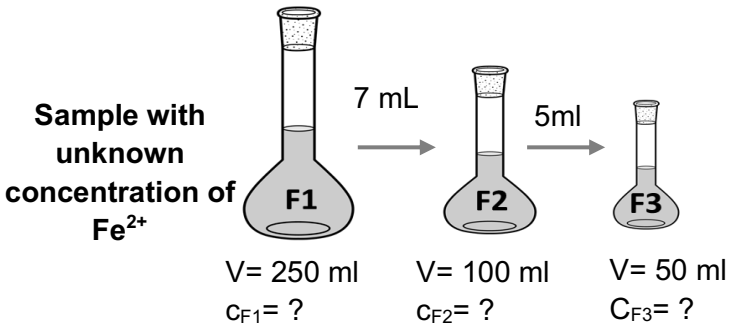
Method:

1. Pipette the calculated volume of water into a test tube using a 5 mL graduated pipette.
2. Add the calculated volume of either solution A or B to the water using a 5 mL graduated pipette.
3. Add the buffer solution using the 5 mL volumetric pipette.

Repeat steps 1 to 3 for the other 7 solutions.

4. Before finalising the calibration samples, dilute the unknown sample F1 to F3 as shown on the following scheme:

   Note: In order to prepare the solution F3 from F2, start by adding 25 mL of the buffer solution and 5 mL of the *o*-phenanthroline solution to the 50-mL volumetric flask F3. Continue the preparation of F3 as shown on the scheme.



5. To finalise the calibration samples, add the *o*-phenanthroline solution to each of the 8 calibration solutions using the 1 mL volumetric pipette.
6. Remove the protective foil from a piece of Parafilm™ and close the test tubes with the Parafilm™ to mix the final solutions by inverting the tubes a few times to homogenise them (make sure to well fix the Parafilm™ with your finger!).

Step 2: Colorimetric determination of the concentration of an Fe^{2+} solution

Measurements:

1. Transfer a part of each of the 8 calibration solutions to individual cuvettes. Each cuvette must be filled to 1/3 at least for correct measurement.
2. Measure the absorbance at λ = 492 nm of the 8 solutions for the calibration curve using the photometer *(operation instructions will be found next to the photometer)*, starting with the blank (β (Fe^{2+}) = 0 mg/L).

☐ **Table 1.2.1.**: Fill in the measured values in **table 1.2.1.** in the ANSWER SHEET!

3. Transfer a part of the unknown sample solution F3 to a cuvette. The cuvette must be filled to 1/3 at least for correct measurement. The width of the cuvette is 1 cm.
4. Measure the absorbance at λ = 492 nm.

☐ **Question 1.2.2.**: Indicate the measured value in the ANSWER SHEET **Question 1.2.2**.

Evaluation:

☐ **Graph 1.2.3.**: Draw a calibration graph (plot the absorbance against the mass concentration) on graph paper → ANSWER SHEET (**Graph *1.2.3***). Label the graph paper using the corresponding sticker (**Graph 1.2.3**)!

☐ **Question 1.2.4.**: Determine the mass extinction coefficient (ε_m) from the graph (1.2.3.), using the Lambert-Beer law, and calculate the molar extinction coefficient (ε) (M(Fe)=55.85 g/mol). Answer the question in the ANSWER SHEET **Question 1.2.4.**

☐ **Question 1.2.5.**: Also calculate the molar extinction coefficient from one of your measured values (Table 1.2.1), again using the Lambert-Beer Law. Answer the question in the ANSWER SHEET **Question 1.2.5**.

☐ **Question 1.2.6.**: Determine the mass concentration of the unknown sample solution F3 (β_{F3}) graphically from the calibration curve (Graph 1.2.3) and calculate its molar concentration (c_{F3}). Answer the question in the ANSWER SHEET **Question 1.2.6**.

☐ **Question 1.2.7.:** Calculate the molar concentration of the unknown sample solution F3 (c_{F3}) using the molar extinction coefficient. Answer the question in the ANSWER SHEET **Question 1.2.7**.

☐ **Question 1.2.8.:** Calculate the corresponding molar concentrations c_{F2} and c_{F1} of the solutions F2 and F1. **Question 1.2.8.:** Answer the question in the ANSWER SHEET **Question 1.2.8**.

Problem 2 – Solve the carcinogen chaos (23 points)

Individual materials and equipment

General use

- **2 × wooden test tube rack** with 20 test tubes.
- **4 × 250 mL beaker** (to collect the finished reactions; to be removed continuously by supervisors to limit exposure)
- **1 × magnetic stirrer/heater** (for Fehling test)
- **1 × 400 mL beaker** filled with 200 mL of water (water bath for the Fehling test)
- **1 × thermometer** (already inserted into the water bath)
- **3 × glass Pasteur pipette** (for unknown liquids) **with bulbs**
- **1 × spatula** (for unknown solid)
- **3 × 200 mL Erlenmeyer flask** (to "park" the Pasteur pipettes for the unknown liquids)
- **1 × 100 mL Erlenmeyer flask** (to "park" the spatula for the unknown solid)
- **1 × 1000 mL Beaker** (to dispose of the used Pasteur pipettes, spatulas and pipettes; labelled "Waste")
- **4 × glass vials with "unknown" substances** labelled "X1", "X2", "X3" and "X4".
- **1 × 100 mL beaker** (for demineralized water)
- **1 × 5 mL graduated plastic pipette** (for demineralized water)
- **1 × pipetting aid** (Brand™) (also for Problem 1)
- **1 × large beaker containing five 15 mL Falcon tubes** with the reagents:
  - "CAN" (40 % ceric ammonium nitrate; 7% HNO_3),
  - "$FeCl_3$" (1 % $FeCl_3$; 0.1% HCl),
  - "Brady" (13% sulfuric acidic ethanol solution of 2,4-dinitrophenylhydrazine (2,4-DNPH) (3%)),
  - "Fehling I" (7% $CuSO_4$)
  - "Fehling II" (alkaline (10% NaOH) potassium sodium tartrate (35%))
  - **and an empty 50 mL Falcon tube** to prepare the final Fehling reagent.
- **1 × plastic multi-rack** (also used for Problem 1) to hold the falcon tubes when pipetting the reagents.
- **1 × spray bottle** with demineralized H_2O (also for Problem 1)
- **permanent marker** (also for Problem 1)
- 1 x cotton glove (to manipulate the heated samples (Fehling))

Individual tests

1) ***CAN* test**

- **5 × test tube**
- **1 × small vial** with control substance (ethanol, labelled “EtOH”)
- **1 × Pasteur pipette** (for ethanol) (to be placed into the beaker “Waste” after usage)
- **1 × 5 mL graduated pipette** (for CAN reagent) (to be placed into the beaker “Waste” after usage)

2) **$FeCl_3$ test**

- **5 × test tube**
- **1 × small vial** with control substance (phenol, labelled “ArOH”)
- **1 × spatula** (for phenol) (to be placed into the beaker “Waste” after usage)
- **1 × Pasteur pipette** (for $FeCl_3$ solution) (to be placed into the beaker “Waste” after usage)

3) ***Brady test (2,4-DNPH test)***

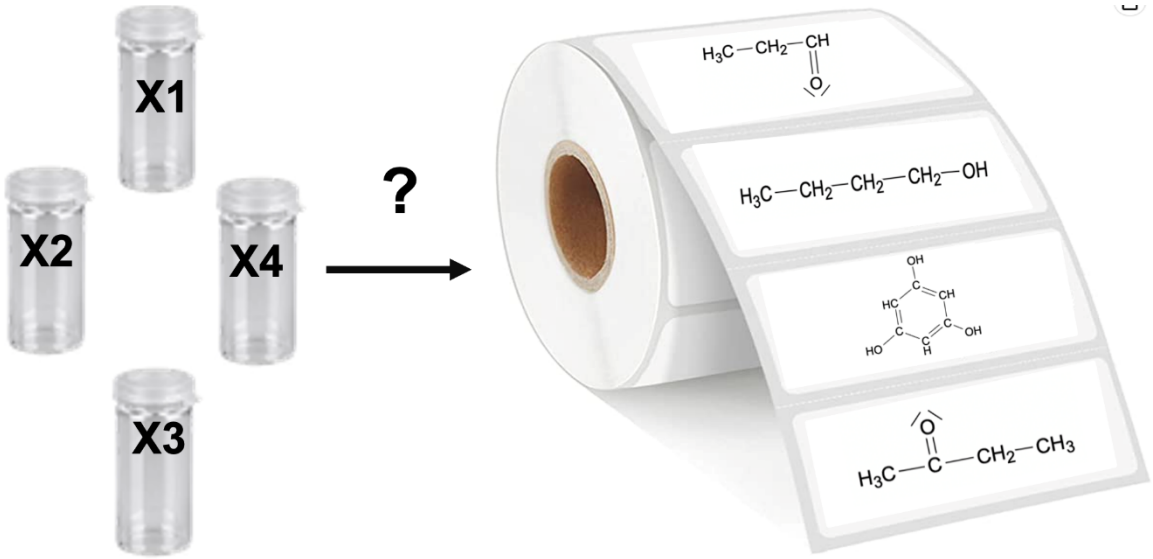
- **5 × test tube**
- **1 × small vial** with control substance (acetone, labelled “Ac”)
- **1 × Pasteur pipette** (for acetone) (to be placed into the beaker “Waste” after usage)
- **1 × 5 mL graduated pipette** (for 2,4-DNPH reagent) (to be placed into the beaker “Waste” after usage)

4) ***Fehling test***

- **5 × test tube**
- **1 × small vial** with control substance (glucose, labelled “G”)
- **1 × spatula** (for glucose) (to be placed into the beaker “Waste” after usage)
- **1 × 5 mL graduated pipette** (for Fehling reagent) (to be placed into the beaker “Waste” after usage)

Introduction

Many organic molecules have carcinogenic properties. So, when working with these substances, it is always extremely important to know what you are dealing with. Now, however, a careless lab technician did not pay attention and forgot to label his substances correctly!
Fortunately, we have some very talented young chemists visiting Luxembourg right now who can fix this mess: by carrying out characteristic test reactions, you must find out which substance is in which container.



The carbon chain of an organic molecule usually carries one or more functional groups that are responsible for its specific properties. Examples of such functional groups are:

- The **carbonyl group**, where an O-atom is attached via a double bond to a C-atom. If this C-atom is linked to alkyl radicals (noted R), it is a **ketone** (R_1-CO-R_2), if it is linked to at least one H-atom, the molecule is called an **aldehyde** (R-CHO or H-CHO).
- The **hydroxyl group**, where an OH-group is attached to a C-atom. If this C-atom belongs to an aromatic system (derived from benzene C_6H_6, noted Ar), the molecule is called a **phenol** (Ar-OH), otherwise it is an **alcohol** (R-OH).

