DENTISTRY

MANUAL LABORATORY CLASSES: BIOCHEMISTRY I

Contents

INTRODUCTION TO SPECTROPHOTOMETRY

Date:

Aims:

1. Determine whether the presented samples of substances absorb UV / visible light
2. Measure the absorbance at different concentrations of the Evans solutions.
3. Solve the case reports.

1. Determine whether the presented samples of substances absorb UV / visible light.

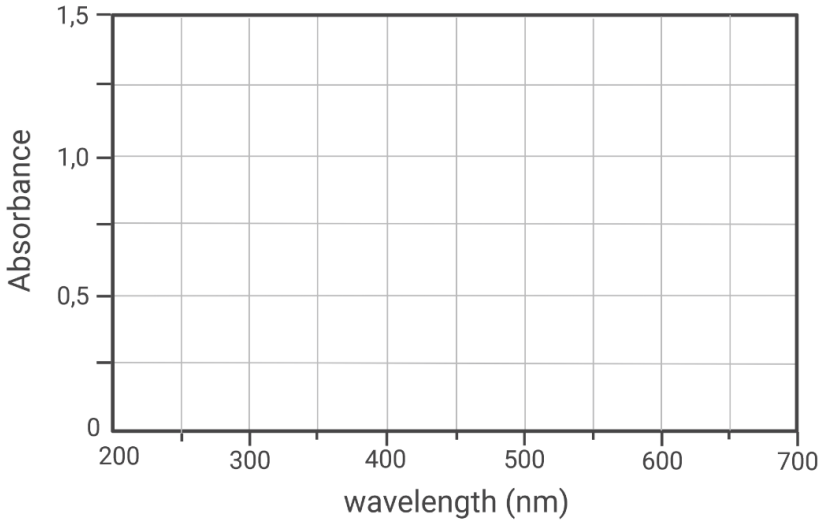
For the samples presented, write down whether they are coloured, if so, estimate approximately their absorption maximum and verify the answer on a spectrophotometer.

Procedure:

- Turn on the spectrophotometer. On the instrument display select Scan mode and load the pre-saved task named “praktikum 1/1.”
- Prepare three clean cuvettes (do not touch clear sides). Fill the first cuvette about two-thirds full with distilled water (this will serve as the blank). Wipe the clear sides of the cuvette with a lint-free tissue and insert it into the instrument. Press Blank to zero the instrument.
- Fill the second cuvette with the glucose sample, insert it and measure the absorbance spectrum. Record the spectrum.
- Repeat the measurement for the albumin and Evans solution samples. Before measuring each new sample, return the cuvette with distilled water to the spectrophotometer and press Blank to re-zero the instrument.

Sample	**Colour Y/N**	**Absorption UV/VIS/None**	**Absorption maximum (nm)**
Glucose			
Albumin			
Evans solution			

Sketch the absorption spectra of all samples on a single graph for comparison.



2. Measure the absorbance and absorption spectra at different concentrations of the Evans solutions.

Procedure:

- Prepare the diluted sample (Sample 2) by making a 5× dilution of the Evans stock solution in a clean test tube. Use an appropriate pipette and suitable pipette tips for the dilution. The undiluted stock Evans solution prepared in Task 1 will be used as Sample 1.
- On the spectrophotometer display select Scan mode and load the pre-saved task named "praktikum 1/2."
- Zero the instrument using the blank: place a cuvette filled with distilled water (clean the clear sides of the cuvette first) into the spectrophotometer and press Blank.
- Pour Sample 1 (undiluted) into a clean cuvette and measure its absorbance spectrum. Then empty the cuvette, rinse it with a small amount of the diluted sample and dry it with a lint-free tissue.
- Only after rinsing and drying, pour Sample 2 into the cuvette and measure its absorbance spectrum.

