LABORATORY PRACTICE IN CHEMISTRY
ERASMUS +
VLADIMIR PRELOG SCIENCE SCHOOL

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Zagreb

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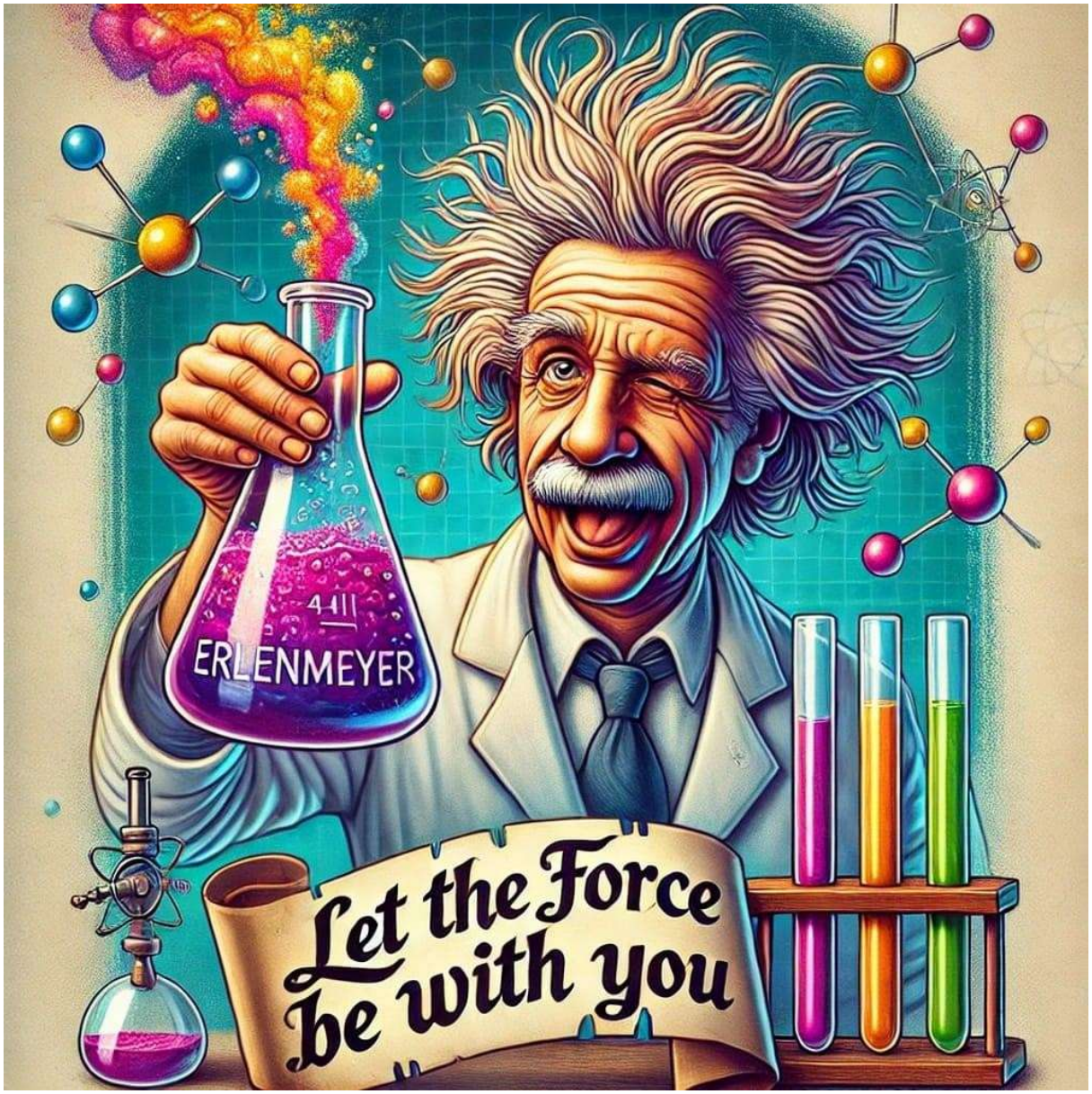
WELCOME TO
VLADIMIR PRELOG
SCIENCE SCHOOL
— ZAGREB, CROATIA


ERLENMEYER
Let the Force
be with you


DO NOT THIX
DO NOT MIX THESE!
SAFETY FIRST SOMETIMES
SAFETY
SAFETY FIRST

1. SAFETY MEASURES

1. **Wear Appropriate Personal Protective Equipment**

- Always wear safety goggles to protect your eyes.
- Lab coats should be worn to protect your skin and clothing.
- Wear gloves to protect your hands when handling dangerous chemicals, biological materials.
- Closed-toed shoes should always be worn.

3. **Never work alone in laboratory**

- In case of an accident there will be no one to help you.

3. **Maintain a Clean Workspace**

- Keep your work area organized. This reduces the risk of accidents and spills.
- Immediately clean up any spills according to the proper procedure.

4. **Understand the Chemicals and Materials**

- Know the properties of any chemicals or materials you are working with, including hazards and proper disposal methods.
- Always read labels and Safety Data Sheets (SDS) before using any chemicals.

5. **No Eating or Drinking in the Lab**

- Eating, drinking, or applying cosmetics in the lab can lead to contamination or accidental ingestion of harmful substances.

6. **Be Aware of Fire Hazards**

- Know how to operate a fire extinguisher and their locations.
- Keep flammable materials away from heat sources, open flames, and sparks.
- When working with a burner, it's important to tie back long hair.
- When working with a burner, never use protective gloves.

7. **Dispose of Waste Properly**

- Dispose of chemicals, biological materials, and waste according to your lab's protocols.
- Use specialized containers for inorganic and organic solutions.

8. **Other**

- Glassware used for measurement should never be heated on an open flame.

- When heating liquids, never point the open end of the test tube towards another person.
- Never smell chemicals by putting the container directly to your nose. Use the hand to direct vapors toward yourself.

First aid

Accidents are possible with any type of work, especially while working in the chemical laboratory. The most common cause of an accident is carelessness, ignorance of the chemical properties of substances and lack of work habits. In student laboratory most accidents occur while pouring acids and basic solutions, working with hot or sharp objects. All of those accidents can be avoided if approached with enough attention and seriousness. However, in case of an accident we need to know how to administer first aid. All injuries can be classified into several groups, namely: burns, cuts, poisoning, explosion, injuries caused by the chemical reagents.

Burns

The most common cause of burns in the chemical laboratory is touching hot objects with bare fingers, such as glass tubes, gas burners, iron tripods, and other heated accessories. Therefore, hot objects and heated laboratory accessories are grasped with laboratory tongs, tweezers or cloth. Injuries caused by grasping hot objects with bare fingers are usually harmless, because they do not create open sores and blisters. These are minor injuries and first aid can be provided immediately. If the skin remains intact, it is necessary to cool the burned area with water for a long time, and then apply ointment against burns. If the wound is more severe seek the medical help immediately.

Cuts

Cuts most often occur when working with glass accessories, and are characterized by external bleeding. The most common are cuts on the fingers and parts of the hand. In the case of minor cuts, it should be checked whether a piece of glass or wood remains in the wound or metal and should be removed with sterilized tweezers if possible or seek medical help. The wound should then be covered with sterilized gauze and bandaged. The bandage should be wraped tightly to prevent further bleeding, but not to interfere with blood circulation. If there was a cut during the injury vein, a tampon made of folded gauze or a compression bandage should be placed on the wound, tied tightly and urgently seek medical help. When providing aid for cut injuries sterilized equipment must be used.

Poisoning

Poisoning occurs by swallowing toxic substances or inhaling toxic fumes. The most important thing is to stop contact with poison. In case of ingestion of toxic substances, vomiting should be induced only if the person is conscious. A poisoned person should drink two to three tablespoons of medical charcoal, which prevents resorption in the intestines. When swallowing concentrated acids and alkalis, do not induce vomiting, but drink larger quantities milk and water, do not use medical charcoal. If you have been poisoned by inhaling toxic vapors, you should go out into the fresh air, if you lose consciousness place the casualty in a lateral position, unfasten parts of clothing that could cause suffocation and possibly apply artificial respiration, heart massage and call a doctor.

Injuries caused by the chemical reagents

Skin injuries are the most common, and eye injuries are the most dangerous, so when working in a chemical laboratory, you should always wear safety glasses.

3) Injuries caused by concentrated acids

Eyes

Wash immediately with lukewarm or cold water. Washing must last at least 5 minutes. Washing must be done immediately, as it is ineffective later. Acids permanently damage the delicate tissue of the eyes. In any case, it is necessary to seek the help of the ophthalmologist without delay.

Skin

The spilled area should be washed immediately with cold water. Washing should last a few minutes. If there are open wounds, you should seek the help of a doctor.If it is only a superficially injured layer of skin, after cooling, apply ointment for burns.

Sulfuric acid should be wiped from the surface of the skin first with a dry cloth, and then washed with water!!

b) Injuries caused by concentrated alkalis

Eyes

Wash immediately with a stream of lukewarm water. Washing must last at least 5 minutes. In any case it is necessary to seek the help of the ophthalmologist.

Skin

Immediately wash the spilled area with cold water. Let the washing last for a few minutes. If they are resulting in open wounds, they should seek the help of a doctor. At the end, smear the injured place with ointment for burns.

Explosions and fires

Explosions and fires most often occur due to improperly assembled equipment or incorrect management of chemical reactions, improper heating of flammable substances, etc. A gas explosion can happen if gas is released uncontrollably from installations or a gas burner. An explosion is created by a mixture of gas and air if there is 5-15% gas in the mixture of gas and air. Natural gas is a mixture of various hydrocarbons, with methane (CH_4) making up more than 90%.

In the event of a fire, all flammable substances should be removed, the main gas supply should be turned off, electrical devices should be switched off, and doors and windows should be closed. Smaller fires in the laboratory can be extinguished with wet cloths, sand, or fire extinguishers.

Fire Extinguishers

Fire extinguishers should be placed near fire-prone areas so that they are visible and accessible. They must be installed at a height of no more than 1.5 meters. After each use, the fire extinguisher must be serviced. Fire extinguishers are classified based on the extinguishing agents they contain, and the types are as follows:

- Powder fire extinguisher „S“
- Carbon dioxide fire extinguisher (most often used)
- Water fire extinguisher
- Halon fire extinguisher
- Foam fire extinguisher

Activation and Operation of a Fire Extinguisher:

1. Bring the extinguisher and shake it.
2. Pull out the safety pin from the movable handle on the valve.
3. Hit the movable handle of the valve with your palm to release it fully, and wait 5 seconds.
4. Approach the fire as much as possible.
5. Release the hose with the nozzle from its seat, aim at the fire, and press the lever every few seconds.
6. Direct the stream from the extinguisher at the source of the fire, not at the flames.

Figure 1. Fire Extinguisher

Signs for Hazard Identification in Laboratory Work (Pictograms)

Pictograms are graphic images that immediately show the user of a hazardous product and what type of hazard is present. With a quick glance, you can see, for example, that the product is flammable or that it might be a health hazard.

Most pictograms have a distinctive red „square set on one of its points“ border. Inside this square is a symbol that represents the potential hazard (e.g., fire, health hazard, corrosive, etc.). Together, the symbol and the border are referred to as a pictogram. Pictograms are assigned to specific hazard classes or categories.

The graphic below shows hazard pictograms. The bold type is the name given to the pictogram; the text inside the brackets describes the hazard.