On the following pages the test reactions for these functional groups are presented. It is up to you to draw the right conclusions!
And no fear: most evidently, the substances given to you are no carcinogenic representatives of their classes.

Approach

Each unknown substance given (X1 to X4) must be tested for the four substance classes presented. We are conducting experiments in order to identify the functional groups present in the compounds. To learn how the reagents indicate a positive test, each time a control substance is given.

General comment on the tests: Depending on the chemical compound and the quantity of the compound used for analyzation, there may be some sort of variance regarding control and test substance, but which will always clearly differ from a negative test. Evaluation of tests is completed after 10 min. The tests are the following:

- ***CAN test: test for alcohols and phenols***

Ceric ammonium nitrate (CAN) $(NH_4)_2[Ce(NO_3)_6]$ forms a red complex with alcohols and phenols, due to the exchange of a nitrate-group NO_3^- for a RO or ArO group:

$$R\text{-}OH + (NH_4)_2[Ce(NO_3)_6] \rightarrow (NH_4)_2[Ce(OR)(NO_3)_5] + HNO_3$$

$$Ar\text{-}OH + (NH_4)_2[Ce(NO_3)_6] \rightarrow (NH_4)_2[Ce(OAr)(NO_3)_5] + HNO_3$$

yellow-orange dark red/ brownish red

- ***$FeCl_3$ test: test for phenols***

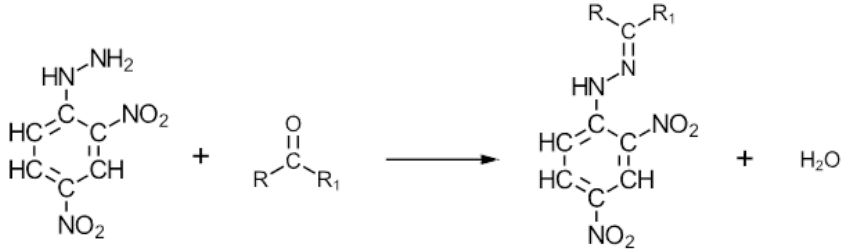
Phenols change the yellowish colour of an aqueous iron (III) chloride solution to blue, purple or lilac. This colour is due to the formation of an anionic complex in which the electrons are delocalised on both the iron atom and the unsaturated system:

$$6\ Ar\text{-}OH + FeCl_3 \rightarrow [Fe(OAr)_6]^{3-} + 3\ Cl^- + 6\ H^+$$

yellowish purple/ lilac

- ***Brady test (2,4-DNPH test): test for aldehydes and ketones***

The carbonyl group reacts with 2,4-dinitrophenylhydrazine to yield a yellow to red precipitate of 2,4-dinitrophenylhydrazone:



2,4-dinitrophenylhydrazine 2,4-dinitrophenylhydrazone (yellow to red precipitate)

- ***Fehling test: test for aldehydes***

The Fehling reagent ($[Cu(C_4H_4O_6)_2]^{2-}$) produces a brick red precipitate due to the reduction of copper(II) to copper(I) in Cu_2O:

$$R\text{-}CHO + 2\ [Cu(C_4H_4O_6)_2]^{2-} + 5\ OH^- \rightarrow R\text{-}COO^- + Cu_2O\downarrow + 3\ H_2O + 4\ C_4H_4O_6^{2-}$$

brick red precipitate

Procedure

Gloves, goggles and a lab coat are mandatory during this task! Gloves must be changed if contaminated.

1. At this point start heating the water bath to 75 °C for the realisation of the Fehling test! *(see next page → 4) Fehling test)*

1) CAN test: test for alcohols and phenols

2. Add to each test tube 1.0 mL CAN reagent using a 5 mL graduated pipette.
3. Dilute with 2.0 mL of demineralized water using the 5 mL graduated pipette.
4. Add the substance to analyse (control and unknown substances X1 to X4)
   - Either 10 drops of liquid by using a Pasteur pipette
   - Or 1 spatula tip of solid using a spatula.

☐ **Table 2.1.1.**: Note your observations in **table 2.1.1.** in the ANSWER SHEET.

2) $FeCl_3$ test: test for phenols

5. Add to each test tube 8 drops of the $FeCl_3$ solution using a Pasteur pipette.
6. Dilute with 2.0 mL of demineralized water using the 5 mL graduated pipette.
7. Add the substance to analyse (control and unknown substances X1 to X4).
   - Either 10 drops of liquid by using a Pasteur pipette
   - Or 2 spatula tips of solid using a spatula

☐ **Table 2.1.2.**: Note your observations in **table 2.1.2.** in the ANSWER SHEET.

3) Brady test (2,4-DNPH-Test): test for aldehydes and ketones

8. Add to each test tube 2,5 mL Brady reagent using a 5 mL graduated pipette.
9. Add the substance to analyse (control and unknown substances X1 to X4)
   - Either 8 drops of liquid by using a Pasteur pipette
   - Or 1 spatula tip of solid using a spatula.

☐ **Table 2.1.3.**: Note your observations in **table 2.1.3.** in the ANSWER SHEET.

*4) **Fehling test: test for aldehydes***

Use the cotton gloves when handling the heated test tubes (e.g. transferring test tubes to rack)!

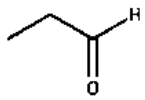
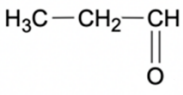
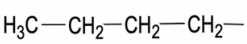
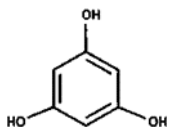
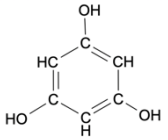
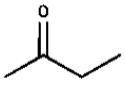
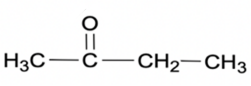
10. Heat the water bath (approx. 200 mL of water in 400 mL beaker) to 75 °C by adjusting the temperature on the magnetic stirrer to the setting 150 and correct, if needed
11. Mix the “Fehling I”- and “Fehling II”- solutions in the empty 50 mL Falcon tube to yield the final mix for the Fehling reaction.
12. Add to each test tube 2.0 mL of the Fehling mix using a 5 mL graduated pipette.
13. Add the substance to analyse (control and unknown substances X1 to X4).
    - Either 20 drops of liquid by using a Pasteur pipette
    - Or 1 spatula tip of solid using a spatula.
14. Heat the test tubes in the water bath.

☐ **Table 2.1.4.**: Note your observations in **table 2.1.4.** in the ANSWER SHEET.

☐ **Table 2.1.5.**: Assign the unknown substances to their correct labels in the ANSWER SHEET (**Table 2.1.5.**).

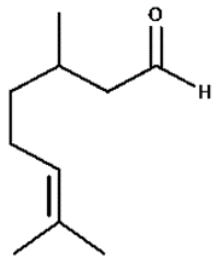
☐ **Question 2.1.6.:** Formulate the reaction scheme for the reaction of the given ketone with the Brady reagent. Answer the question in the ANSWER SHEET **Question 2.1.6.**

In organic chemistry, different formulas, showing different detailed information, are used. The formulas shown in the introduction are called structural formulas. A much faster method to draw molecules are the skeletal formulas since there the C-atoms and the C-H-bonds are not explicitly written out. The following table shows both types of formulas.

skeletal formula	structural formula
	
	
	
	

The following molecules are widely used as fragrances.

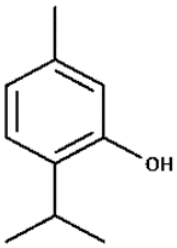
☐ **Table 2.1.7.**: Indicate in **table 2.1.7.** in the ANSWER SHEET whether these substances would react with the reagents you have just studied or not.



citronellal

citrus fruits

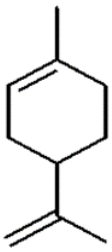
mosquito repellent



thymol

thyme

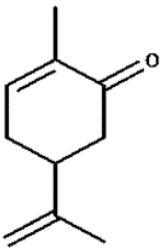
disinfectant, fungicide, bactericide



limonene

citrus fruits a.o.

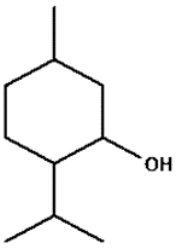
fragrance, insecticide



carvone

caraway, spearmint

flavouring, germination inhibiting



menthol

mint

flavouring, disinfectant

Problem 3: Ionizing radiation

Introduction

Radioactivity and radon

Radioactivity originates from the decay of unstable nuclei of certain atoms. Discovered by Henri Becquerel in 1896, radioactivity involves the spontaneous emission of particles or electromagnetic radiation from these nuclei. Among its forms are alpha decay, beta decay, and gamma decay.

There is always a certain amount of natural radioactivity present which e.g. originates from cosmic rays or weak radioactive substances in the environment. This natural radioactivity is detected as so-called *background radioactivity*.

Radon, a colourless and odourless noble gas, emerges as a decay product of uranium and thorium. When radon escapes the Earth's confines and enters the air, it can be inhaled. Its accumulation indoors, especially in poorly ventilated spaces like basements, heightens health risks. The invisible threat of radon lies in its potential to induce lung cancer. Upon inhalation, its decay products irradiate the lung tissue, potentially causing DNA damage and elevating the risk of lung cancer.

Radon entering homes and workplaces thus poses a significant challenge. As radon seeps through the ground and accumulates indoors, building occupants face elevated exposure risks. To address this, national and international bodies have established guidelines and regulations. Radon testing, often accompanied by mitigation techniques like improved ventilation and specialized construction materials, aims to minimize exposure risks.

Radioactive activity

The activity A of a particular radioactive material is defined as the decay rate, i.e., the number of radioactive decays happening per second. We can measure a quantity that is proportional to A with a Geiger-Müller counter. This detector counts the number of decays that occur in front of its window. The SI unit for the activity is Becquerel (Bq), 1 Bq= 1 decay per second. For the experiments in this task, we present the activity in number of decays per minute.

Quadratic law of distance

When any kind of radiation (radioactive particles or electromagnetic waves) is incident on a surface S, its intensity is defined as the power per unit area. As the distance d from a point-like source of radiation increases, the intensity I decreases. The relationship between intensity and distance can be shown to follow an inverse square law if there is no or negligible absorption present:

$$I = \frac{k}{d^2}$$

where k is a constant. This means that if the distance increases by a factor 2, the intensity decreases by a factor 4.

Exponential laws in radioactivity

The activity A of a radioactive source decreases exponentially over time. It means that after a certain time $t_{1/2}$, called half-life, the activity is $A/2$. After $2 \cdot t_{1/2}$, it is $A/4$, after $3 \cdot t_{1/2}$, $A/8$ and so on (see Figure 3.1 below).