	Evans solution (mL)	**Water (mL)**	**Total volume (mL)**	**Absorption maximum (nm)**	**Absorbance in the abs. maximum**
Sample 1	2 ml	-----	2 mL		
Sample 2 Evans solution diluted 5x			2 mL		

Compare the absorption spectra, the absorption maxima, and the absorbance at the absorption maximum for the diluted and undiluted solutions and record the values in the table.

Conclusion:

Summarize the results of your measurements. The following questions may help you evaluate the data:

Evaluation and interpretation of results — Task 1:

How accurate were your estimates of the absorption maxima?
How is the colour of the Evans solution related to its absorption spectrum?
Give examples of other organic substances in the body that do and do not absorb visible light.

Evaluation and interpretation of results — Task 2:

How did the absorbance and the absorption maximum change when the Evans solution was diluted?

3. Case studies

You will receive a case card from the teaching assistant.

1) Based on your knowledge and the information given in the case study, briefly explain the mechanism by which sunscreens provide protection.

2) Using your knowledge of molecular absorption spectra, explain the changes in colour of a bruise (haematoma).

OPTICAL METHODS – SPECTROPHOTOMETRY

Date:

Aims:

1. Verify the validity of the Beer–Lambert law using a calibration series of Fe^{2+} solutions
2. Determine the Fe^{2+} concentration in an unknown sample using calibration curve
3. Compare mathematical approaches suitable for concentration determination

1. Verify the validity of the Beer–Lambert law using a calibration series of Fe^{2+} solutions.

Principle of the assay: In dilute solutions Fe^{2+} is almost colourless. It reacts quantitatively with 2,2'-bipyridine (dipyridine) to form a red complex with an absorption maximum at 520 nm.

Following the procedure below, prepare a **calibration series by diluting the Fe^{2+} stock solution** (**350 µmol·L^{-1}**).

Procedure:

- Prepare eight 50 mL volumetric flasks. Label them 1–8 as shown in the table. Samples 1–6 will form the calibration series. Samples 7 and 8 are unknowns for Task 2.
- Using a glass pipette, add the appropriate volume of the Fe^{2+} stock solution to flasks 2–6 (see the table for volumes).
- Using a glass pipette, add the appropriate volume of the unknown sample(s) into flasks 7 and 8.
- Using the dispenser, add the dipyridine reagent to all flasks, then add acetate buffer. Pour distilled water into each flask to about 1 cm below the calibration mark. Using a dropper, fill each flask precisely to the mark with distilled water. Close each flask with a stopper and mix thoroughly.
- Measure and record the absorbance of each sample at 520 nm. Measure in order from the lowest concentration sample to the highest. Measure the diluted unknown(s) last. Important: between measurements do not rinse the cuvette with water; instead rinse it with a small volume of the sample that you will measure next.

Sample No	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Fe^{2+}solution (mL)	**-**	**2**	**5**	**10**	**15**	**20**	**-**	**-**
Unknown sample (mL)	**-**	**-**	**-**	**-**	**-**	**-**	**5**	**0.5**
Reagent (mL)	**0.5**							
Acetate buffer (mL)	**5**							
Water	**fill flask below the mark**							
Absorbance								
Concentration Fe^{2+} calculated (µmol/L)								

Results:

- Create a table containing the calculated concentration and measured absorbances in Excel spreadsheet.
- Construct the calibration curve in Excel. Create a scatter plot using your data. Select a point in chart, right click and select Add trendline item. Add a linear trendline to the plot. In trendline options select linear trendline and check field Display Equation on chart and Display R-squared value on chart.

Equation:

R-squared value:

- Evaluate your calibration line: examine its linearity and identify any outliers (points that lie clearly off the regression line). If necessary, remove outliers and state the reasons for their removal in the conclusion. After any removal, recalculate and record the regression equation and the correlation coefficient.

Equation:

R-squared value:

2. Determine the Fe^{2+} concentration in an unknown sample using calibration curve.

Since the concentration in the unknown sample was not known beforehand, you prepared two dilutions of the sample simultaneously (e.g. 10x and 100x).