	**Exploding bomb** (for explosion or reactivity hazards)		**Flame** (for fire hazards)		**Flame over circle** (for oxidizing hazards)
	**Gas cylinder** (for gases under pressure)		**Corrosion** (for corrosive damage to metals, as well as skin, eyes)		**Skull and Crossbones** (can cause death or toxicity with short exposure to small amounts)
	**Health hazard** (may cause or suspected of causing serious health effects)		**Exclamation mark** (may cause less serious health effects or damage the ozone layer*)		**Environment*** (may cause damage to the aquatic environment)

Figure 2. Pictograms and meaning of corresponding pictograms

Where will I find the pictograms?

Pictograms will be on the product supplier labels of the hazardous products.

Figure 3. Pictograms on the bottle of hazardous products.


OH
CO_2
OH
OH
Vegetble
scraps
Nitrogen
compoueds
COMPOSTIG
COMPOS

2. RESEARCH PAPER

2.1. TOPIC 1: COMPOSTING

Group Work: Composting

Topic: Composting Viewed from Different Aspects

Objective: To explore and analyze the composting process and demonstrate its impact on the environment, agriculture, economy, and society. Based on the research, create a presentation that will clearly and concisely display the key information about composting.

Task Distribution (Aspects of Composting)

Guidelines for the Research Paper:

1. **The Composting Process**
    - Explain the basic composting process (what it is, what happens in the soil, which microorganisms are involved).
    - List what can be composted (organic waste: kitchen, garden, industrial waste, etc.).
    - Highlight the conditions needed for successful composting (temperature, moisture, air).
2. **Ecological Aspect of Composting**
    - Explain the benefits of composting for the environment (waste reduction, methane emission reduction in landfills, soil enrichment).
    - Consider composting as an alternative to reduce the need for chemical fertilizers.
    - Mention the role of composting in preserving soil biodiversity and sustainable resource management.
3. **Social and Economic Aspect of Composting**
    - Explore the economic benefits of composting (waste disposal cost reduction, compost market, employment in the composting industry).
    - Analyze social benefits (increased awareness of sustainable development, community education, and engagement).
    - Discuss challenges in implementing composting in urban and rural areas.
4. **Composting in Agriculture and Horticulture**
    - Investigate how composting improves soil quality in agriculture (fertilizer, soil structure improvement, fertility increase).
    - Consider the application of compost in horticulture (plant growth, reduced need for chemical pesticides and fertilizers).

- Provide examples of successful compost applications in agriculture (e.g., in organic farming).

5. **Composting in Urban Areas**
   - Explore how composting is implemented in urban areas.
   - Consider urban composting initiatives, including household composting, community composting, and compost use for urban gardens.

Instructions for Creating the Presentation:

- Tools for creating the presentation: PowerPoint, Google Slides, Prezi, or any other presentation tool according to personal preferences.
- Slide Structure:
  - The opening slide should include the topic title, the names of the group members, and the date.
  - Include images, diagrams, charts, and other visual elements to make the presentation clearer and more interesting.
  - End with a slide summarizing conclusions and suggestions.
- Group Work: Each group member should actively participate in researching and presenting their part. Groups should collaborate on integrating all parts of the presentation.

Recommended Research Materials:

- Books and articles on sustainable waste management.
- Websites and videos of environmental protection organizations.
- Professional journals and reports on ecological and agricultural composting.

Expected Outcome:

The presentation should clearly, concisely, and visually present all aspects of composting. By the end of the task, the group should be able to argue the importance of composting as an ecological and social solution for sustainable development.

Good luck with the work, research, and creating the presentation!

2.2. TOPIC 2: WASTEWATER TREATMENT

Group Work: Wastewater Treatment

Topic: Wastewater Treatment Viewed from Different Aspects: Processes in Slovakia and Croatia

Objective: To explore and compare wastewater treatment methods in Slovakia and Croatia, analyze the benefits and challenges of these processes, and create a presentation based on the research.

Task Distribution (Aspects of Wastewater Treatment)

Guidelines for the Research Paper:

- **Basic Wastewater Treatment Processes**
  - Explain the basic methods of wastewater treatment (mechanical, biological, chemical treatment).
  - Discuss the importance of each phase of treatment for environmental preservation and human health.
  - Consider different technologies such as sedimentation, filtration, activated sludge, biofilters, and microorganism use.
- **Wastewater Treatment in Slovakia**
  - Explore the wastewater treatment methods used in Slovakia.
  - Discuss the specifics of Slovakia's wastewater treatment system.
  - Mention key successes and challenges in wastewater management in Slovakia.
  - If possible, include data on wastewater treatment plants in Slovakia and their environmental impact.
- **Wastewater Treatment in Croatia**
  - Explore the wastewater treatment system in Croatia.
  - Discuss the technologies used in Croatia (differences compared to Slovakia).
  - Mention specific challenges in Croatia (e.g., infrastructure aging, geographical or financial barriers).
  - Include data on key wastewater treatment plants in Croatia and compare them with Slovakia's.
- **Ecological and Social Aspects of Wastewater Treatment**
  - Explain the ecological benefits of wastewater treatment (reducing water pollution, protecting ecosystems).
  - Discuss the social and economic impacts (employment, resource conservation, sanitation).

    - Compare how Slovakia and Croatia manage the social and ecological challenges related to wastewater.
- **Impact of Wastewater Treatment on the Environment**
    - Analyze the impact of treated wastewater on the environment, including water quality in rivers, lakes, and groundwater.
    - Consider the aspects of treated water reuse in agriculture, industry, and urban areas, along with challenges regarding safety and sustainability.

Instructions for Creating the Presentation:

- Tools for creating the presentation: PowerPoint, Google Slides, Prezi, or any other presentation tool according to personal preferences.
- Slide Structure:
    - The opening slide should include the topic title, the names of the group members, and the date.
    - Include images, diagrams, charts, and other visual elements to make the presentation clearer and more interesting.
    - End with a slide summarizing conclusions and suggestions.
- Group Work: Each group member should actively participate in researching and presenting their part. Groups should collaborate on integrating all parts of the presentation.

Recommended Research Materials:

- Scientific papers, reports, and studies.
- Official websites related to environmental protection in Slovakia and Croatia.
- Video materials on wastewater treatment plants and their technologies.
- Statistical data on water quality and treatment systems in both countries.

Expected Outcome:

The presentation should clearly display the wastewater treatment process in Slovakia and Croatia, compare different approaches and technologies, and provide insights into the ecological, economic, and social benefits and challenges.

Good luck with the work, research, and creating the presentation!

2.3. TOPIC 3: BIODIESEL PRODUCTION

Group Work: Biodiesel Production

Topic: Biodiesel Synthesis Processes Through Researching Different Technologies and Principles of Production Viewed from Various Aspects.

Objective:

Task Distribution (Aspects of Biodiesel Production)

Guidelines for the Research Paper:

1. **Basic Types of Biofuels**
    - Research different types of biofuels, such as bioethanol, biodiesel, biogas, and bio-liquefied gas. Understand what they are made from, what raw materials are used (e.g., plant oils, waste, algae, agricultural residues), and their advantages and challenges in application.
2. **Technologies and Production Processes**
    - Consider different technologies and processes for biofuel production, including fermentation for bioethanol production, transesterification for biodiesel production, and anaerobic digestion for biogas production.
    - Research the most promising and efficient technological innovations.
3. **Ecological Impacts of Biofuel Production**
    - Investigate the environmental impact of biofuel production, including water consumption, greenhouse gas emissions, biodiversity impact, and land use.
4. **Economic Sustainability and Biofuel Market**
    - Consider the economic aspects of biofuel production, including raw material costs, infrastructure, subsidies, and competition with fossil fuels.
    - Study global biofuel markets, policy development, and economic incentives for companies investing in biofuels.
5. **Future and Innovations in Biofuel Production**
    - Research trends and innovations in biofuel production, such as the use of algae, advanced second-generation biofuels (produced from non-food raw materials like lignocellulose), and potential new technologies to improve production efficiency and reduce environmental impacts.

Instructions for Creating the Presentation:

- Tools for creating the presentation: PowerPoint, Google Slides, Prezi, or any other presentation tool according to personal preferences.

- Slide Structure:
    - The opening slide should include the topic title, the names of the group members, and the date.
    - Include images, diagrams, charts, and other visual elements to make the presentation clearer and more interesting.
    - End with a slide summarizing conclusions and suggestions.
- Group Work: Each group member should actively participate in researching and presenting their part. Groups should collaborate on integrating all parts of the presentation.

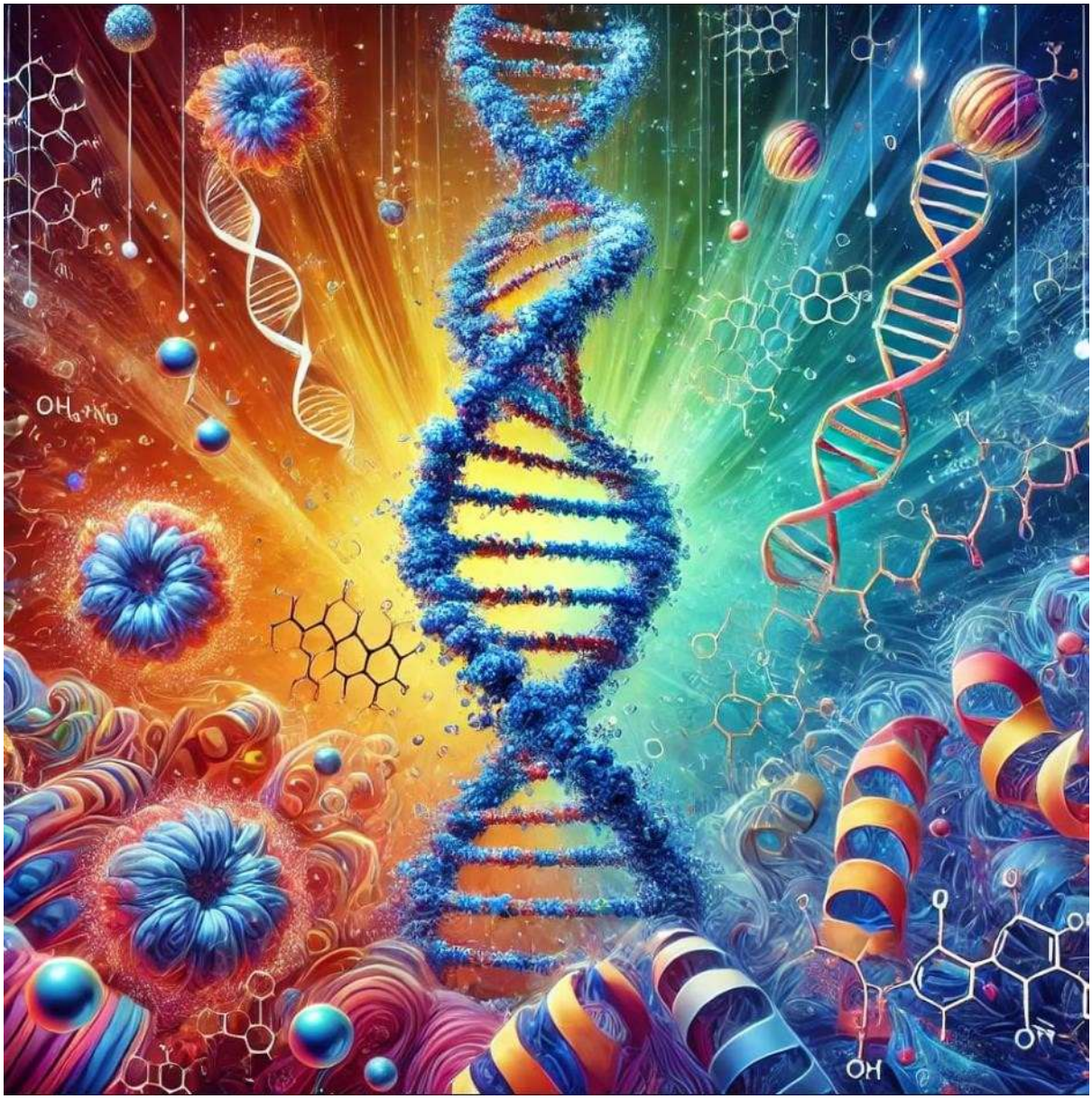
Recommended Research Materials:

- Scientific papers, reports, and studies.
- Official websites related to biodiesel production.
- Available video materials.