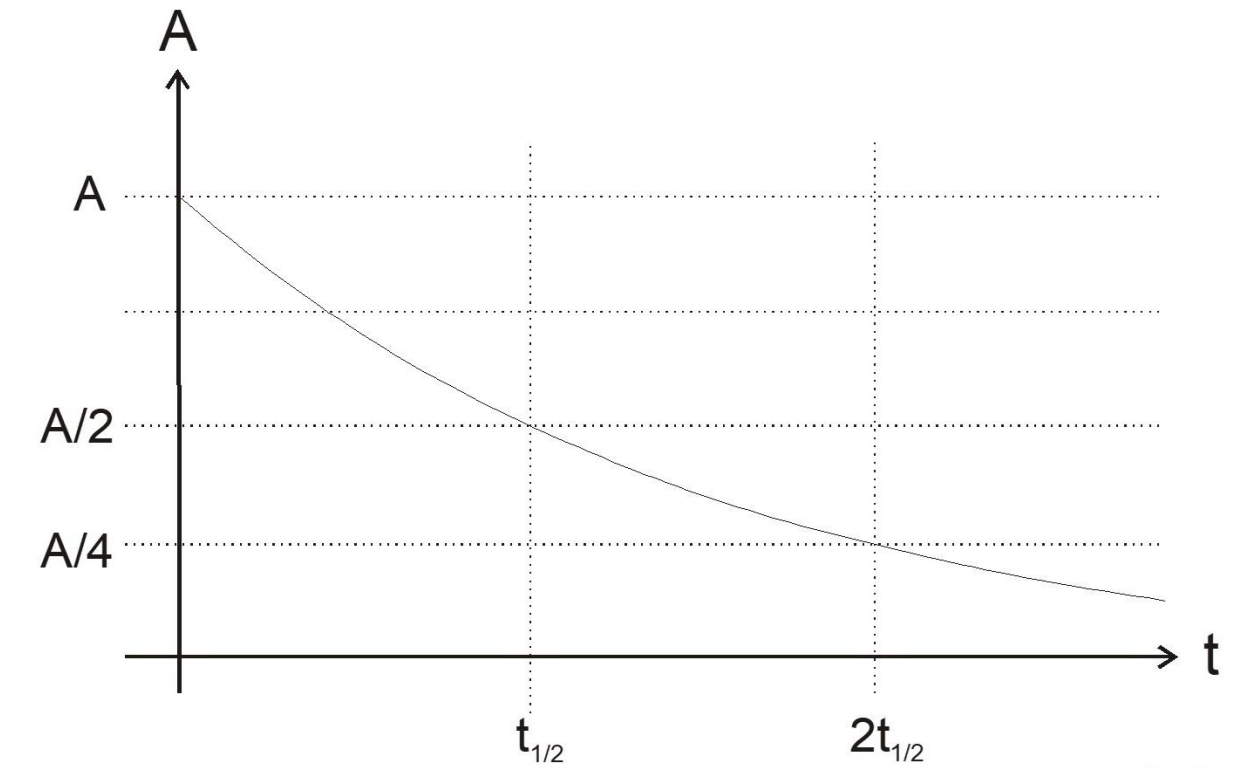


Figure 3.1: Radioactive activity versus time

The intensity of radioactive radiation travelling through a material also decreases exponentially with thickness. Consider radiation with an intensity I that travels a certain distance D through a

material. If the outgoing intensity is $I/2$ for a certain distance $D_{1/2}$, then it is $I/4$ for a distance $2D_{1/2}$, and so on.

Note: All experiments are done in the *Main Lab*, except for some steps in Problem 3.1. which will be done in the *Balloon Lab*.

Problem 3.1. Evidence for the existence of radon (23 points)

HINT: Problem 3.1. requires a waiting period after setup. Therefore, it is recommended to start with steps 1) to 7) of Problem 3.1. before starting Problem 3.2. and Problem 3.3.

Materials:

- **Balloons**
- **Balloon holder**
- **Fleece blanket**
- **Scissors**
- **Stopwatch**
- **Geiger-Müller counter**
- **Clip**

Radon (Rn-222) has a half-life of 3.8 days. It decays by α-decay into polonium (Po-218, half-life 3 minutes). This is followed by further decays with short half-lives (see Figure 3.2 below).

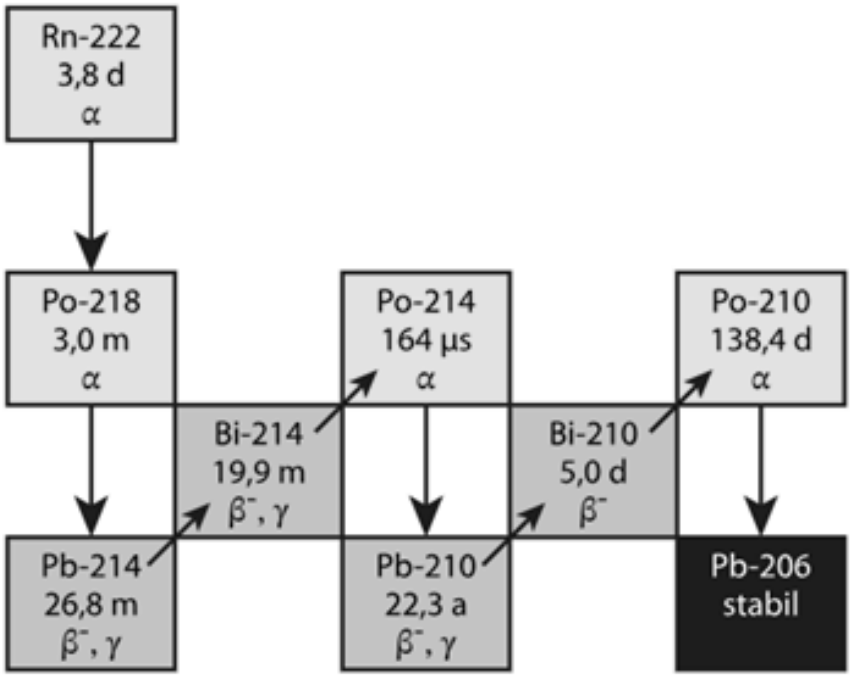


Figure 3.2: Radioactive series of radon, half-lives of the nuclei and types of radioactivity. Units in the picture: d=days, m=minutes, a=years.

In this experiment, you will expose a negatively charged balloon to the air for 30 minutes. During this time, some of the daughter nuclides (atoms formed from the decay) of radon gas will accumulate on the balloon's surface.

Afterwards, you will release the air from the balloon, thus reducing its surface area, which spatially concentrates these radioactive elements previously attracted to its surface. You will then use a Geiger-Müller counter to measure the combined activity of these elements on the balloon's surface. You will monitor the change of this activity during a time interval of 150 minutes. It is important to note that the measured activity is not from a single radioactive element (as shown in Figure 3.2) but rather a combined effect that we call "effective activity". In a first approximation, this effective activity also exhibits an exponential decay, at least in the first three hours.

The following steps need to be completed in the room *Balloon Lab*:

1) Find the station with your country's name.
2) Inflate a balloon with your breath or with one of the pumps provided and close the outlet of the balloon with a knot.
3) Electrically charge the inflated balloon by rubbing it with the fleece blanket provided or against your hair..

> **Important:** In order to charge the balloon as much as possible, rub it *forcefully* with the fleece blanket. Only the area where the blanket (or your hair) and the balloon touch becomes charged. **Verify that the balloon is well charged**! You can do so by approaching it with your hand without touching it; if it is well charged, it should be attracted to your hand.

4) Once charged, place the inflated balloon on the provided holder. To stabilize the position of the balloon, attach the clip to the knot (see Figure 3.3).

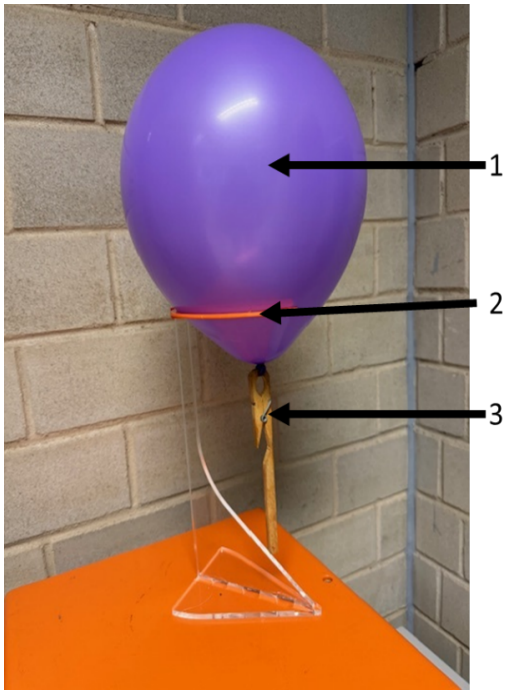


Figure 3.3: 1 = Balloon; 2 = Holder; 3 = Clip

5) Start the stopwatch. Leave the balloon undisturbed on the holder in the *Balloon Lab* for 30 minutes.

During the waiting of 30 minutes, perform the following two steps in the *Main Lab*:

6) Familiarize yourself with the use of the Geiger-Müller counter for the measurements of radioactive activities, referring to the instructions (see Appendix p.T26). Don't touch the window of the sensor.

   Note: The measurement time is pre-set to 1 minute. Do not change this setting!

7) Determine the *background radioactivity*: Measure the background activity A_0 by placing the Geiger-Müller counter onto your desk (sensor window downwards) without any sample. Perform this measurement three times and determine the mean value of A_0.

☐ **Table 3.1.1.:** Record the values in **table 3.1.1.** on the ANSWER SHEET.

After the waiting time of around 30 minutes, go back to the *Balloon Lab* and bring the balloon along with the stopwatch to your desk in the *Main Lab*. When transporting and handling the balloon, touch its surface as little as possible!

8) Reset the stopwatch and start it again.

9) Carefully cut the knot of the balloon with the provided scissors to release the air. **Hold the balloon firmly with two fingers to prevent it from flying away!** Put the empty balloon onto the desk and place the window of the Geiger-Muller counter

on it. The entire balloon should be covered by the counter. **Once in place, the position of the window of the Geiger-Müller counter on the empty balloon should not be changed anymore!**

10) Wait until the stopwatch displays 10 minutes. By doing so, you assure that the contribution of Po-218 to the measured activity can be neglected. Reset the stopwatch and start it again. This is the starting point in time for all following measurements.