Substitute the absorbance values of the unknown sample (flasks/tubes 7 and 8) into the final (adjusted) calibration-line equation and calculate the Fe^{2+} concentration in the original unknown sample. Compare the two calculated values. If they differ, choose the value you consider correct and justify your choice in the conclusion.

When calculating the concentration, do not forget to account for the dilution factor (if you measured the absorbance of a 10× or 100× diluted sample, convert the result back to the concentration of the original sample).

Calculation:

Concentration of an unknown sample (units): ……

3. Compare mathematical approaches suitable for determination of concentration of Fe^{2+}

As an alternative calculation, use the method of the nearest standard. Choose the standard whose absorbance is closest to the absorbance of the unknown sample and calculate the concentration in the sample by direct proportion from the standard's absorbance and concentration.

Selected standard:

Calculation:

Concentration of an unknown sample: ……

Conclusion:

Summarize the results of your measurements. The following questions may help you evaluate the data:

- *Was your calibration curve linear across the entire concentration range? Did you remove any outlying points? If so, why?*
- *Which of your two dilutions of the unknown sample produced the correct result? What is the difference between the two results? Is the magnitude of this difference significant?*
- *If the absorbance of the unknown sample still falls outside the linear range of the calibration curve even after dilution, what is the correct course of action?*
- *What are the advantages and disadvantages of the method used in Task 2 compared with the calculation method used in Task 3?*

ELECTROCHEMICAL METHODS – POTENTIOMETRY

Date:

Aims:

1. Master the calibration of a pH meter.
2. Determine the concentration of acetic acid in vinegar.

1. Calibration of the pH meter

Procedure: First, the assistant will guide you on how to use the pH meter. Next, you will calibrate the instrument, which is essential before measuring pH. To calibrate, use two standard solutions with known pH values that match the expected range of your sample. In this case, you will use a phthalate buffer (pH 4.0, red) and a phosphate buffer (pH 7, green). The specific steps for calibration can be found in the manual for each pH meter on your bench.

2. Determine the concentration of acetic acid in vinegar

Procedure:

- Fill the burette with NaOH solution (titrant) up to the mark.
- Using a graduated cylinder, measure **20 mL of distilled water** into a 50 mL beaker. Then add **1.4 mL of vinegar** using a pipette and place a stir bar into the beaker. This creates a 15.3x diluted vinegar solution.
- Place the beaker on a magnetic stirrer and position the burette so that its tip is above the beaker. Immerse the glass electrode into the solution, ensuring the frit that connects the inner and outer parts of the electrode is submerged. Then turn on the stirrer.
- Once the pH stabilizes, record the value and note it in the table.
- Add **1 mL** of NaOH from the burette and after the pH stabilizes, record the new pH. Continue adding 1 mL of NaOH from the burette and recording until you reach a total volume of 19 mL. Record the pH after each addition!
- Then add **0.5 mL** of NaOH at a time and record pH, until you reach 20 mL.
- From 20 mL onwards, switch to using a pipette and add **0.2 mL** of NaOH and record pH, until you reach 22 mL.
- After 22 mL, revert to using the burette, adding **0.5 mL** of NaOH until you reach 23 mL. Finally, add **1 mL** of NaOH until a total volume of 25 mL is reached (see the table on the next page).

Results:

mL NaOH	0	1	2	3	4	5	6	7	8	9	10	11
pH												

mL NaOH	12	13	14	15	16	17	18	19	19,5	20	20,2	20,4
pH												

mL NaOH	20,6	20,8	21	21,2	21,4	21,6	21,8	22	22,5	23	24	25
pH												

- From the measured values, plot a graph showing dependence of pH (Y-axis) on the amount of added titrant NaOH (X-axis). Mark the equivalence point in the graph.
- Based on the determined volume of the titrant needed to reach the equivalence point, calculate the molar concentration of acetic acid (don't forget to account for the dilution). Convert this concentration into mass concentration (g/100 mL), as indicated on the label, and then compare the calculated value with the one provided by the manufacturer. (Molar mass of acetic acid: 60.052 g/mol)