Expected Outcome:

The research results should include identifying and evaluating the raw materials for biodiesel production, technical efficiency, and biodiesel quality based on the production method. Consider the environmental impact and economic viability of biodiesel production. Also, consider potential challenges and barriers in the biodiesel industry.

Good luck with the work, research, and creating the presentation!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3. PROTEINS LECTURE AND PROTEINS IN ACTION

Experiment 1

Equipment and chemicals: 3 test tubes, test tube rack, mortar and pestle, spatula, *w*(NaOH) = 10 %, *w*($CuSO_4$) = 1 %, lactase, proteinase and amylase solutions, distilled water, 3 droppers.

NaOH solution		 
$CuSO_4$ solution		

Step 1

Grind the lactase pill in the mortar. Transfer part of the powder into a test tube and add 1 mL of distilled water. Record your observations.

_____.

Step 2

Pour 1 mL of the prepared proteinase and amylase solutions into the second and third test tubes. Then add 1 mL of sodium hydroxide solution to each test tube and gently shake.

Question 1: Are there any visible changes in the test tubes after adding sodium hydroxide?

_____.

Step 3

Carefully add 1-2 drops of solution X to each test tube and shake. Record your observations.

_____.

Question 2: Can you conclude what solution X is? What is its role in the experiment?

_____.

Question 3: What is this reaction called?

_____.

Task 2: Make a conclusion: Which group of compounds do the substances (enzymes) in the test tubes belong to?

_____.

Task 3: Based on the diagram of the spatial structure of enzymes shown in Figure 4, what can you conclude about enzyme structure?

_____.

Task 4: Use the general structural formula to show one building unit in this polymer structure.

_____.

Question 4: Explain why polymer chains fold into secondary and tertiary structures?

_____.

TASK 5: In Figure, name the bonds and interactions in the marked places and define the individual structures.

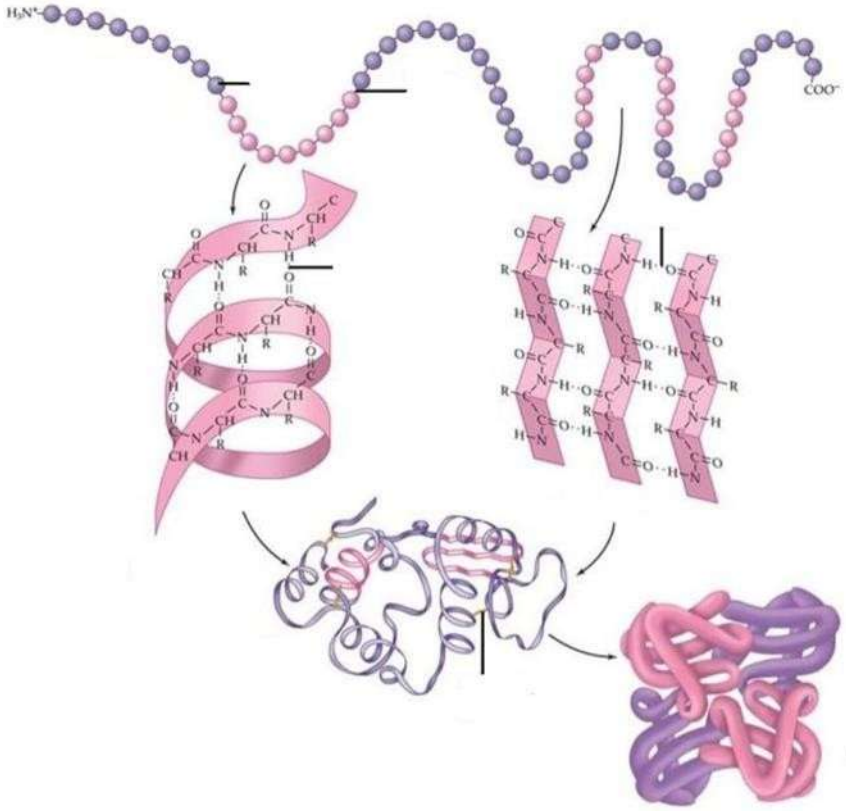


Figure 4. Protein structure

Experiment 2

Equipment and chemicals: 5 test tubes, test tube rack, starch solution, Lugol's iodine, distilled water, amylase solution, buffers of different pH values (3, 5, 7, 8, 10).

Step 1

Pour 7 mL of starch solution into a test tube, add 1 drop of Lugol's iodine, and shake. Record your observations.

_____.

Question 5: What does Lugol's iodine detect? Explain the observed effect. Make a structural sketch of the created ion.

_____.

Step 2

Divide the starch solution into 5 test tubes equally. Add 2 mL of buffers of varying pH values to each test tube. Sketch the setup.

Step 3

Assign someone to measure reaction times. Add 2-4 drops of amylase solution to the first test tube and start timing. Record the reaction time until the color disappears. Repeat for the remaining test tubes. Calculate reciprocal values for the recorded times and enter them into the table.

Table 1: Reaction time for different pH buffers

pH Value	Reaction time	Reciprocal value
3		
5		
7		
8		
10		

Task 6: Describe the changes in the test tubes and draw a conclusion.

_____.

Task 7:

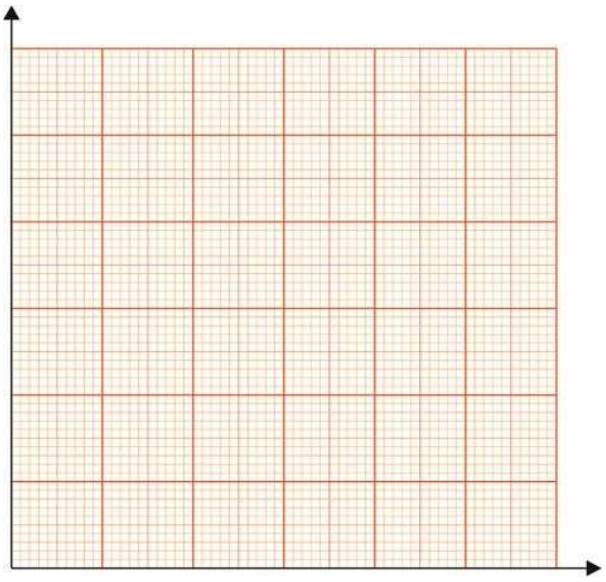


Figure 5. The reciprocal reaction time values against the pH of each reaction mixture.

Demonstration Experiment

Equipment and chemicals: 3 test tubes, test tube rack, beaker, water heater, Fehling's reagent, dropper, starch solution, Lugol's iodine, amylase solution.

Setup

The teacher prepares identical mixtures (amylase, starch, and Lugol's iodine) in two test tubes, adding Fehling's reagent. A third test tube contains starch, Lugol's iodine, and Fehling's reagent without amylase. One of the first two test tubes and the third are placed in a hot water bath (40°C), while the other remains at room temperature. After 4 minutes, the test tubes are observed.

Task 9: Sketch the test tubes and record observations.

_____.

Task 10: Recall the two components of Fehling's reagent and write the reaction equation with glucose.

_____.

Experiment 3

Equipment and chemicals: Protease source (pineapple extract), collagen with red beads, 3 test tubes, test tube rack, water heater, ice, glass beaker, 5 mL measuring cylinder.

Step 1

Pour 1 mL of collagen into each test tube. Place the first in hot water, the second in ice, and the third at room temperature. Add 3 drops of fresh pineapple extract to each test tube, shake, and observe after 1.5 minutes.

Task 11: Describe the observations and conclude which test tube shows the weakest reaction.

_____

Task 12: Hypothesize the effect of canned pineapple extract on red-labelled collagen.

_____

Question 8: What explains the different enzyme activities in fresh versus canned pineapple?

_____

Step 2

Propose an experiment to test this hypothesis using available materials.

_____

Conclusion:


9NA DNA DNA PROTEIN PROTEIN
PROTEIN
DNA

4. DETERMINATION OF MOLECULAR WEIGHT USING SDS-PAGE

Principle: When proteins are denatured, they bind SDS in a nearly constant mass ratio (1.4 g SDS per 1 g of protein). The resulting SDS-protein complexes acquire uniform charge density and migrate based on their molecular mass during electrophoresis. It has been established that, under these conditions, the graphical relationship between log10 M_r and R_f values (the distance a protein travels relative to the distance a dye travels) is strictly linear. Therefore, a set of proteins with known molecular masses is used to calibrate the gel system. These proteins are separated and visualized alongside the analyzed samples. The R_f values of the markers are calculated and plotted on a semilogarithmic scale against molecular masses to create a calibration curve. The R_f values of the sample proteins are then used to determine their molecular masses from the calibration curve.

Theoretical Background: SDS-PAGE is a method that uses polyacrylamide gels to separate proteins based on size. Proteins are denatured by SDS (sodium dodecyl sulfate), which imparts a negative charge to the molecules, enabling them to migrate toward the positive electrode under an electric field. Larger proteins migrate more slowly through the gel, while smaller proteins migrate faster. This allows size estimation based on migration profiles relative to molecular markers.

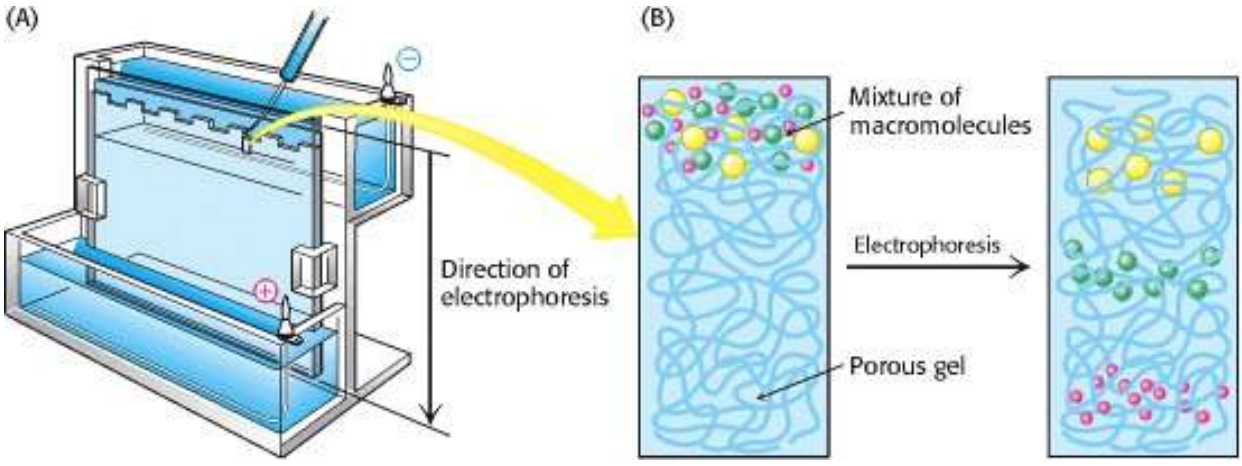


Figure 6. (A) Application of samples onto the gel.(B) Separation of a mixture of macromolecules (Adapted from J. M. Berg et al., Biochemistry, 5th ed.)