You are now ready to start the activity measurements:

11) Note the time t displayed on the stopwatch. Measure the activity A. Perform 3 measurements (A_1, A_2, A_3) without a break in between and calculate the mean value for the measured activity A_{mes}. Don't forget to subtract the background activity A_0. A_{mes} should be much bigger than A_0.

☐ **Table 3.1.2.:** Record the measured and calculated values in **table 3.1.2.** on the ANSWER SHEET.

In case that the measured activity A_{mes} is about equal to the background activity A_0, it may be that your balloon wasn't well charged. You have enough time to retry with a second balloon. You can also buy measurement data for 3 penalty points. In this case, you will not receive any points for the measurements table (3.1.2).

12) Repeat the activity measurement (step 11) every 15 minutes for a duration of 150 minutes.

Use the time between the measurements to perform Problems 3.2. and 3.3.!

☐ **Graph 3.1.3**: After finishing the measurements, use the data from Table 3.1.2. to create a plot of the activity A as a function of time t (**Graph 3.1.3.**).
Use the provided **graph paper** and **label it with the correct sticker (Graph 3.1.3.)**.

☐ **Question 3.1.4.:** Estimate the effective radioactive half-life of the decay products of radon gas on the balloon by using graph 3.1.3. Record your value on the ANSWER SHEET **Question 3.1.4**.

☐ **Question 3.1.5.:** The decay of which elements contribute mainly to the decrease of the effective activity that you measure during the activity measurements in steps 11)-12)? (see Figure 3.2.) Tick **(✓)** the cell(s) under the different nuclei mentioned on the ANSWER SHEET **Question 3.1.5**.

☐ **Question 3.1.6.:** Assume that the balloons have a perfect spherical shape and that you have three different balloons with diameters $d_1 = 5\,cm$, $d_2 = 10\,cm$ and $d_3 = 20\,cm$. Assume also that the density of the radioactive ions on the balloon's surface is the same everywhere. If the activity measured by the Geiger-Müller counter for the balloon with diameter d_1 is $A_1 = 100\,counts/min$ at a certain time t (air has streamed out), calculate A_2 and A_3 at the same time for the 2 other balloons, also without air. Don't forget to consider the background activity A_0; use A_0=30 counts/min. Record the calculated values on the ANSWER SHEET **Question 3.1.6**.

☐ **Question 3.1.7.:** Have a look at the graph in Figure 3.2.
Now consider a hypothetical case where we assume that all elements in figure 3.2., apart from Rn-222, are present in equal quantities on the balloon at the beginning of your measurements. No new elements will be collected on the balloon during the measurements. Which elements are mainly present on the balloon's surface after 3 hours? Tick **(✓)** the cell(s) under the different nuclei mentioned on the ANSWER SHEET **Question 3.1.7**.

Problem 3.2. Law of distance (15 points)

Material

- **Iron rod**
- **Ruler**
- **Lamp**
- **Light detector**
- **Amplifier**
- **Ammeter**
- **Cables**

The intensity of radioactive radiation decreases with the distance from the source. For safety reasons, we investigate the relationship between *intensity and distance* using light instead of radioactive radiation. This can be done since light waves are electromagnetic waves just like the radiation in the case of the radioactive γ decay. The only difference is the frequency (or wavelength) of the electromagnetic waves.

The intensity of a point source is inversely proportional to the square of the distance (quadratic law of distance), see introduction.

Experimental setup:

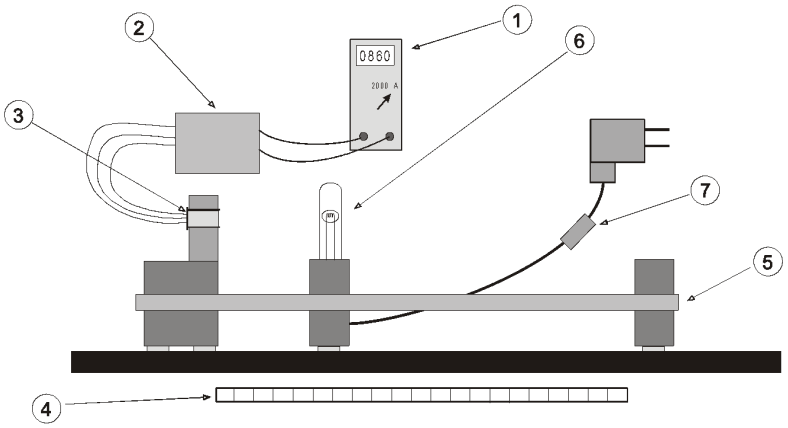


Figure 3.4: 1 = Ammeter; 2 = Amplifier; 3 = Light detector; 4 = Ruler; 5 = Iron rod; 6 = Lamp; 7 = Switch for Lamp

Set up the experiment as shown in Figure 3.4. The ammeter is set to a measuring range of 2000 µA.

A small halogen lamp serves as the light source. The light detector produces an electric current when illuminated. This current is proportional to the intensity of the light which falls onto the detector. Since the current is very small, it needs to be amplified to be measured by the ammeter.

Please note: Even in absolute darkness, a small current is measured by the setup. This is because the amplifier is not an "ideal instrument". Furthermore, light from the outside or from the illumination in the lab will also fall onto the detector. These two influences result in a so-called dark current I_0 that must be considered for the evaluations. To determine the current I_L produced by the lighting by the halogen lamp in your setup, I_0 must be subtracted from the total current measured.

- When the lamp is switched off, measure the dark current I_0.

☐ **Table 3.2.1.**: Insert the value in **table 3.2.1.** on the ANSWER SHEET.

- When the light is on, measure the current intensity I for 30 different distances between $d = 20\ cm$ and $d = 80\ cm$. Determine I_L. Calculate $1/d^2$ (cm^{-2}).

☐ **Table 3.2.2.:** Record your results in the measurement **table 3.2.2.** on the ANSWER SHEET.

☐ **Graph 3.2.3.:** On a sheet of millimeter graph paper create a plot of I_L as a function of $1/d^2$ (**Graph 3.2.3.**). Draw a line through the measurement points in Graph 3.2.3. but only through the points that in a first approximation fulfil the quadratic law of distance. Use the provided **graph paper** and **label it with the correct sticker (Graph 3.2.3.)**.

☐ **Table 3.2.4.:** Determine the minimum value d_{min} for which the quadratic law of distance holds. Insert the value in **Table 3.2.4**

☐ **Question 3.2.5.:** If instead of a point-like source, you were to use a planar light source and a detector pointing towards the plane, which of the following statements would be true? Tick (✓ → ***Yes)*** the correct cells on the ANSWER SHEET (Question 3.2.5.).

As a function of distance *d*:

- The intensity would decay slower than with a point-like source
- The intensity would decay faster than with a point-like source
- The decay of the intensity is the same as for a point-like source

Problem 3.3. Absorption of radiation (12 points)

Material

- **Iron rod**
- **Light detector**
- **Cables**
- **Ruler**
- **Amplifier**
- **Absorption plates**
- **Lamp**
- **Ammeter**

The intensity of the radioactive radiation that passes through a plate decreases with the thickness of the plate. For safety reasons, just as with Problem 3.2., we investigate the relationship *between the transmitted intensity and the plate thickness* using electromagnetic waves from the visible range instead of radioactivity. There are 10 plastic plates of the same thickness available.
We use the same experimental setup as for Problem 3.2. In order to have a higher intensity, keep a constant distance $d = (10 \pm 0.5)\ cm$. At this distance, the light source can no longer be looked upon being point-like. For the absorption experiment, this does not play a role.

- Successively place (by holding them in your hand) 1, 2, 3, ..., 10 plates of the same thickness of 2 mm in the light beam and determine each time the current I_L. Do not forget to consider the dark current!

☐ **Table 3.3.1.:** Record measurement results in **table 3.3.1** provided on the ANSWER SHEET.

☐ **Graph 3.3.2.:** Create a plot of I_L as a function of the number of plates (**Graph 3.3.2.**). Draw a best-fit curve through your measurement points by hand. Determine the point, where I_L has halved from the initial value, so that you are able to answer **question 3.3.3.** Use the provided **graph paper** and **label it with the correct sticker (Graph 3.3.2.)**. You will only receive one sheet of graph paper for this plot.

☐ **Question 3.3.3.:** Determine the lowest number $N_{1/2}$ of the plate for which less than half of the intensity of the initial value is transmitted. Record your value on the ANSWER SHEET **Question 3.3.3**.

☐ **Question 3.3.4.:** If we had used gamma radiation instead of light, consider plates made of the following materials as absorbers. Assuming that all plates are the same size, sort the materials by how strongly they absorb radiation. Mark the best absorber with 1 and the worst with 4 on the ANSWER SHEET **Question 3.3.4.**

- Iron
- Lead
- Glass
- Air

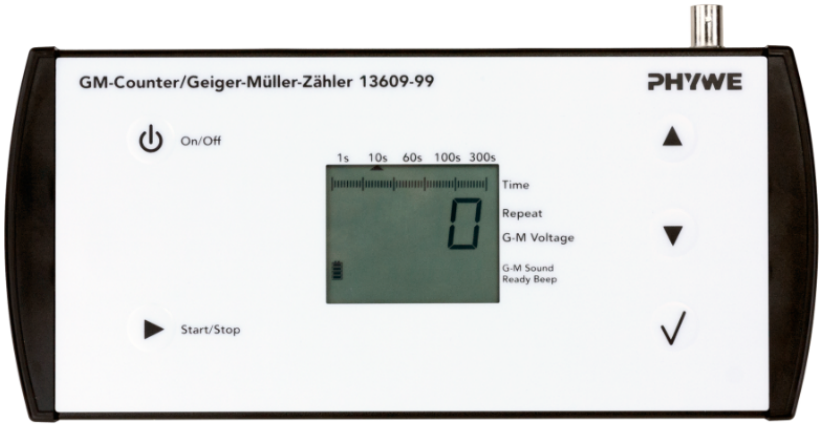
☐ **Question 3.3.5.:** Imagine that a material for shielding radioactive radiation has a thickness $D_{1/2} = 2\ cm$ for absorbing half the radiation. Which of the following thicknesses is sufficient to reduce the radiation to less than 5% of its initial value? Tick (✓) the correct cell(s) on the ANSWER SHEET **Question 3.3.5.**

- 8 cm
- 9 cm
- 7 cm
- 10 cm

APPENDIX – Use of the Geiger-Müller counters

There are two types of Geiger-Müller counters available.

Model A



Model A has a button labelled "Start/Stop". This is the only button that you need to press.
The measurement time is pre-set to 60 seconds. You can start a measurement by pressing the Start/Stop button. This erases the displayed value of the last activity measurement and starts a new measurement. The measurement will automatically stop after 60 seconds.