Calculations:

Equivalence point – NaOH consumption (mL):	
Acetic acid concentration (mol/L):	
Acetic acid concentration (g/100 ml):	

Conclusion:

Summarize the results of your measurements. The following questions may help you evaluate the data:

- *What exactly is pH? On what principle does the pH meter you are using operate?*
- *What is the chemical formula of acetic acid? Is it a strong or a weak acid, and why?*
- *Evaluate and explain what might cause a discrepancy between your measured value and the value stated by the vinegar manufacturer on the label.*

BUFFER SOLUTIONS

Date:

Aims:

1. Prepare an acetic buffer with various pH values. Calculate pH values using Henderson-Hasselbalch equation and compare your calculation with experimental values
2. Determination of the buffering behaviour of an acetate buffer

1. Preparation of acetic buffer with various pH values

Procedure:

- Label five beakers and dispense **10 mL of distilled water** into each using a dispenser.
- Pipette **10 mL of acetic acid** (c = 0.8 mol·L^{-1}) into the first beaker and mix thoroughly.
- Withdraw 10 mL from beaker No. 1 and transfer it into beaker No. 2; mix thoroughly.
- In the same way withdraw 10 mL from beaker No. 2 and transfer it into beaker No. 3; mix. Then transfer 10 mL from 3 → 4 and 4 → 5. Finally withdraw 10 mL from beaker No. 5 and discard it (this ensures that all beakers contain the same final volume of 10 mL). The result is a geometric dilution series across beakers 1–5 (each step is a 2× dilution).
- To each beaker pipette **10 mL of sodium acetate** (c = 0.1 mol·L^{-1}).
- Measure the pH of each beaker using a pH meter. Calibrate the pH meter first using standard buffer solutions.
- Calculate the resulting concentrations of the acid and the salt in each beaker. Remember that after mixing the concentrations of the acid and the salt are half of their original (pre-mixing) concentrations. Check your results with the teaching assistant or laboratory technician.
- Then calculate the pH in each beaker by substituting into the Henderson–Hasselbalch equation (pK_a of acetic acid = 4.76) and enter the values in the table.
- Compare the theoretical (calculated) pH values with the measured values.
  Note: Do not discard the buffers — you will use them again.

Calculations:

Results:

Buffer No	**1**	**2**	**3**	**4**	**5**
CH_3COOH (mol/L)					
CH_3COONa (mol/L)					
pH values calculated					
pH values measured					

2. Determination of the buffering behaviour of an acetate buffer

Procedure:
First prepare two titration flasks with acetate buffer no. 3 from the previous exercise, in which the concentrations of the acid and its conjugate base are equal (see the table). You will estimate the buffer capacity first by titration with NaOH (c = 0.05 mol·L^{-1}) and then with HCl (c = 0.05 mol·L^{-1}).

- Fill the one burette with NaOH and another one with HCl to the chosen mark (remember to place a beaker under the burette while filling and to remove the filling funnel immediately after the burette is filled).
- Prepare the first titration: pipette **5.0 mL of buffer No. 3** and **5.0 mL of distilled water** into a clean titration flask, add one drop of phenolphthalein, and titrate with NaOH solution, adding it dropwise while swirling until a persistent pale-pink endpoint is reached.
- Prepare the second titration: pipette **5.0 mL of buffer No. 3** and **5.0 mL of distilled water** into a titration flask, add one drop of **bromophenol blue**, and titrate it with HCl solution, adding it dropwise while swirling until a persistent pale-yellow endpoint is reached.
- For the third determination, choose the buffer (from your samples) that you judge to have the better ability to maintain constant pH upon addition of NaOH. Pipette 5.0 mL of that buffer and 5.0 mL of distilled water into a titration flask, add a suitable indicator again, and titrate with NaOH dropwise.
- Compare the volumes of titrant consumed in the different titrations and draw conclusions about the buffer capacity from your measurements.