Exercise Title: Electrophoresis of Proteins in SDS Polyacrylamide Gel (SDS-PAGE)

Objective: In this exercise, students will learn how to perform protein electrophoresis using SDS polyacrylamide gel (SDS-PAGE). The goal is to separate proteins based on their molecular weights, understand the method's principle, and learn to interpret the results.

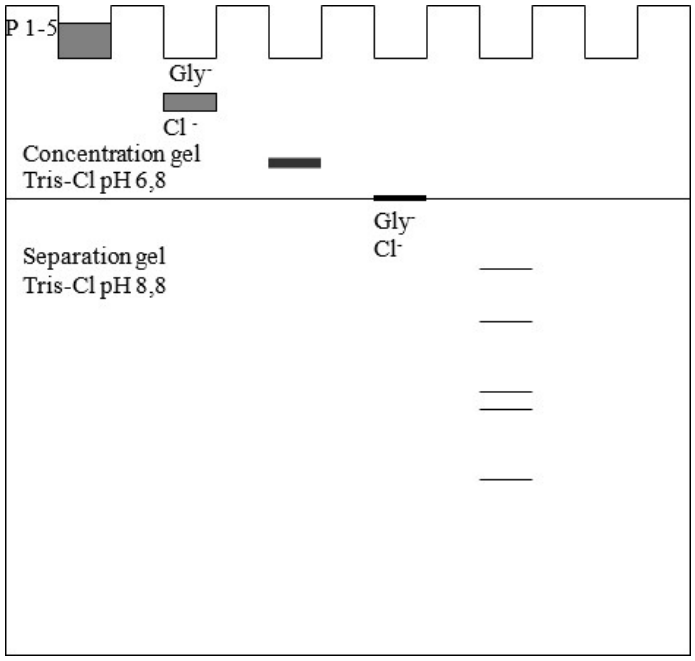


Figure 7. Concentration of proteins into a narrow starting zone in the concentration gel and upon entering the separation gel.

Procedure:

1. Assemble the apparatus for pouring gels.
2. According to the data from the table below, prepare the gel mixture for separation and, after polymerizing the gel, prepare the mixture for the concentration gel. At the end of the procedure, do not forget to insert the comb for creating wells.

Table 2. Separation gel (*w*=10%)

10 % gel	V/mL
mQ H_2O	2
Tris-HCl (c = 1,5 mol dm^{-3}, pH=8,8)	1,25
acrylamide / bisacrylamide, 30 %	1,65
SDS, 100%	0,05
APS, 10%	0,05
TEMED	0,004

Table 3. Concentration gel (*w*=5%)

5 % gel	V/mL
mQ H_2O	2,01
Tris-HCl (c=0,5 mol dm^{-3}, pH=8,8)	0,375
acrylamide / bisacrylamide, 30 %	0,503
SDS, 100%	0,03
APS, 10%	0,03
TEMED	0,001

3. After the gel polymerization, carefully remove the comb and place the gel into the electrophoresis tank, then fill it with running buffer above the level of the gel.
4. The first marker sample is applied by the teacher into one of the wells so you can observe the procedure.
5. Apply the prepared protein samples, which have been thermally treated in the presence of SDS detergent, into the wells of the gel using a micropipette (each person applies their own sample).
6. Then, close the electrophoresis tank and start the program for protein separation.
7. Turn off the electrophoresis device when the blue dye reaches the bottom edge of the gel, which can be easily monitored.
8. Next, remove the gel and place it in the staining chamber with Coomassie Brilliant Blue G-250 (CBB) stain.
9. After 2 minutes, pour out the stain and begin rinsing the gel with distilled water several times, removing excess dye from the gel using the provided blotter.
10. When you are satisfied with the clarity of the protein bands in the gel, you can scan or photograph the gel and include it in your data analysis document.

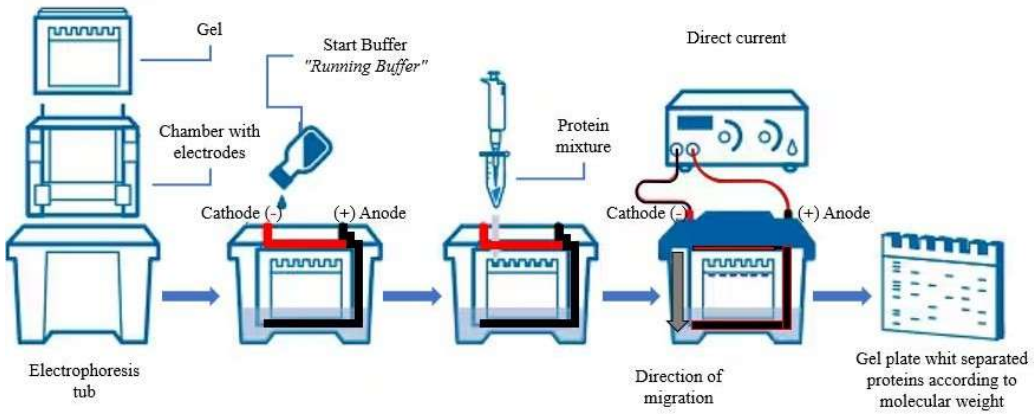


Figure 8. Layout of the prepared apparatus and procedure for separation and visualization of SDS proteins by discontinuous electrophoresis on a polyacrylamide gel.

Table 4. Results of electrophoresis and solving problems related to the molar mass of proteins.

Protein Code	Protein Name	Molecular Weight (kDa)	Protein role
α			
β			
x			
y			
z			

Task:

The molecular weights of the proteins and their names are missing in the table above. Only the labels α, β, x, y, and z are provided. During the electrophoresis process, you need to determine which proteins are involved and what their roles are using the given tasks and texts. After electrophoresis and gel staining, everyone should be able to determine the corresponding molecular weights of the proteins.

Conclusion:

5. CHROMATOGRAPHY

Chromatography is a process for separating pure substances from homogeneous liquid or gaseous mixtures. The method is based on the differing speeds at which ions or molecules carried by a solvent travel across the stationary phase. The stationary phase is typically a solid carrier with a large surface area (e.g., silica gel, calcium carbonate, or chromatography paper). The mobile phase, which is the solvent or more commonly a mixture of solvents, moves through the stationary phase.

Individual components of the mixture travel at different speeds due to various physical processes, thus separating from one another. This process is known as developing a chromatogram. The chromatogram is developed in a closed container to ensure the atmosphere remains saturated with solvent vapors. The line reached by the solvent is called the solvent front.

The position of zones on the chromatogram is determined by the R_f value for each component. The R_f value is characteristic of a specific substance in a precisely defined chromatographic system.

R_f value = distance traveled by component ÷ distance traveled by solvent

$$\boldsymbol{R}_f = \frac{d_1}{d}$$

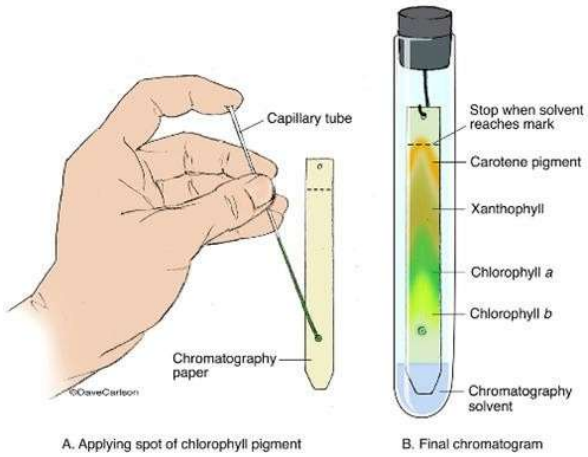


Figure 9. Chromatography on paper.

Exercise Title: Paper Chromatography

Work task: Separate plant pigments by chromatography and calculate R_f value

Equipment and chemicals: plant material, mortar with pestle, knife, 100 mL Erlenmeyer flask, beaker, 10 mL measuring cylinder, test tube, capillary, funnel, filter paper, spinach leaves or other herbs, ethanol, petroleum ether, acetone, chromatography paper.

Precautions:

ethanol		
petroleum ether		
acetone		

Procedure:

1. Chop spinach leaves (or another plant) finely with a knife, then thoroughly crush them in a mortar with a pestle. Add 2-3 mL of ethanol and mix well with the pestle (it is possible to heat the mixture slightly and then cool it to room temperature).
2. Carefully filter the resulting mixture, collecting the filtrate in a test tube. Seal the test tube tightly with a rubber stopper.
3. Prepare the mobile phase (chromatography solvent) by mixing 100 mL of benzine, 25 mL of petroleum ether, and 20 mL of acetone.
4. Cut the chromatography paper into a strip of appropriate dimensions, tapering one end of the paper. On the prepared strip of chromatography paper, mark a baseline using a pencil, 1.5–2 cm from one end of the strip. Ensure the dimensions of the paper strip match the size of the test tube so that the tapered end of the paper reaches the bottom of the test tube.

5. Immerse a glass capillary into a chlorophyll solution. Remove the capillary and touch the chromatography strip at the middle of the starting line to create a circle of solution about 2-3 mm in diameter, no larger. Wait for 5 minutes for the solution sample to dry. Repeat the procedure. Record your observations on the strip.

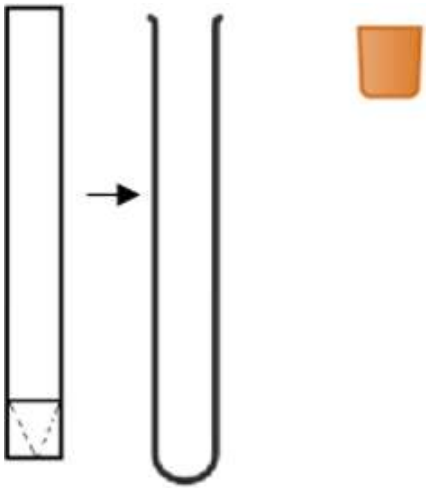


Figure 10. Chromatography setup

6. Pour the chromatography solvent into a test tube. Attach the chromatography paper strip to a holder or fold it over the edge of the test tube and immerse it in the solvent, ensuring that the starting line with the sample does not touch the solvent. Seal the test tube.

Record your observations in the test tube after immersing the strip in the solvent.

7. Chromatography takes approximately 30 minutes. After 30 minutes of chromatography, the solvent will have risen 5-6 cm on the paper strip. Remove the strip from the test tube and detach it from the holder. Immediately mark the position of the solvent front (the height the solvent has reached, d) with a pencil. Dry the strip in the air with gentle movements or using a hairdryer.

8. Mark the most intense part of each spot with a pencil as a single point. Use a triangle ruler (or straightedge) to measure the distances traveled by each spot (d1, d2..) and the solvent from the starting line.