Model B

Model B has a button labelled "START/STOP" and a button labelled "RESET".
The measurement time is pre-set to 60 seconds. Before you start a measurement, press the "RESET" button to erase the displayed value of the last activity measurement. You can then start a measurement by pressing the "START/STOP" button. The measurement will automatically stop after 60 seconds.

Problem 4 – Effect of UV light on cell growth (16 points)

Materials and equipment

On the shared bench:

- Transilluminator for UV radiation
- Safety goggles anti-UV
- Shaker under heating lamp
- Gloves in different sizes (lab entrance)

On your bench:

- Pasco Spectrophotometer for OD600nm analysis
- Ipad + charger
- Single-use Microcuvettes (50x), only for Biology purpose
- Microcuvette holder (black)
- Starting solution of E. coli “EC” (20mL) in a tube of 50 mL (1x)
- Microtubes of 1.5 mL “MT” (20x)
- LB medium for blanking “LB” (10mL)
- Micropipettes (P10, P100, P1000)
- Tips in red box (for P10 micropipettes)
- Tips in yellow box (for P100 micropipettes)
- Tips in blue box (for P1000 micropipette)
- Sunscreen “SUN” (1 syringe) and Body lotion “BL” (1 syringe)
- Small Petri dishes (4x)
- Spreader (2x)
- Petri dish holder (2x)
- Rack for the small Petri dishes
- Rack for microtubes of 1.5 mL
- Paper tissues
- Permanent marker
- Timer
- Waste bin for tips/ tubes
- Safety goggles

Introduction

To better understand the biological effects of UV light, you will first use a whole organism approach and monitor the cell growth of the bacteria *Escherichia coli (E. coli)* over a specific time interval. Bacterial growth refers to the increase in the number of cells which is achieved by asexual reproduction. The ability to divide is one of the most important properties of a living cell, but it consumes energy and is complex. For example, in addition to the actual cell division, new cell building blocks need to be produced beforehand. Hence the UV radiation has a multitude of potential targets that could results in a damaged cell that is unable to divide. In Problem 4, you will not only test how UV radiation affects the cell growth but also test two different cosmetic products to see if they reduce the damage caused by the UV radiation. The first one is sunscreen and the second is a body lotion. In practice, absorption measurements are usually used to determine cell growth.

At any time, the students will have to wear the adequate protective equipment: lab coat closed, gloves (to be changed every hour or after potential contamination) and safety goggles. Should you have any question, raise the gold card so it's visible to a supervisor. They will then come to your bench to assist you. Don't come to the shared bench to ask questions to the supervisor.

Problem 4.1.: Experimental set-up and UV exposure

4.1.1. Experimental set-up

1. Label the lid of the 4 small Petri dishes with the numbers 1 to 4. The numbers correspond to the following treatments:

    1 - No lotion + no UV exposure
    2 - No lotion + UV exposure
    3 - Sunscreen with a sun protection factor of 50 (SPF50) + UV exposure
    4 - Body lotion + UV exposure

2. Turn the dish labelled "3" upside down and place the Petri dish holder over it. Make sure that the teeth of the dish holder line up with the opening in the lip of the bottom of the dish (see figure 4.1a).

3. Apply 1 ml sunscreen from the syringe labelled "SUN" to the dish labelled "3" and use a spreader to evenly distribute the sunscreen.

4. Place the spreader at one end of the holder and while maintaining contact with the holder, move the spreader to the other end of the dish holder (see figure 4.1b). Make sure that the sunscreen is evenly distributed on the dish (see figure 4.1c). If this is not the case, use the spreader again or add more sunscreen. If done, remove the dish holder (figure 4.1d).

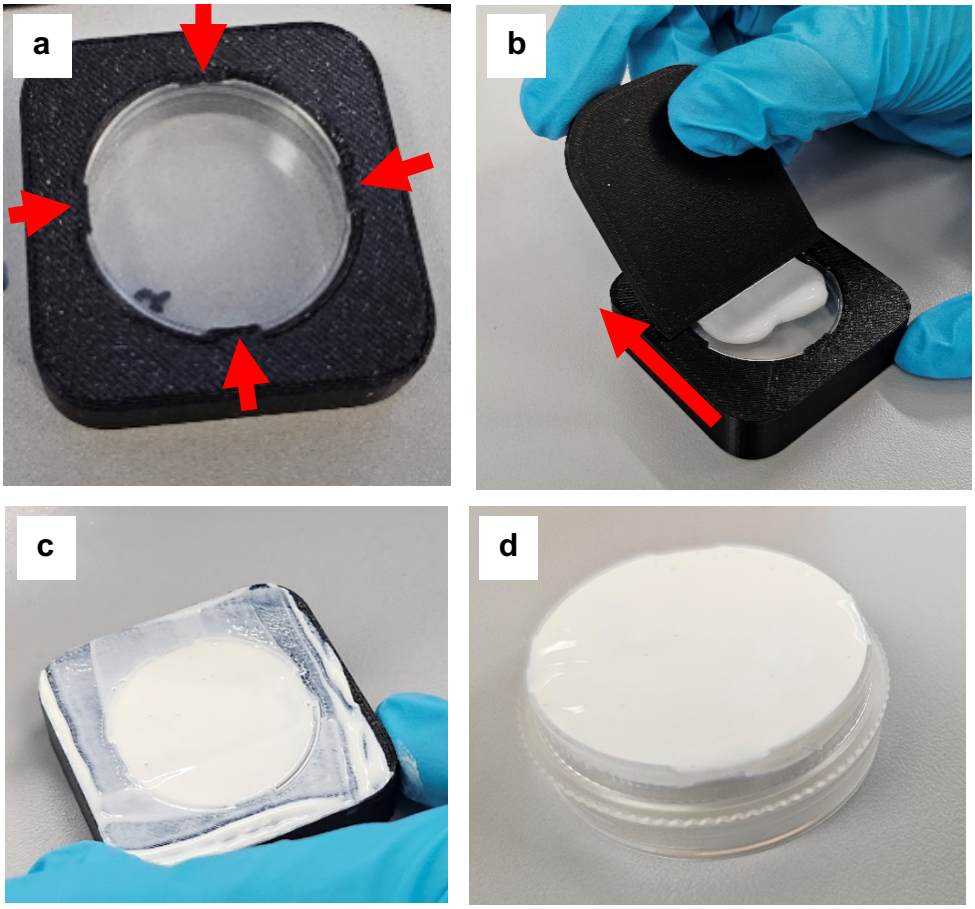


Figure 4.1. Application of sunscreen and body lotion.

5. Repeat the same steps for the dish labelled “4” but add body lotion (syringe labelled “BL”) instead. Use a new Petri dish holder and spreader.

6. Place the four dishes into the rack. Pay attention that the sample that won’t be exposed to UV needs to be placed in the circle with the bottom. You need to flip the Petri dishes with sunscreen and body lotion over again, so they are facing downwards (See figure 4.2.).

Figure 4.2. Rack with the 4 dishes

4.1.2 Preparation of starting E. coli solution

To be able to make meaning ful comparisons between the treatments it is important that the starting concentration of *E. coli* is the same for all treatments. Imagine you are provided with a theoretical stock solution of *E. coli* that has an optical density (OD) of 0.9.

- ☐ **Question 4.1.1.:** Calculate the correct volume to take from the stock solution to end up with an OD of 0.2 in 20 ml of solution. Show your calculation in the ANSWER SHEET **Question 4.1.1.**

Optical density:
The optical density measures the degree of light scattering caused by the bacteria within the culture. The more bacteria are present in the solution, the more light will be scattered and you will end up with a higher OD value. The OD is directly proportional to the concentration. The OD is measured at 600 nm.

Make sure to read the following instructions on how to use the spectrophotometer **before** continuing with the steps on page T32.

How to use the spectrophotometer

Sample microcuvettes for light absorption measurements

All microcuvettes have two different sides – one pair of opposing sides has a transparent side, called the optical window, and the other pair is denting inwards. The light beam needs to pass through the transparent sides. Thus, attention should be paid that no fingerprints, drops of solution or scratches are found on transparent sides.

To avoid errors when handling microcuvettes, you should only touch the upper part of the cuvette. Any drops of liquid remaining on the transparent part of the microcuvette should be carefully blotted with a piece of paper.

Filling the microcuvette

The cuvette needs to be filled with 1 mL of solution. The filling of the microcuvette with the sample solution is carried out using a micropipette so that no air bubbles form in the cuvette.

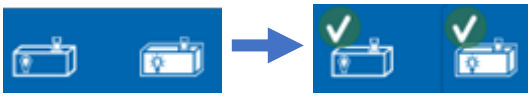
Inserting the microcuvette into the spectrophotometer

The sample is always placed between the light source and the detector so that the light beam passes through the transparent sides of the cuvette from the light source to the detector. On all the cuvettes, there is a small arrow at the top of one of the transparent sides. Therefore, the cuvettes shall always be placed in the apparatus with the arrow pointing in the direction of the light, marked as a white light source on the machine.

Setting the zero point/blank measurement

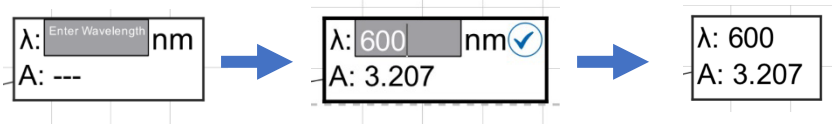
With the spectrophotometer it is possible to independently set the absorbance measurement wavelength. It is then possible to use a sample with only the solvent to take a “blank” measurement and subtract the measured value from all following sample absorption values. After calibration, the spectrophotometer does the subtraction automatically. Thus, not only water but also other compounds, including those which have a colour to begin with, can be used as a solvent for the preparation of solutions of the substance to be detected.

To calibrate the machine, insert your blank and press first the symbol of a dark spectrophotometer. Let the calibration run and afterwards press the symbol of a white spectrophotometer. Both symbols should now have a check mark.



Selecting your wavelength

Click on the grey square with the text ”Enter Wavelength”, enter the value “600” and press Enter.



Taking a measurement

To take a measurement, place your sample in the machine and press the red button **twice!** (red arrow, see figure 4.3). The measured absorbance will be displayed in the box (blue arrow, see figure 4.3).