Results:

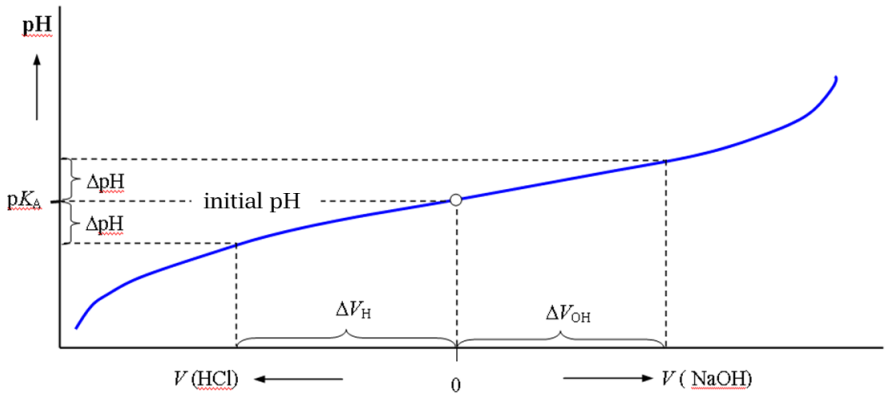
Buffer No	**3**	**3**	**..** *choose*
Water (mL)	5	5	5
Buffer (mL)	5	5	5
Colour indicator	drop	drop	drop
Volume of HCl used (mL)	-----		-----
Volume of NaOH used (mL)		-----	

Conclusion:

Summarize the results of your measurements. The following questions may help you evaluate the data.
Note: For a precise determination of buffer capacity, pH must be measured continuously during the titration with a pH meter, and the amount of titrant that produces a one-unit change in pH should be read from the titration curve. This exact determination is not part of this practical.

- *Describe the initial state before titration (point 0 on the graph — see below). Which buffer components are present in the solution?*
- *On the graph, mark the initial pH value and the points corresponding to ΔV_HCl and ΔV_NaOH.*
- *Describe what happens to the pH when a small amount of NaOH is added to the buffer. Write the chemical reaction.*
- *Describe what happens to the pH when titrating with HCl. Write the chemical reaction.*

- *How does the buffer capacity change if we double the concentrations of both the acid and the salt in the buffer?*
- *Which of the buffers you prepared has the highest capacity against addition of acid and base?*



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Problems to solve:

- *Derive the composition (relative amounts) of the phosphate buffer that occurs in blood. Dissociation constants of phosphoric acid: pK_{a1} = 2.16; pK_{a2} = 7.21; pK_{a3} = 12.67.*

- *In blood, the bicarbonate buffer system is an important buffering system. State which components it consists of. Calculate the ratio of these components at physiological pH ≈ 7.4, given that $pK_a(CO_2/H_2CO_3)$ = 6.1. Which type of added substance (an acid or a base) does this system buffer better?*

FACTORS AFFECTING LABORATORY RESULTS

Date:

Aims:

1. Experimentally test your pipetting accuracy when preparing solutions by dilution.
2. Evaluate the precision and trueness of your work; demonstrate understanding of the sources and types of error.

1. Testing pipetting precision in the process of solution preparation

Procedure:

Each subgroup will use 96-well microplate, $KMnO_4$ stock solutions R1 = 0.04 M and R2 = 0.004 M, pipettes and appropriate tips: yellow, P2–20 µL, P20–200 µL, blue P200–1000 µL.