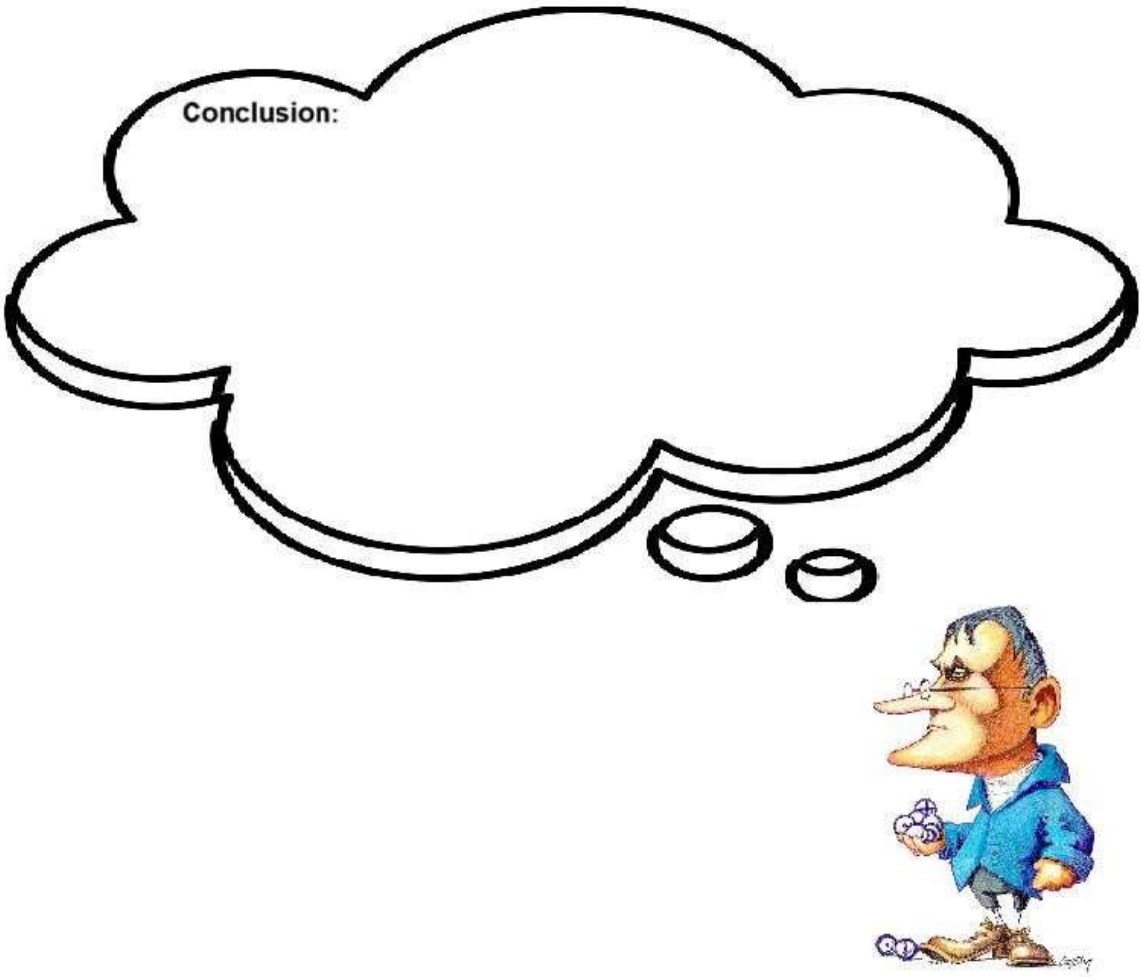
Calculate the R_f values for each spot.

Measurement results:

Solvent path length (d) _____cm

Table 5. Results of chromatography

Pigment spot	I	II	III	IV		
Path length of the pigment (d_1,d_2,d_3) /cm						
R_f value						



6. DETERMINATION OF BIOLOGICAL OXYGEN DEMAND (BOD) AND CHEMICAL OXYGEN DEMAND (COD) IN WASTEWATER

Exercise Title: Biochemical Oxygen Demand (BOD)

INTRODUCTION:

Oxygen enters the water through absorption from the air or as a product of photosynthesis during the growth of algae. The decomposition of organic substances in water decreases the oxygen concentration, which indicates water pollution.

Oxygen in water is determined using the Winkler method, where oxygen oxidizes manganese (II) hydroxide to manganese (IV) hydroxide in an alkaline medium. When the solution is acidified in the presence of potassium iodide (KI), an equivalent amount of iodine is released. It is then titrated with a sodium thiosulfate solution, using starch as an indicator.

An important indicator is the decrease in oxygen content in water, known as Biochemical Oxygen Demand, short BOD.

BOD is defined as the amount of oxygen consumed for the oxidation of impurities in water during biochemical processes. The process should happen in a neutral environment, and oxygen consumption is checked after 5, 20, or 100 days. It is labelled as BOD_5, BOD_{20}, or BOD_{100}. Most commonly, BOD_5 is determined.

Equipment: Winkler bottle (100 mL) or volumetric flask (100 mL), water bath, oxygen meter.
Chemicals: Water sample (wastewater), Manganese (II) chloride solution $w(MnCl_2)$ = 30%, sodium hydroxide solution $w(NaOH)$ = 32%, hydrochloric acid solution $w(HCl)$ = 25%, sodium thiosulfate solution $c(Na_2S_2O_3)$ = 0.05 mol dm^{-3}, potassium iodide, starch solution, distilled water.

WORK DESCRIPTION:

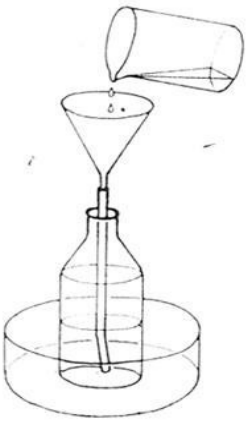


Figure 11. Filling Winkler bottle

Determination of Oxygen

This method of determining oxygen is interfered with by the presence of iron salts, organic substances, nitrites, sulfides, sulfites, and other reducing and oxidizing substances.

Procedure on the 1st day:

1. Fill a Winkler bottle with the test water by using a tube that reaches the bottom.
2. Put glass balls into the Winkler bottle and record the volume they take up.
3. Continue filling the bottle until 2 to 3 volumes of the test water overflow.
4. Remove the tube when there are no more air bubbles in the bottle.
5. Use a pipette to add 2 mL of manganese (II) chloride solution and 2 mL of alkaline potassium iodide solution to the bottom of the Winkler bottle.
6. Carefully seal the bottle (make sure no bubbles remain).
7. Turn the bottle several times to mix its contents.
8. Wait until the precipitate forms in the presence of oxygen, manganese (II) hydroxide (5 to 10 min). After the precipitate settles, the bottle is carefully opened, a small amount of clear liquid is poured out, and 3 mL of concentrated HCl is added.
9. Transfer the contents of the Winkler bottle to a 250 mL Erlenmeyer flask.
10. Rinse the Winkler bottle 2-3 times with distilled water to transfer all the precipitate to the flask.

11. Add 0.5 g of potassium iodide powder and 2 mL of hydrochloric acid to the flask.
12. Allow the mixture to stand for 10 minutes. If any precipitate remains, gently shake the flask. The mixture turns orange-brown due to liberated elemental iodine.
13. Titrate the released iodine with sodium thiosulfate solution until the solution turns yellow.
14. Titrate the released iodine with sodium thiosulfate solution until the solution turns yellow.
15. Add 1-2 mL of starch solution. The solution turns blue.
16. Continue titration, adding sodium thiosulfate drop by drop until decolorization occurs.

Procedure for determining BOD_5 ( )

· Repeat the above procedure with water which has been kept for 5 days at room temperature

· The Winkler bottle is filled to the top with water, sealed, and submerged in a water bath with the stopper facing downward to prevent air contact.

· The oxygen content of the water sample is determined and recorded.

· The oxygen content is measured again.

· The data is recorded. The difference between the initial and final oxygen content represents BOD_5.

The following equations represent these reactions:

$Mn^{2+} + 2OH^- \rightleftarrows Mn(OH)_2$

$Mn(OH)_2 + ½\ O_2 \rightleftarrows MnO(OH)_2$

$MnO(OH)_2 + 4H^+ \rightleftarrows Mn^{4+} + 3\ H_2O$

$Mn^{4+} + 2\ I^- \rightleftarrows Mn^{2+} + I_2$

$I_2 + 2\ S_2O_3^{2-} \rightleftarrows 2\ I^- + S_4O_6^{2-}$

Record all of your observations in the table.

Table 6. Analysis Results:

Water source / measured parameters	Sample 1 _____	Sample 2 _____	Sample 3 _____	Sample 4 _____
Temperature				
Initial oxygen content				
Oxygen content after 5 days (BOD_5)				
Oxygen content after 12 days (BOD_{12})				

CHEMICAL OXYGEN DEMAND (COD)

Exercise Title: Chemical Oxygen Demand

INTRODUCTION:

Chemical Oxygen Demand (COD) is marked as the amount of oxidizing agent consumed during the titration of a water sample. It is usually determined by titration with potassium permanganate solution, which is why this analysis is also known as potassium permanganate consumption.

Based on the consumption of potassium permanganate, the pollution of water by organic and inorganic substances can be assessed. A higher consumption indicates contamination and a complete chemical and microbiological water analysis is needed.

Equipment:
Erlenmeyer flasks (100 mL and 300 mL), 5 mL graduated cylinder, pipettes (50 mL and three way safety bulb), two 50 mL burettes, burner, wire gauze, tripod, funnel, boiling stones.

Chemicals:
Water sample, sulfuric acid solution (w = 0.25%), oxalic acid solution ($H_2C_2O_4$, c = 0.005 mol/dm³), potassium permanganate solution ($KMnO_4$, c = 0.002 mol/dm³).

H_2SO_4		
$H_2C_2O_4$		
$KMnO_4$		

Procedure:

1. Transfer 50 mL of the water sample by pipette into a 300 mL Erlenmeyer flask, add 2.5 mL sulfuric acid, boiling stones, and heat to boiling.
2. After boiling for 2–3 minutes, add 7.5 mL of potassium permanganate solution from a burette to the still-hot solution.
3. Cover the flask with a funnel and carefully continue heating for another 10 minutes, ensuring the liquid boils gently.
4. Add 7.5 mL of oxalic acid solution from a burette to the boiling solution. A colourless solution should result. If discoloration does not occur immediately, continue heating until it becomes colourless.
5. The colourless, still-hot solution is titrated with potassium permanganate solution until the first colour change from colourless to pale pink.