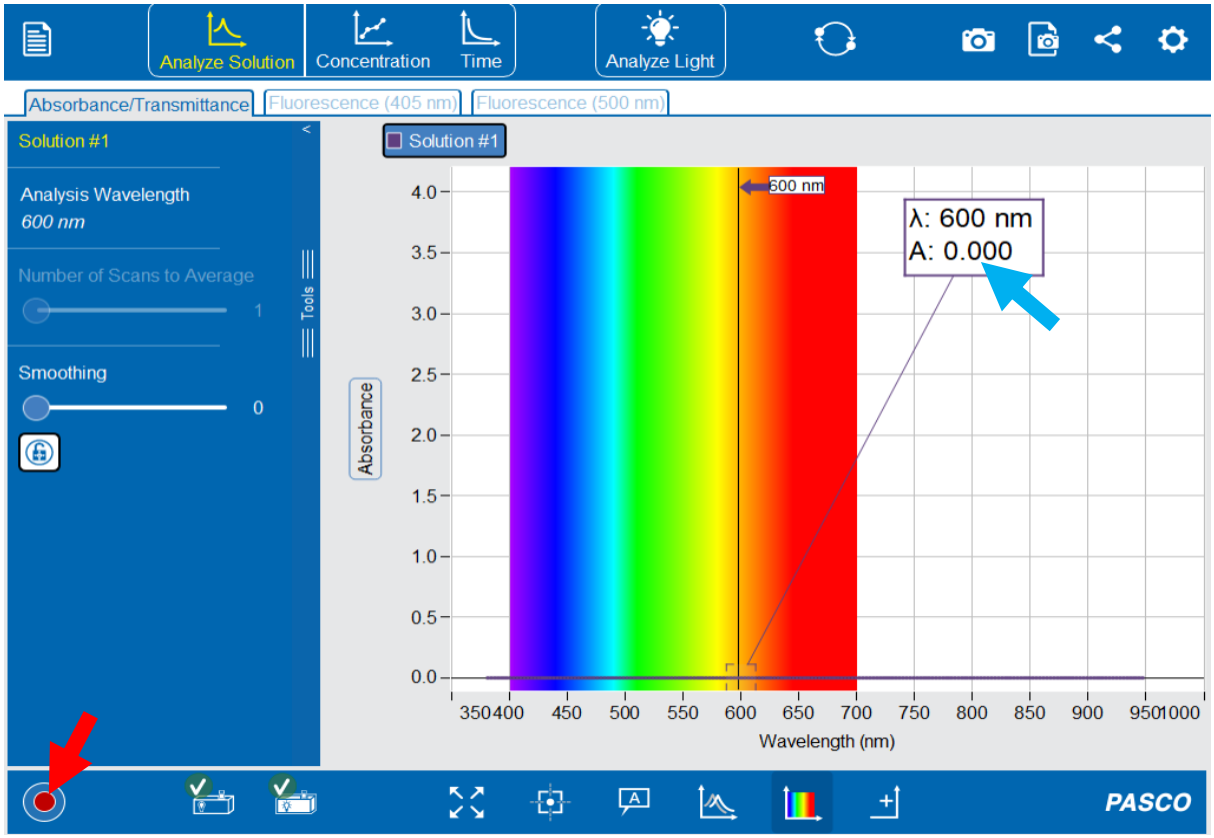


Figure 4.3. Interface of the spectrometer

1. Determine the zero point by using 1 mL of the appropriate blanking solution. Keep the microcuvette in case you need to recalibrate the machine.
2. Gently invert the tube "EC" several times to resuspend the *E. coli* bacteria.
3. Measure using a new microcuvette the OD of the *E. coli* cells from tube "EC".

☐ **Question 4.1.2.**: Enter the measured OD into your ANSWER SHEET **Question 4.1.2** and pour the 1 mL *E. coli* solution back into the original tube.

4.1.3 UV exposure

Don't forget to wear safety goggles when manipulating the transilluminator and turn it on only when the cover is properly closed.

1. Add 4 mL of your bacterial culture to each petri dish.
2. **You need to wear the safety googles at this point!!** Carefully bring your samples over the shared bench, a supervisor will place them on the transilluminator for you. Start a 15 min timer as soon as your samples are placed on the transilluminator.
3. After 15 minutes UV exposure return to the shared bench and ask the supervisor for your samples.

Problem 4.2.: Growth analysis by determination of $OD_{600\ nm}$

1. First, transfer 1 mL of bacterial culture from each dish to 4 different microtubes, that you will label according to the set-up. Always use a new pipette tip when you switch between samples. Leave them on the bench in the microtube rack. You will use these tubes during section **5.2.1. Extraction of DNA from bacteria**.

2. Transfer 1 mL bacterial culture from of each Petri dish to 4 different microcuvettes. Don't forget to label them.

3. Determine $OD_{600\ nm}$ for each experimental condition using the spectrophotometer.

   Table 4.2.1.: Fill in **table 4.2.1.** in the ANSWER SHEET.

4. Transfer the bacterial culture immediately back to the correct Petri dish.

5. Carefully put the holder containing the dishes on the orbital shaker situated under a warming light and start a timer.

6. Repeat the OD determination for time points 30 min, 60 min, 90 min by repeating steps 2 to 5. Do not stop the timer, when you remove the samples from under the warming light. Let it run continuously throughout the experiment. The determination of the OD needs to be done withing 5 min after you collected the samples from under the warming light.

   Graph 4.2.2.: Plot your data and draw a growth curve ANSWER SHEET 4.2.2.

   Questions 4.2.3. & 4.2.4.: Answer the questions on your ANSWER SHEET 4.2.3 & 4.2.4.

Problem 5 – Effect of UV light on genetic material (34 points)

Materials and equipment

On the shared bench:

- Transilluminator for UV radiation
- Safety goggles anti-UV
- Heat block
- Nanodrop
- Micropipette P2 + tips
- Tissue
- Agarose gel chamber + gel + TAE running buffer
- Micropipette P20 + tips
- Power supply
- Microscope + holder for phone
- Gloves of different sizes (lab entrance)

On each team bench:

- Small centrifuge
- Vortex
- 750 µL of Human cancer cells “HC” (1 microtube)
- Microtubes of 1,5mL (5x)
- Small blue tubes of 200 µL (5x)
- Micropipettes (P10, P100, P1000)
- Tips in red box (for P10 micropipette)
- Tips in yellow box (for P100 micropipette)
- Tips in blue box (for P1000 micropipette)
- PM solution “PM” (1 microtube)
- Distilled water “H2O” (1 microtube)
- Rack for 15 mL and 50 mL tubes
- Microscope
- Glass counting slide
- Glass coverslip
- Trypan blue dye solution “TB” (1 microtube)
- Calculator
- DNA samples in small red tubes for gel electrophoresis
- DNA ladder (1 small red tube)
- Blue loading dye “LD” (1 small transparent tube)
- Liquid waste bin “LW” (1 tube)
- Rectangular glass bowl & wash bottle for cleaning of the counting slides
- Waste bin for tips/ tubes

Introduction

In cancer research, most of the experimental procedures are divided into cell assay, cell counting, genetic material extraction and quantification, PCR, agarose gel electrophoresis… During this problem 5, you will work on the various crucial stages of cellular and molecular biology used in cancer research laboratory, using either real human cancer cells (skin cancer cells) or model organisms, in this case the *E. coli* bacterium.

Current cancer research relies heavily on the use of human models and cells to understand the underlying mechanisms of the disease, develop new therapies, and evaluate their efficacy. These models and cells are invaluable tools enabling researchers to explore various aspects of cancer, from genetic mutations to response to treatments.

Problem 5.1.: Cell counting using a microscope

Determination of the total concentration of cancer cells in the microtube "HC" (as a number of cells per mL).

Only for human cancer cells!

Preparation of your cell counting slide

The cell concentration is determined by the number of cells in a specific volume of liquid medium. The result of the count is expressed in cell concentration, i.e. in number of cells per millilitre.

The cell counting is performed by counting the cells under the microscope, using a special counting slide, such as the Neubauer counting slide (see Fig.5.1.). The slide consists of a grid engraved on the central platform that will facilitate the counting of the cells, two grooves separating the central platform from the mounting support and two mounting supports to hold a cover slip in place.

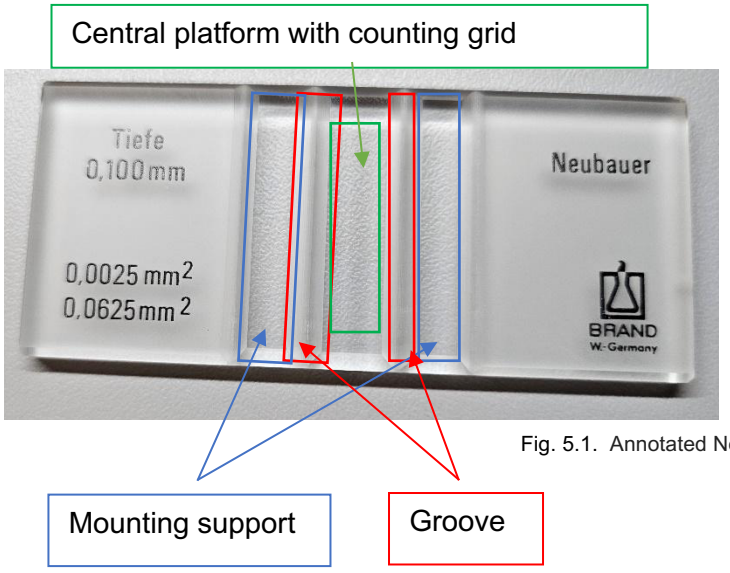


Fig. 5.1. Annotated Neubauer counting slide

USE GLOVES FOR ANY EXPERIMENTS DEALING WITH CELL MANIPULATION!!!

1. Place the Neubauer counting slide on a flat surface.

2. Add 2 µL of water on each of the two cover slip mounting supports (see Figure 5.2a).

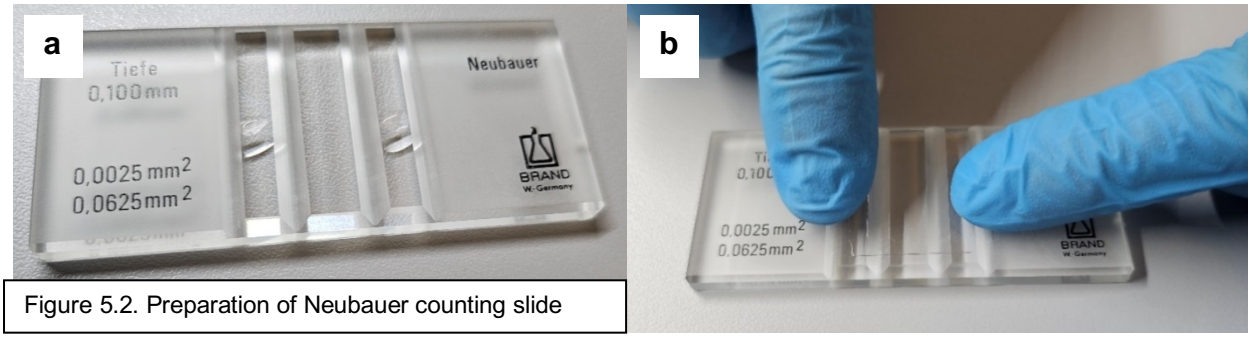


Figure 5.2. Preparation of Neubauer counting slide

3. Place the cover slip, in portrait mode on the mounting support. With your two thumbs, placed on the edges of the slip, gently press down while slightly moving back and forth until you feel resistance to that action (see Figure 5.2b).