Each student in a subgroup will pipette samples into two columns (see the table, blue and yellow columns). One student will perform the serial dilutions into the green columns (9–12).

a) Accuracy of small-volume pipetting (blue columns)

- In the first column assigned to you (wells A–H), pipette **158 µL of distilled water** into each well, add **2.0 µL** of R1 (0.04 M $KMnO_4$) to each well. Mix the samples by repeatedly aspirating into the tip and dispensing, then discard the tip.

b) Accuracy of larger-volume pipetting (yellow columns)

- In the second column assigned to you (wells A–H), pipette **140 µL of distilled water** into each well. Add **20 µL** of R2 (0.004 M $KMnO_4$) to each well. Mix the samples by repeatedly aspirating into the tip and dispensing, then discard the tip.

c) Serial (two-fold) dilution series (green columns 9–12)

- Pipette **80 µL of distilled water** into each well of columns 9–12 (wells A–H), then discard the tip.
- Add **80 µL of R2** (0.004 M $KMnO_4$) into each well of column 9 (A9–H9). Mix the samples by repeatedly aspirating into the tip and dispensing, then discard the tip. This is the starting concentration for the serial dilution.
- Transfer **80 µL** from A9 → A10, mix A10, then repeat the same transfer from B9 → B10, C9 → C10, … H9 → H10. Mix the samples by repeatedly aspirating into the tip and dispensing, then discard the tip.
- Transfer **80 µL** from A10 → A11, mix A11, and repeat for B10 → B11, … H10 → H11.
- Pipette distilled water into column 12 — this column will serve as the blank.
- Measure the absorbance of all wells in the plate at **520 nm** using the plate reader.

	1	2	3	4	5	6	7	8	9	10	11	12
	Student Nr.1		Student Nr.2		Student Nr.3							
A	2 µl R1	20µl R2	2 µl R1	20µl R2	2 µl R1	20µl R2			80µl R2	80µl R9	80µl R10	water
B	2 µl R1	20µl R2	2 µl R1	20µl R2	2 µl R1	20µl R2			80µl R2	80µl R9	80µl R10	water
C	2 µl R1	20µl R2	2 µl R1	20µl R2	2 µl R1	20µl R2			80µl R2	80µl R9	80µl R10	water
D	2 µl R1	20µl R2	2 µl R1	20µl R2	2 µl R1	20µl R2			80µl R2	80µl R9	80µl R10	water
E	2 µl R1	20µl R2	2 µl R1	20µl R2	2 µl R1	20µl R2			80µl R2	80µl R9	80µl R10	water
F	2 µl R1	20µl R2	2 µl R1	20µl R2	2 µl R1	20µl R2			80µl R2	80µl R9	80µl R10	water
G	2 µl R1	20µl R2	2 µl R1	20µl R2	2 µl R1	20µl R2			80µl R2	80µl R9	80µl R10	water
H	2 µl R1	20µl R2	2 µl R1	20µl R2	2 µl R1	20µl R2			80µl R2	80µl R9	80µl R10	water

2. Evaluate the precision and trueness of your work

- For the samples prepared by method (a), method (b) and method (c), calculate: the mean, the standard deviation (SD and SEM) and the coefficient of variation (CV %).
- Calculate the theoretical concentration of $KMnO_4$ in the measured samples (c_{theor}).
- Using the supplied calibration curve, determine the experimentally measured concentration (c) of $KMnO_4$. Substitute the average absorbance into the calibration-line equation and calculate the concentration.
- Calculate the relative error (bias) compared with the theoretical value. $\text{bias}\ (\%) = \frac{\bar{c} - c_{\text{teor}}}{c_{\text{teor}}} \times 100$