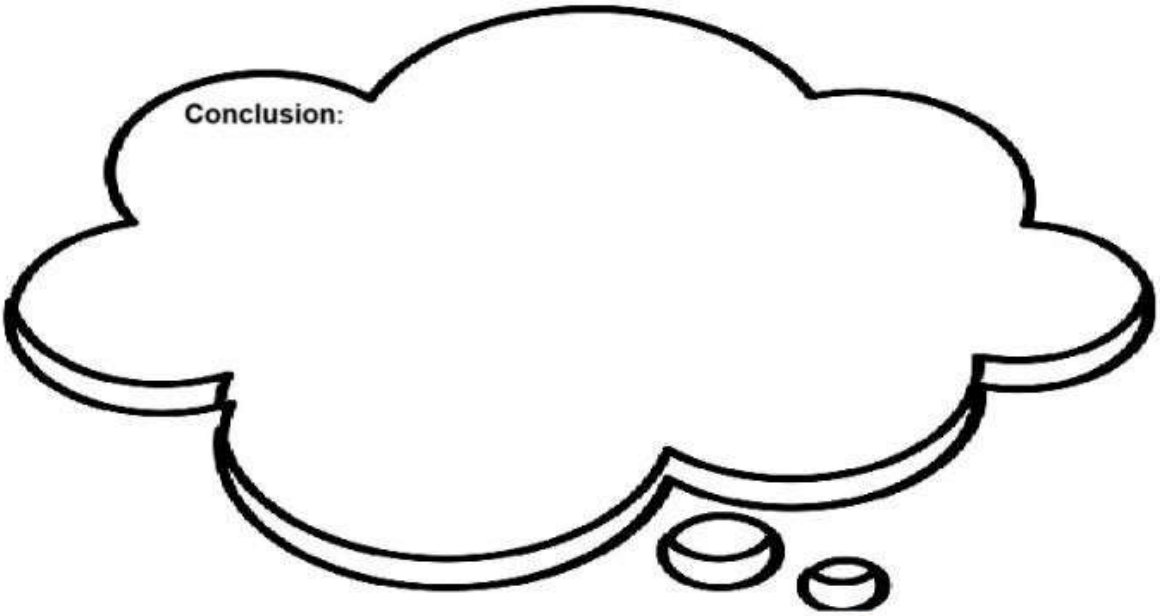
6. Record the volume of potassium permanganate used (V_1) and add it to the previously added volume ($V_1 + V_2$).
7. For a control, add 7.5 mL oxalic acid solution to a 100 mL Erlenmeyer flask, followed by 2.5 mL sulfuric acid, and titrate with potassium permanganate solution until pale pink.
8. Record the potassium permanganate volume as V_3. This volume accounts for the oxidation of oxalic acid used in the experiment, not for the oxidation of organic substances in the water.
9. Subtract this value from the total potassium permanganate volume to determine the amount used for the oxidation of organic substances in water: $V(KMnO_4) = V_1 + V_2 - V_3$.

Table 7. Analysis Data:

Water source / measured parameters	**Sample 1** _____	**Sample 2** _____	**Sample 3** _____	**Sample 4** _____
Sample volume				
Oxidizing agent concentration				
$KMnO_4$ consumption (volume)	**V_1 =** **V_2 =** **V_3 =**	**V_1 =** **V_2 =** **V_3 =**	**V_1 =** **V_2 =** **V_3 =**	**V_1 =** **V_2 =** **V_3 =**
$V(KMnO_4) = V_1 + V_2 - V_3$				

$$\gamma = \frac{m(\mathrm{KMnO_4})}{V(\mathrm{H_2O})} = \frac{n(\mathrm{KMnO_4}) \cdot M(\mathrm{KMnO_4})}{V(\mathrm{H_2O})}$$

$$\gamma = \frac{c(\mathrm{KMnO_4}) \cdot c(\mathrm{KMnO_4}) \cdot M(\mathrm{KMnO_4})}{V(\mathrm{H_2O})}$$



7. BIOFUEL SYNTHESIS

Biodiesel is a mixture of methyl esters of fatty acids (FAME; Fatty Acid Methyl Esters) from oils and fats of various sources, and is most commonly produced industrially through a chemically catalyzed transesterification reaction. Biodiesel represents an environmentally friendly alternative to conventional fossil diesel fuel. On the other hand, there are several more environmentally friendly alternatives to conventional industrial biodiesel production, such as replacing chemical catalysts with enzymes and using waste vegetable oils and animal fats instead of fresh ones. In this project, the chemical synthesis and biocatalyzed synthesis of biodiesel will be carried out from one type of waste vegetable oil.

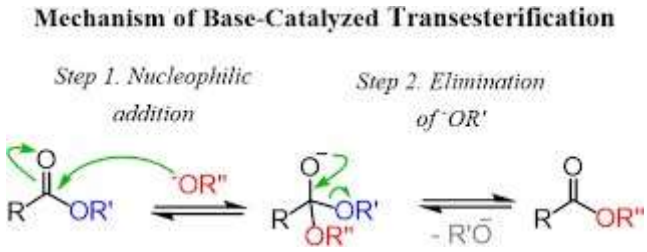


Figure 12. Biodiesel Synthesis through Transesterification from Sunflower Oil - Base Catalyzed.

EQUIPMENT: Water bath, digital scale, glass beaker (250 mL), magnetic stirrer, magnetic stirrer with heater, plastic beaker, graduated cylinder (100 mL), graduated cylinder (250 mL), stand, clamp, three-neck round-bottom flask, glass stopper, Liebig condenser, thermometer, funnel, aluminum foil, separating funnel, glass beaker (400 mL), glass container with stopper.

CHEMICALS: Methanol, KOH (solid), sample of sunflower oil.

CH_3OH		
KOH		

PROCEDURE

Preparation of Methanol and KOH Solution

Add 60 mL of methanol and 3 g of KOH to a glass beaker. Place the beaker with the solution on the magnetic stirrer until all the KOH has dissolved in the methanol.

WORK DESCRIPTION:

Transesterification

Set up the heating apparatus: place the three-neck round-bottom flask (500 mL) on the magnetic stirrer with a heater (aluminum foil). Attach the Liebig condenser to the middle neck of the flask (used for condensing methanol). Place a thermometer in the second neck to monitor the reaction temperature. Add 250 mL of sunflower oil to the flask. Heat the sunflower oil to 50 °C. Using a funnel, add the methanol and KOH solution through the third neck. Allow the mixture to react for 1.5 hours. Afterwards, use the separating funnel to separate the biodiesel layer from the glycerol and other impurities. After separation, it is necessary to measure the volume of the obtained product and transfer it to a glass bottle with a cap.

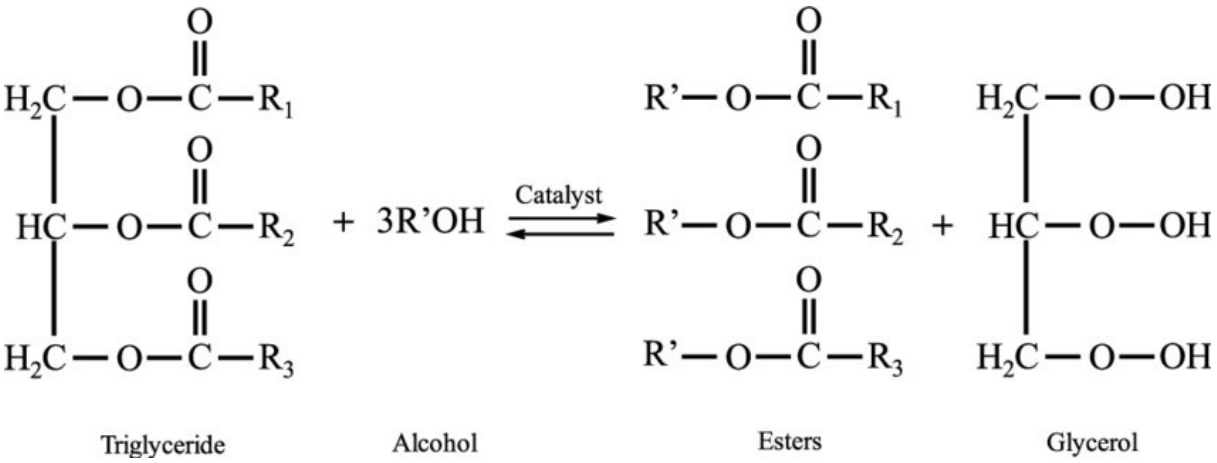


Figure 13. Representation of the Transesterification Reaction with KOH Catalyst

Synthesis of biodiesel from edible oil catalyzed by lipases from Burkholderia cepacia bacteria

Lipase from *B. cepacia* (BCL; Burkholderia cepacia Lipase) is an extracellular, heat-resistant lipase that is tolerant to various organic solvents, including short-chain alcohols (such as methanol). Due to its resistance to methanol, BCL is highly useful in biodiesel synthesis, as it prevents enzyme inactivation caused by methanol, which is a common issue in enzymatic biodiesel synthesis. The optimal temperature for BCL is 40 °C, but it also shows high activity at temperatures up to 60 °C.

For the biodiesel synthesis from waste edible vegetable oils and animal fats, a commercial Amano Lipase from *B. cepacia* bacteria (AMANO Pharmaceutical Co., Japan) was used as a catalyst for industrial applications. Industrial enzymes are specifically produced for industrial use, with their native properties enhanced through protein and genetic engineering.

Work Description: Set up the apparatus as in chemically catalyzed biodiesel synthesis. The reaction mixture consists of: 163.63 g of oil, 20.35 g of methanol, and 16.36 g of lipase. 2.86 g of lipase was prepared by dissolving it in Britton-Robinson buffer (pH = 10). The buffer was prepared by mixing equal volumes of boric, phosphoric, and acetic acids with concentrations of 4 mol/dm³. A pH value of 10 was obtained by adding sodium hydroxide solution with a concentration of 0.2 mol/dm³. The synthesis time lasts 5-6 hours. After the synthesis is complete, the reaction mixture is transferred to a separating funnel, where it separates into two phases: • the lipophilic phase (upper layer) and • the hydrophilic phase (bottom layer).

The lipophilic layer contains biodiesel, i.e., a mixture of methyl esters of fatty acids, and unreacted triacylglycerols from the oil, while the hydrophilic layer contains glycerol, water from the oil or fat and the buffer, lipase, and unreacted methanol.


BIOFUEL

8. BIODIZEL PROPERTIES

Exercise Name: PYCNOMETRIC DETERMINATION OF BIODIESEL DENSITY

Task: Determine the density of the given sample using a pycnometer

Equipment:
Pycnometer, funnel, dropper, analytical balance, thermometer, small pieces of filter paper

Chemicals:
Liquid sample for density determination, distilled water

Procedure:

1. Weigh the clean and dry pycnometer on an analytical balance and record its mass ($\boldsymbol{m}_1$).
2. Fill the pycnometer with the liquid sample, weigh it, and record the mass ($\boldsymbol{m}_2$). **NOTE:** Fill the pycnometer with the liquid in small portions to avoid air bubbles, as they can lead to inaccurate results. When the pycnometer is about half full, seal it with its stopper. The stopper will expel excess liquid through the capillary in its center. Allow a droplet to form at the top of the stopper; do not wipe it off. Carefully dry the outside of the pycnometer with filter paper (it must be completely dry). Before placing the pycnometer on the balance, wipe off the remaining droplet at the top of the capillary. Weighing must be done quickly, as the liquid evaporates rapidly through the capillary, which can cause errors in the results.
3. After weighing the sample, thoroughly wash and rinse the pycnometer with distilled water.
4. Fill the pycnometer with distilled water in the same manner as with the sample and record the mass of the pycnometer filled with distilled water ($\boldsymbol{m}_3$).
5. Empty the pycnometer and leave it to dry.
6. Calculate the density of the sample.

Measurement Results:

- Atmospheric pressure: p =..... Pa

- Laboratory temperature: t =.....°C

Table 8. Measurements results.