   The mounting supports are 0.1 mm taller than the central platform. Thus, by adding a cover slip, a chamber with a known volume is created.

Check of your Neubauer counting slide

☐ **Question 5.1.1.:** Once you are satisfied with your preparation of the counting slide, raise your golden card to alert the supervisor to check your preparation. If your preparation does not fulfil the required criteria, the supervisor will ask you to prepare it again. If the supervisor is satisfied, they will add a stamp in your ANSWER SHEET Question 5.1.1 to validate the counting slide. If the second try is still not successful, you won't get any marks for this question, but you can continue to try to prepare the slide without getting stamp and without calling the supervisor anymore.

Transfer of cancer cells onto the Neubauer counting slide

1. Carefully resuspend the cells in the tube labelled "HC" by pipetting up and down 3 times using a P1000 micropipette set to 1 mL.

2. Take a new microtube and add 20 µL of trypan blue. Trypan blue is a dye commonly used to visually separate living cells from dead cells. Live cells have their cell membrane still intact and exclude the dye. The cells appear unstained under the microscope. Dead cells cannot exclude the dye and appear blue under the microscope (see figure 5.3).

3. Add to the same tube 20 µL of your resuspended cells.
4. Carefully pipette up and down 3 times the mix without creating bubbles.

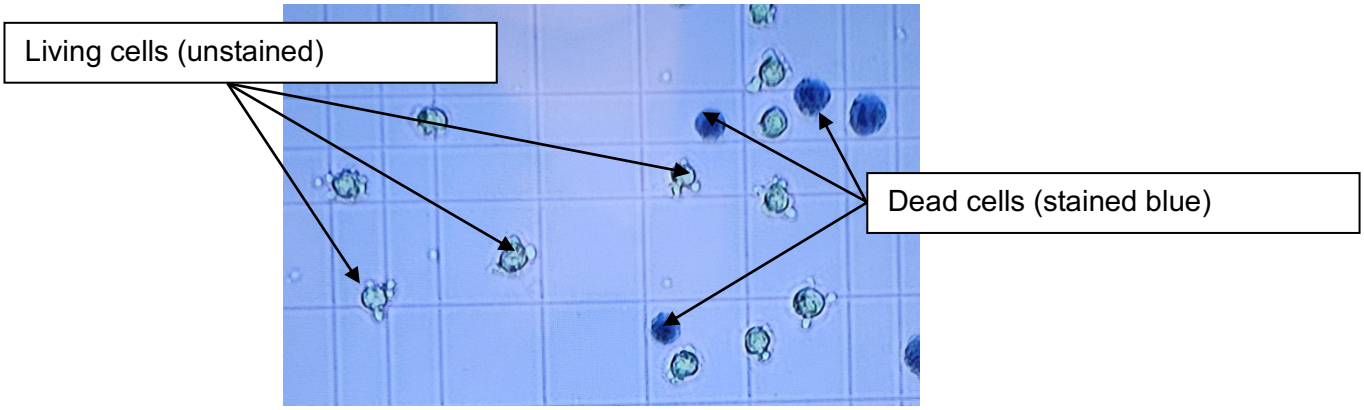


Figure 5.3. Living and dead cancer cells under the microscope.

5. Transfer 25 µL of the trypan blue-cell suspension you just prepared to the chamber of the Neubauer slide by carefully placing the tip of the pipette slightly inclined near the coverslip on the central grid platform (see figure 5.4). The filling must be done carefully in one go, without air bubbles and it is important not to overfill the chamber. In case air bubbles are visible or if the liquid has spilled over the edges into the channels, the chamber must be cleaned and refilled (see Remarks). You have enough solutions to clean and reset the counting slide twice.

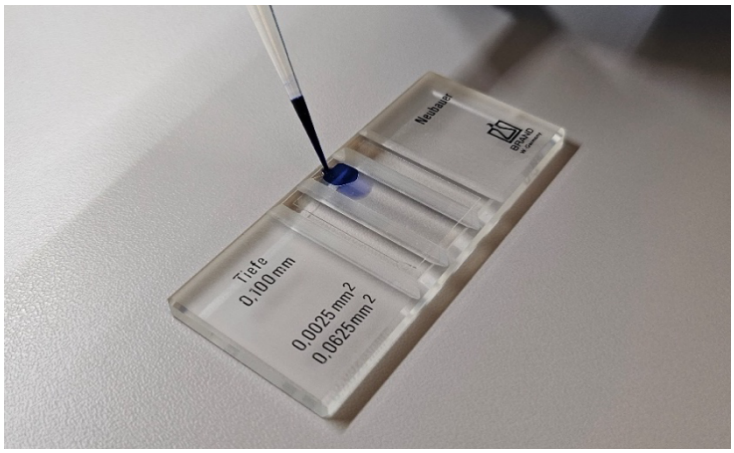


Fig. 5.4. Filling of the Neubauer counting slide

Remarks:

If you have to clean your counting cell to reuse it, keep your gloves on, hold the chamber in your hands above the rectangular glass bowl and after gently sliding off the coverslip (be careful not to cut yourself with the coverslip), rinse it with the wash bottle. Both the coverslip and counting slide need to be rinsed. Then wipe/dry the counting slide and the coverslip gently with the paper tissue.

Cell counting

The Neubauer counting slide has a counting grid that is 3 mm x 3 mm in size. The grid is divided into 9 square subdivisions. Each subdivision is thus 1 mm x 1 mm in size. The cover slip and the counting grid are separated by a gap of 0.1 mm. The central square, that you will use for counting, is further subdivided into 16 smaller squares separated by triple lines. The dimension of a small square is 0.25 mm by 0.25 mm.

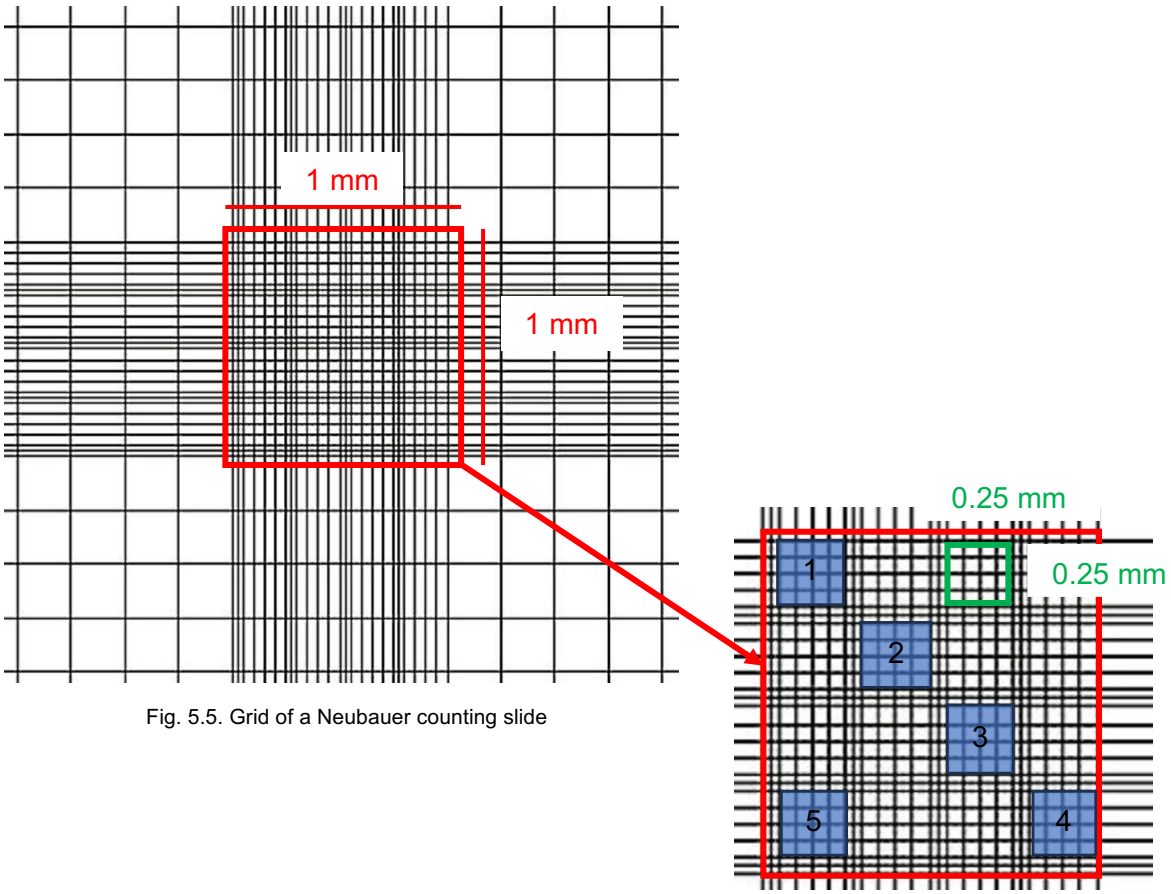


Fig. 5.5. Grid of a Neubauer counting slide

Using the 4x objective, locate the central grid (red square in figure 5.5.) and check the homogeneity of the distribution of cancer cells to be counted (if the distribution is poor, start again see Remarks section above).

☐ **Question 5.1.2.:** Once you are satisfied with your counting slide, bring it **TOGETHER** with your ANSWER SHEET to a supervisor on the shared bench. They will take a picture of your counting slide, add a stamp to your ANSWER SHEET **Question 5.1.2** and return the slide to you. Start with the cell counting immediately.

1. Use the 10x objective or higher to carry out the counting.

Count the cancer cells contained in 5 blue squares of the grid, as shown in figure 5.5.

Counting cells that touch the grid line

To avoid double counting, researchers usually only count cells touching 2 out of 4 outer lines in each square. Hence, we only count cells touching the **upper** and **left triple lines** and ignore the cells touching the right and bottom triple lines (see figure 5.6.).