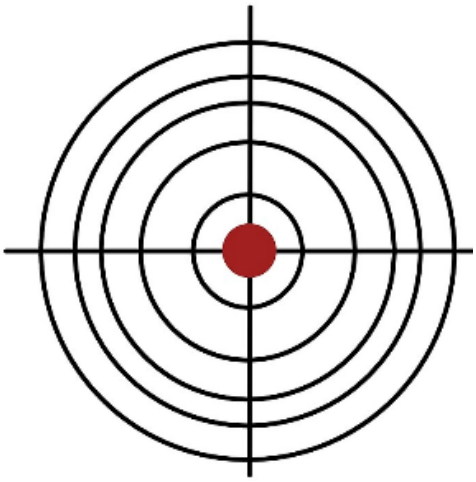
	Absorbance						
	Method a)			Method b)			Method c) (column 11)
	Student Nr. 1	Student Nr. 2	Student Nr. 3	Student Nr. 1	Student Nr. 2	Student Nr. 3	
A							
B							
C							
D							
E							
F							
G							
H							
Mean							
SEM							
SD							
CV							
c KMnO4 (measured)							
c KmnO4 (theoretical)							
Bias							

Conclusion:

Summarize the results of your measurements; the following questions may help you evaluate the data.

- *Describe the differences between the results obtained by individual students. Discuss possible sources of error you observed and propose measures to avoid them.*
- *What type of errors is reflected by the coefficient of variation (CV)? What type of errors is reflected by the bias? How do standard deviation (SD) and bias differ?*
- *Based on the bias and CV, decide which of the solution-preparation methods is the most precise and which is the most accurate in your hands.*
- *For each method used to determine the concentration of $KMnO_4$, state which types of error it may be affected by, how you would detect those errors, and suggest ways to eliminate them.*

How would you evaluate your work? Plot the results of your measurements obtained with methods a), b) and c) on a target (bull's-eye) diagram 😊*. Indicate the mean and the standard deviation.*



SPECIFICITY OF ENZYMATIC ACTIVITY AND EFFECT OF THE ENVIRONMENT

Date:

Aims:

1. Determine the pH optimum for β-fructofuranosidase.
2. Assess the effect of temperature on the catalytic activity of β-fructofuranosidase.
3. Assess the effect of enzyme concentration on catalytic activity of β-fructofuranosidase.
4. Determine the substrate specificity of β-fructofuranosidase.

Principle:

Your task is to observe and influence the chemical reaction in which sucrase (invertase) hydrolyses sucrose. The substrate is the disaccharide sucrose (c = 0.029 mol·L^{-1}). The enzyme is β-fructofuranosidase (invertase, sucrase) obtained from yeast (the enzyme preparation was made by grinding 0.5 g of baker's yeast in 200 mL of distilled water). If the enzyme is active, sucrose is cleaved to glucose and fructose. After addition of Fehling's reagent and heating, glucose reduces the cupric salt ($CuSO_4$) to brick-red cuprous oxide (Cu_2O).

Baker's and brewer's yeast contain Saccharomyces cerevisiae. S. cerevisiae commonly occurs in foods and can occasionally be part of the human microbiota (less frequently than S. boulardii). It is widely used as a model eukaryotic organism in molecular biology. S. cerevisiae is a facultative pathogen and can cause infections in immunocompromised individuals.

1. Determination of the pH optimum for β-fructofuranosidase

Procedure:

- Into three labelled test tubes dispense the reagents in the volumes and order indicated using a dispenser and micropipette. (Mix the enzyme preparation well before each use.)
- Shake the tubes and incubate them in a water bath at 37 °C.
- Meanwhile prepare a stock solution of Fehling's reagent in a beaker by mixing Fehling I and Fehling II 1:1. Prepare only the amount you will actually need.
- After the incubation, add Fehling's reagent with a pipette and boil the samples in a water bath.
- Record the colour of the solutions in the table and determine which pH gives the optimal activity of β-fructofuranosidase.

	1.	**2.**	**3.**
Citrate-phosphate buffer pH	1 mL pH 4.0	1 mL pH 7.0	1 mL pH 8.0
Sucrose	2 mL	2 mL	2 mL
Enzyme	1 mL	1 mL	1 mL
Mix and incubate at 37 °C in a water bath for 15 min 			
Fehling´s reagent	2 mL	2 mL	2 mL
Mix and boil in the beaker water bath			
Colour			

Observations:

Briefly summarise the outcome of your experiment. Based on your results explain the effect of pH on the enzyme-catalysed reaction.