Mass of the empty pycnometer (m_1) / g	
Mass of the pycnometer filled with the sample (m_2) / g	
Mass of the pycnometer filled with distilled water (m_3) / g	
Water temperature (t) / °C	
Density of water at the given temperature (ρ) / g·cm^{-3}	
Density of air at the given temperature (ρ) / g·cm^{-3}	

$$\rho(\text{sample}) = \frac{m(\text{sample})}{V\ (\text{sample})} + \rho(\text{air}) = \frac{m_2 - m_1}{V(\text{sample})} + \rho(\text{air})$$

$$V(\text{sample}) = V(\text{pycnometer}) = \frac{m_3 - m_1}{\rho(H_2O) - \rho(\text{air})}$$

Figure 14. Pycnometer

Conclusion:

Exercise Title: Viscosity

Work task: Determine the absolute and relative viscosity value of the sample

Equipment: graduated pipettes, Ostwald viscometer, stopwatch, thermometer, syringe bottle, pipette bulb, stand with gripper

Chemicals: sample for viscosity determination, distilled water

Procedure:

Measure the temperature of the water and the sample.

In a well-cleaned viscometer, pipette a precisely determined volume of the tested liquid through arm c (the volume depends on the size of the viscometer).

Using a bulb pipette, draw the liquid into arm d slightly above the upper mark and let the Repeat the measurement for each given sample, as well as for distilled water, at least three liquid flow out of that arm.

Using a stopwatch, measure the time it takes for the liquid to drop from the upper to the lower mark (from a to b).

times.

Before adding another sample, the viscometer should be rinsed well with that sample two to three times.

Determine the densities of the samples with a pycnometer or read them from the tables.

Read the absolute viscosity of water from the table.

By comparing the two liquids, we obtain an expression for the absolute viscosity of the sample:

$$\eta_t = \eta_v \frac{\rho_t}{\rho_v} \frac{t_t}{t_v}$$

Expression for the relative viscosity of the sample:

$$(\eta_t)_{rel} = \frac{\eta_t}{\eta_v} = \frac{t_t}{t_v} \frac{\rho_t}{\rho_v}$$

Quantities with the index v refer to the standard liquid (water), and quantities with the index t refer to the tested liquid (sample).

Measurement results:

Table 9. Biodizel viscosity

sample number	*t* / s			
	1	2	3	mean value
1				
2				
3				
4				
5				
distilled water				

sample temperature (t_t) = _____ °C

water temperature (t_v) = _____ °C

water density (ρ_v) = _____ g /cm^3

sample density (ρ_t) = _____ g /cm^3

water viscosity at measurement temperature (η_v) = _____ mPa s

viscosity of the sample at the measurement temperature (η_t) =
_____ mPa s

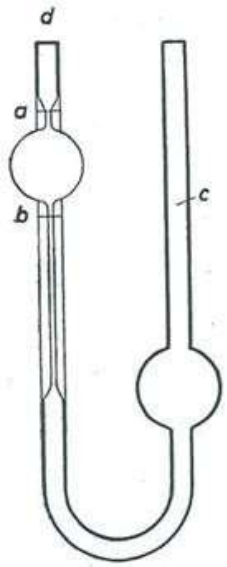
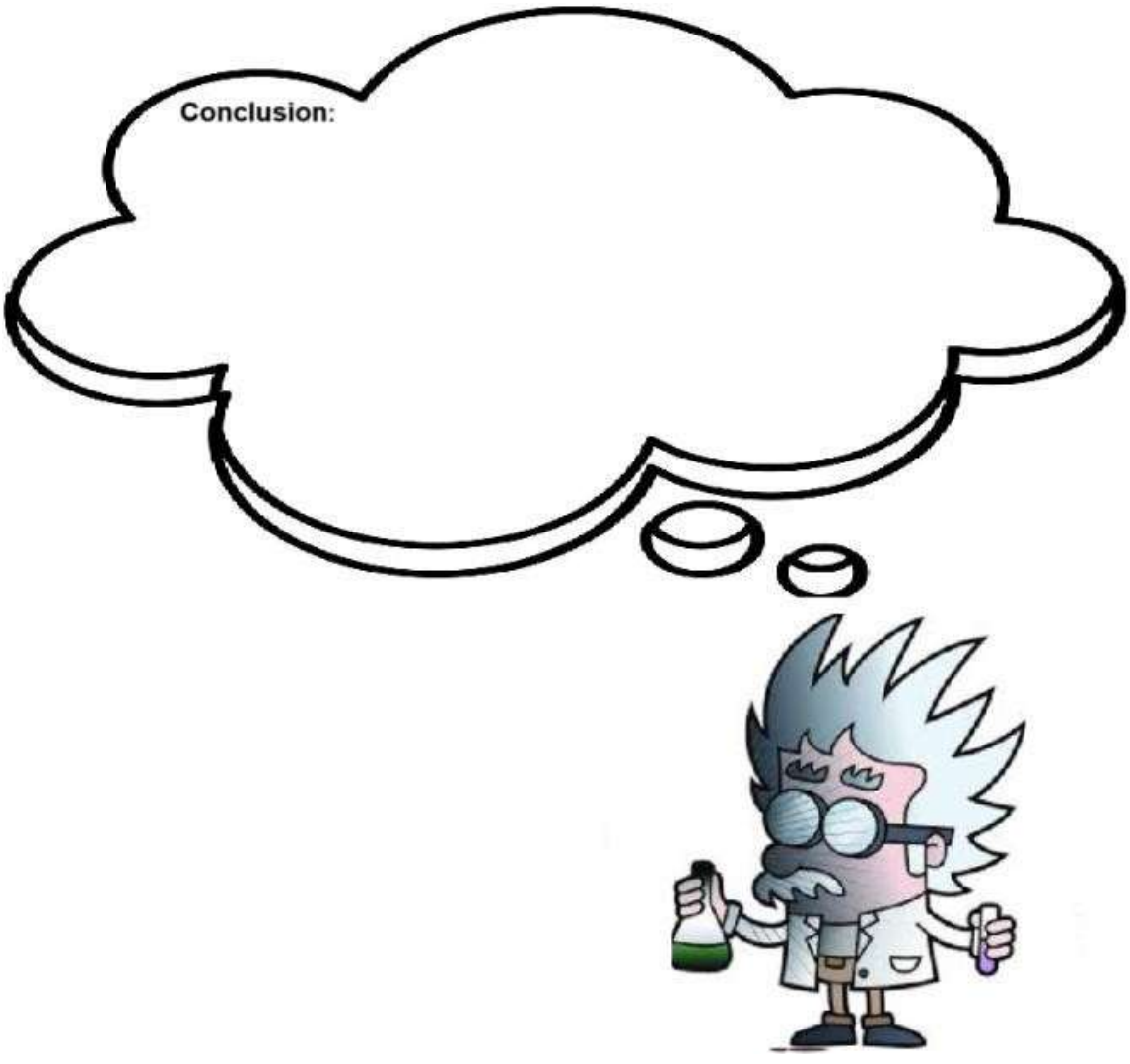
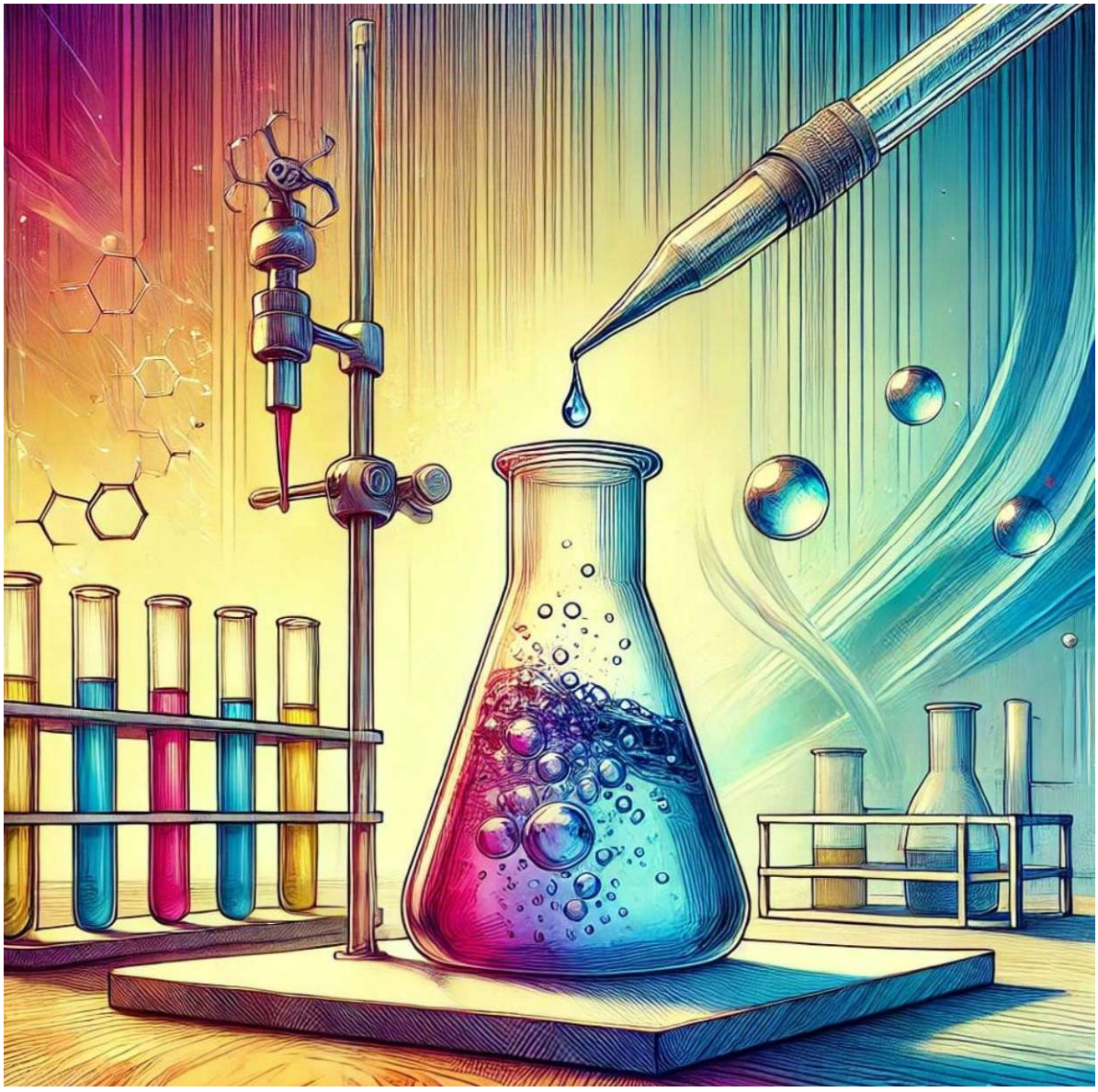


Figure 15. Ostwald viscometer





6. APPLICATIONS OF CONDUCTIVITY MEASUREMENTS

Conductivity measurement is used to determine ion concentrations, for example in water-softening devices. It is used to distinguish between weak and strong electrolytes, measure the degree of dissociation, determine the solubility of slightly soluble salts, and monitor acid-base titrations. A device for conductometric titration consists of a conductivity measurement cell immersed in the beaker where the titration is carried out. An important fact to remember throughout the discussion is that the molar conductivity of H^+ (aq) and OH^- (aq) is very high.

Let us consider changes in conductivity when a strong base (like an aqueous solution of sodium hydroxide) is titrated with a strong acid (like hydrochloric acid). At the beginning, the solution contains Na^+ and OH^- ions, and the conductivity is high. As the titration progresses, some OH^- ions are replaced by Cl^- ions. As a result, the conductivity decreases sharply (Fig.XY). At the equivalence point (when just enough acid has been added to neutralize the base), all OH^- ions are replaced by Cl^- ions, and the solution shows the conductivity typical of a sodium chloride solution in water. Beyond the equivalence point, further addition of acid causes.