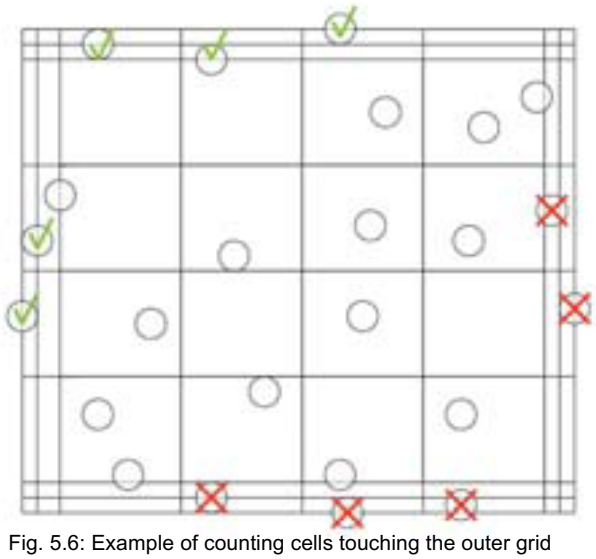


Fig. 5.6: Example of counting cells touching the outer grid

☐ **Table 5.1.3.:** Report your cell counting in your ANSWER SHEET **table 5.1.3.**

Question 5.1.4.: What is the percentage of living cells?

Clearly show your calculations in the ANSWER SHEET 5.1.4.

Question 5.1.5.: What is the concentration of living cells in your tube "HC"?

The concentration should be calculated in cells per millilitre. Clearly show your calculations in the ANSWER SHEET 5.1.5.

Question 5.1.6.: What is the total number of living cells in your tube "HC"?

Clearly show your calculations in the ANSWER SHEET 5.1.6.

Problem 5.2. Extraction of genetic material and determination of DNA concentration

5.2.1. Extraction of DNA from bacteria

1. Use the 4 microtubes (previously collected in *Problem 4.2. Growth analysis by determination of $OD_{600\ nm}$*, containing the bacterial suspension from the 4 experimental conditions.
2. Spin samples in the mini centrifuge for 5 min. Make sure that your samples are properly placed in the centrifuge i.e. distribute the weight evenly on both sides.
3. Discard the supernatant with the pipette into the liquid waste (LW) without touching the pellet. Always use a new pipette tip when you switch between samples.
4. Add 50 µL of the reagent labelled PM to each tube.

   Question 5.2.1.: What is the role of the PM solution? Answer the question in ANSWER SHEET **Question 5.2.1.**

5. Close the microtubes and vortex (10 sec) until the pellet is dissolved.
6. Heat the samples in a heat block (shared bench) at 100 °C for 10 min. Use your timer.
7. Meanwhile label a 2nd set of microtubes for the genomic DNA stock.
8. After the 10 min in the heat block, vortex the samples for 3 sec and spin the microtubes again in the minicentrifuge for 5 min.
9. Transfer 30 µL of the supernatant into the second set of microtubes. Do not touch the pellet!
10. Label a 3rd set of microtubes for diluted DNA.

5.2.2. Determination of DNA concentration

1. Dilute your four DNA samples by adding 5 µl of your DNA sample and 45 µl of water to the microtubes labelled in point 10 of **5.2.1**.. You will have a final total volume of 50 µl.
2. Vortex briefly the diluted samples (1 sec) and spin them down shortly (5 sec).
3. Determine the DNA concentrations and the purity of your diluted DNA samples using the Nanodrop spectrophotometer. The Nanodrop can be found on the shared bench. The Nanodrop is a UV spectrophotometer able to determine the concentration and purity of

nucleic acids such as DNA & RNA in small samples (2 µL) by measuring the absorbance at 260 nm as this is the peak of absorbance for nucleic acids.

Fig. 5.7: Example of a Nanodrop

4. Lift the arm and clean both pedestals with a new laboratory wipe. It is not necessary to set the zero point. This was already done.

5. Pipette 2 µL of the sample solution onto the lower pedestal and lower the arm. The machine will automatically take the measurement and display the result. Measure the DNA concentration of your 4 samples as well as the 260/280 ratio. It is important to wipe both pedestals with a laboratory wipe between the samples.

260 nm corresponds to the peak absorbance of DNA.

280 nm corresponds to the peak absorbance of protein.

☐ **Table 5.2.2.:** Fill in **table 5.2.2.** in the ANSWER SHEET.

Problem 5.3. Preparation of DNA samples for PCR

The PCR, or Polymerase Chain Reaction, is a molecular biology technique that enables a specific DNA sequence to be amplified exponentially. It was developed by Kary Mullis in 1983 and revolutionised molecular biology by enabling the rapid and specific production of large quantities of DNA from very limited samples.

First you need to calculate the volume of DNA samples you need to take in order to start the PCR experiment from 400 ng of template DNA.

- ☐ **Table 5.3.1. (1st column):** Calculate the volume in µL you need to take to have 400 ng of template DNA in each sample. Round to 1 decimal place. Fill in the table with the results of your calculations in ANSWER SHEET **table 5.3.1.**

- ☐ **Table 5.3.1. (2nd column):** Calculate the volume of water you need for each sample, to have a total final volume of 20 µL for each sample. Fill in the table with the results of your calculations in ANSWER SHEET **table 5.3.1.**

1. Pipette the appropriate volumes of water and DNA into each small blue tube according to your pipetting scheme.
2. Vortex briefly the diluted samples (1 sec) and spin them down shortly (5 sec).
3. Go to the shared bench with your small, labelled tubes containing 400 ng of DNA and measure the DNA concentrations using the nanodrop.

- ☐ **Table 5.3.1. (3rd column):** Fill in the table in ANSWER SHEET table 5.3.1. with your results.

- ☐ **Table 5.3.1.:** Once you have added all your results to the ANSWER SHEET table 5.3.1 Please ask the supervisor for a stamp and signature to certify your results.

Problem 5.4. Preparation for gel electrophoresis and DNA loading

Gel electrophoresis is a technique used to separate DNA fragments according to their size. DNA samples are loaded into wells at one end of a gel, and an electric current is applied to pull them through the gel. DNA fragments are negatively charged, so they move towards the positive electrode which is at the other end of the gel. Because all DNA fragments have the same amount of charge per mass, small fragments move through the gel faster than large ones. When a gel is stained with a DNA-binding dye and placed under blue or UV light, the DNA fragments can be seen as **bands**, each representing a group of same-sized DNA fragments.

You will find on your bench a holder containing 6 small red tubes labelled 0-5.

Each tube contains (or not) a 1500 bp (base pairs) DNA fragments corresponding to the PCR products of the following samples:

- Tube 0: Ladder (A mix of DNA fragments of known lengths (in bp) that serve as a reference)
- Tube 1: Negative control without DNA
- Tube 2: Sample 1 (no UV exposure)
- Tube 3: Sample 2 (no lotion + 15 min UV exposure)
- Tube 4: Sample 3 (sunscreen SPF50 + 15 min UV exposure)
- Tube 5: Sample 4 (body lotion + 15 min UV exposure)

Be careful to change your tips between each sample!

1. Add 4 µL of blue loading dye (LD) to the PCR samples (tubes 1-5) No need to add to the tube 0.
   - ☐ **Question 5.4.1.:** What is the role of the loading dye? Answer the question on the ANSWER SHEET **Question 5.4.1**.

2. Mix each sample by pipetting up and down 2-3 times.

Take your samples to the shared bench to load the gel electrophoresis. The gel and buffer are already added to the chamber.

add instructions from the next page here

3. Starting with the left well, load 8 µl of your DNA ladder (tube 0) into the first well of the gel.

4. Load 20 µl of each sample into a separate well of the gel. You should not leave any empty wells between the samples and the samples should be placed as follows (see figure 5.8):

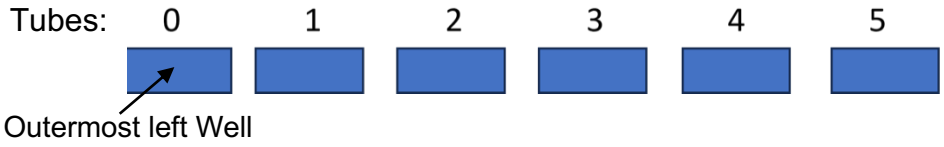


Figure 5.8. Loading schematic of the gel electrophoresis

☐ **Question 5.4.2.:** Once you are done with loading the gel, inform a supervisor so they can start the gel electrophoresis. You will also receive a stamp in your ANSWER SHEET **Question 5.4.2**.

Loading of the gel

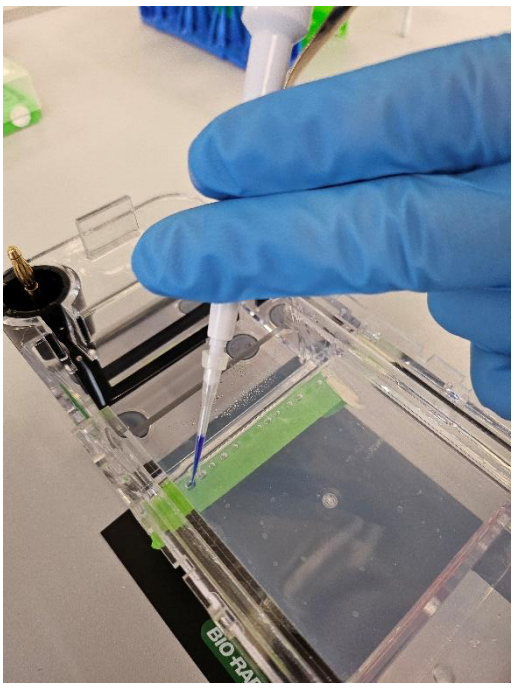


Figure 5.9. Loading of the gel

1. A stable position is important as the wells are relatively small. Rest your elbows on the table.
2. Place the pipette tip just inside the desired well. Do not puncture the well!
3. Dispense your sample slowly and press the plunger of the pipette down to the first stop only. If you press all the way down, you will introduce extra air might dislodge your sample from the well.
4. Remove the pipette tip from the well and hold the plunger down until the pipette tip is no longer in contact with the liquid.

Problem 5.5. Analysis of PCR results

- On the last page of the answer sheet, you can find an APPENDIX, that shows an agarose gel. Draw on this paper the expected result of the gel electrophoresis. Once you are done, remove the paper from the answer sheet and bring the piece of paper and your answer sheet to a supervisor. The supervisor will add a stamp to 5.5.1 in your ANSWER SHEET to certify that they received the drawing. Furthermore, they will hand you the theoretical results of the gel electrophoresis that you prepared previously.

 Analyse the results to answer the MCQ in ANSWER SHEET **5.5.2, 5.5.3, 5.5.4, 5.5.5.**