Presence of H^+ (aq) ions in the solution causes the conductivity to increase sharply. The equivalence point is observed as a distinct minimum on the conductivity graph. This titration technique is useful when solutions are coloured, making the use of indicators impossible.

Let us now consider the titration of a weak base with a strong acid (Fig. XY) At the beginning, the conductivity is low because the base is only weakly dissociated. Up to the equivalence point, the addition of acid leads to the formation of a fully dissociated salt, and conductivity increases. Beyond the equivalence point, when H^+ (aq) ions are present in the solution, the conductivity rises sharply.

Conductometry is one of the primary methods for determining equivalence points in weak acid/weak base titrations (e.g., for the titration of an aqueous ammonia solution with acetic acid). Initially, only the weakly dissociated base is present. As the weak acid is added, a small number of OH^- ions are replaced, and conductivity increases because a salt (a strong electrolyte) is formed (Fig. XY). After the equivalence point, the addition of the weakly dissociated acid does not cause a significant increase in the number of conducting ions present. The distinct change in the slope at the equivalence point enables its easy determination.

Exercise Title: Conductometric Titration

Task: Determine the molar and mass concentration of the nitric acid solution through conductometric titration with sodium hydroxide.

Apparatus and Chemicals: Automatic burette, 10 mL bulb pipette, two 100 mL beakers, conductometer, magnetic stirrer, magnetic stir bar, syringe bottle, sodium hydroxide solution of known concentration, given sample of nitric acid of unknown concentration.

Introduction:

Titrations in which the equivalence point is determined by measuring the specific electrical conductivity of the reaction mixture. The equivalence point corresponds to the reaction between reactants according to the stoichiometric ratios defined by the appropriate chemical reaction, i.e., at the equivalent ratio of reactants.

The instrument called a conductometer measures the resistance (R / Ω) that the electrolyte between two electrodes (typically platinum) offers to the passage of electric current. The reciprocal value of resistance is the electrical conductivity (G / S; S – Siemens).

$$G = \frac{1}{R}$$

If the area and the distance between the electrodes are known, the cell constant for conductivity measurement can be calculated as:

$$c = \frac{l}{A}$$

where l is the distance between the electrodes and A is the surface area of the electrodes. Modern devices include a set of predefined conductometric cells, so the electrical conductivity (κ / S cm^{-1}) is directly read from the instrument.

$$\kappa = \frac{1}{R} \cdot c$$

Work Description:

1. Pipette 10.00 mL of the given sample solution (unknown molar concentration of nitric acid) into a 250 mL beaker.
2. Place the magnetic stir bar in the beaker, place the beaker with the sample solution on the magnetic stirrer, and add enough distilled water so that the electrode's tip is completely submerged in the solution. The magnetic stir bar mustn't touch the electrode.
3. Turn on the magnetic stirrer. Read the initial conductivity value. Add 0.5 mL of titration solution (sodium hydroxide of known concentration), read the conductivity, and record it in the table.
4. Also, make sure to record the exact molar concentration of the sodium hydroxide solution (to 4 or 5 decimal places).
5. Continue titration until the measured conductivity value is close to the initial value. Stop the titration and quickly rinse the electrodes.
6. After the experiment, rinse the combined electrode thoroughly with distilled water. Leave the electrode in a beaker with distilled water.
7. Plot the following graph of conductivity (κ) vs. volume of titrant added. The titration volume at the equivalence point can be determined both graphically and mathematically.

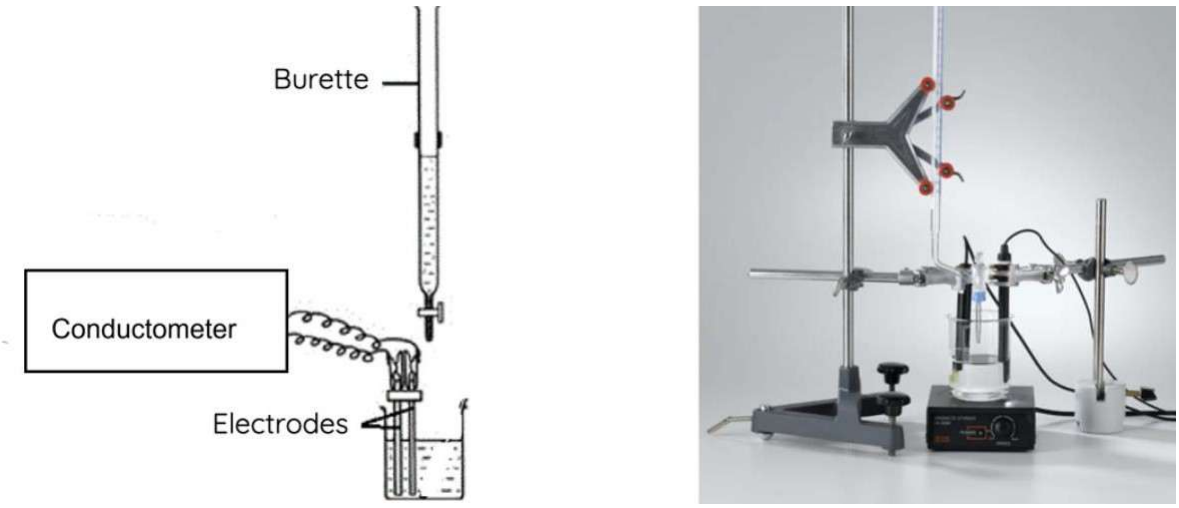


Figure 16: Apparatus for conductometric titration.

Table 10. Conductometric titration for determining the molar concentration of nitric acid, with a standardized solution of sodium hydroxide of known molar concentration, c(NaOH) = _____mol dm^{-3} at 25 °C.

V(NaOH) / mL	κ / mS cm^{-1}	V(NaOH) / mL	κ / mS cm^{-1}

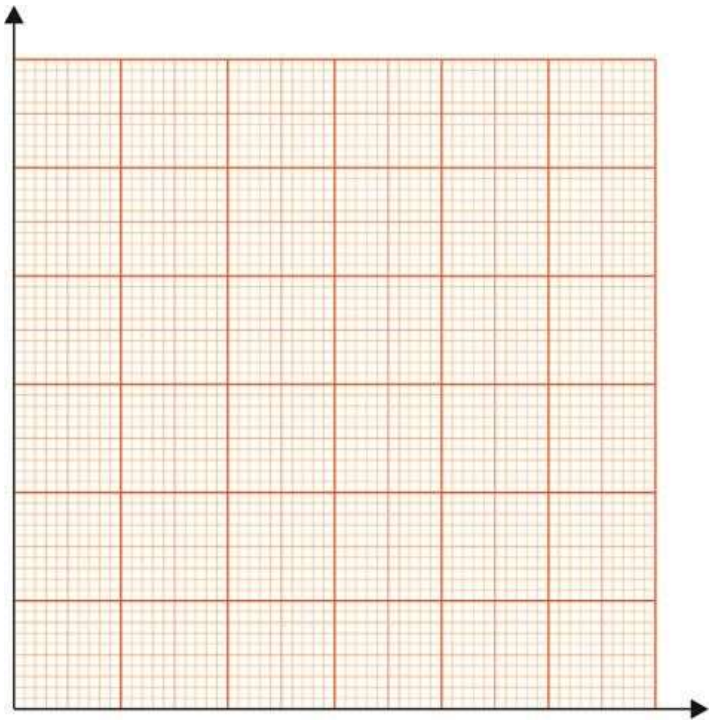
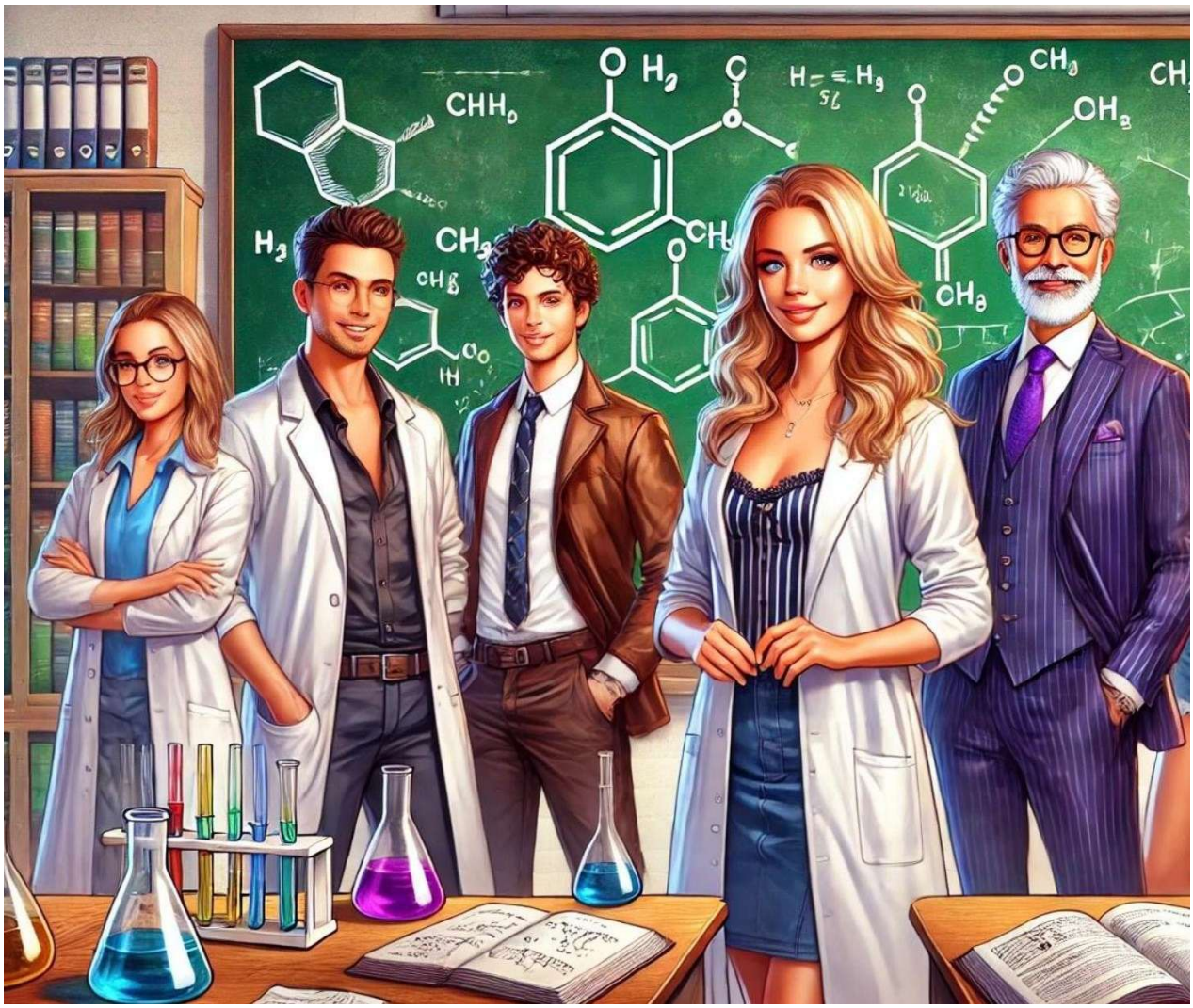


Figure 17. Conductometric titration for determining the molar concentration of nitric acid, with a standardized solution of sodium hydroxide of known molar concentration.

Conclusion:



HOPE YOU HAD A GREAT TIME!!!