If you observe that enzyme activity is negative (i.e. no activity or an apparent decrease) at extremely acidic pH, how would you verify whether the enzyme has been denatured irreversibly or is only temporarily inactivated?

2. Effect of temperature

Procedure:

- Into four labelled test tubes dispense buffer (at the previously determined optimal pH) and sucrose in the volumes shown, using a dispenser and pipette.
- Shake the tubes and incubate them for 5 minutes at the indicated temperatures (ice bath, room temperature, 37 °C water bath). Boil the contents of the last tube.
- Then add 1 mL of the enzyme preparation to each tube, shake and return the tubes to the respective temperatures.
- After the incubation add Fehling's reagent and boil in a water bath.
- Record the colour of the solutions and describe enzyme efficiency at the tested temperatures.

	1.	**2.**	**3.**	**4.**
Citrate-phosphate buffer	1 mL	1 mL	1 mL	1 mL
Sucrose	2 mL	2 mL	2 mL	2 mL
	Ice bath	Room temperature	37 ^{o}C	Boiling water
Mix and incubate at appropriate temperature for 5 min.				
Enzyme	1 mL	1 mL	1 mL	1 mL
Mix and incubate at appropriate temperature for 15 min.				
Fehling´s reagent	2 mL	2 mL	2 mL	2 mL
Mix and boil in the beaker water bath				
Colour				

Observations:

Briefly summarise the result of your experiment. Which temperature caused irreversible changes in the enzyme structure?

3. Effect of enzyme concentration

Procedure:

- Into four labelled test tubes dispense buffer, sucrose, enzyme preparation and distilled water in the volumes shown using a dispenser and pipette; mix and incubate in a water bath.
- After incubation add Fehling's reagent and boil.
- Record the colour and describe how changing the enzyme concentration affected activity.

	1.	**2.**	**3.**	**4.**
Citrate-phosphate buffer	1 mL	1 mL	1 mL	1 mL
Sucrose	2 mL	2 mL	2 mL	2 mL
Enzyme	1 mL	0,5 mL	0,1 mL	-
Distilled water	-	0,5 mL	0,9 mL	1 mL
Mix and incubate at 37 °C in a water bath for 15 min				
Fehling´s reagent	2 mL	2 mL	2 mL	2 mL
Mix and boil in the beaker water bath				
Colour				

Observations: Briefly summarise the result of your experiment.

4. Determination of enzyme specificity

Procedure:

- Into three labelled test tubes dispense citrate-phosphate buffer at the optimal pH, then add the substrates and enzyme as indicated.
- After mixing add enzyme preparation, shake and incubate in a 37 °C water bath.
- After incubation add Fehling's reagent with a pipette and boil in a water bath.
- Record the colour and determine which substrate(s) the enzyme acts on.

	1.	**2.**	**3.**
Citrate-phosphate buffer	1 mL	1 mL	1 mL
Sucrose	2 ml	-	-
Starch	-	2 mL	-
Gelatin	-	-	2 mL
Enzyme	1 mL	1 mL	1 mL
Mix and incubate at 37 °C in a water bath for 15 min			
Fehling´s reagent	2 ml	2 ml	2 ml
Mix and boil in the beaker water bath			
Colour			

Observations:

Briefly summarise the results.

Explain substrate specificity of the enzyme — how and why the enzyme binds only certain molecules.

What is the chemical difference between sucrose and starch?

Why does sucrase cleave sucrose but not starch?

Conclusion

First carefully consider and draw the structures of the substrates and products of the enzymatic reaction. Sketch the structural formulas. Explain the principle of the colour change from blue to brick-red observed when detecting the product of sucrose breakdown.

D-glucose
– open-chain form

α-D-glucopyranose – cyclic form
- circle the hemiacetal hydroxyl group

D-fructose
– open-chain form

β-D-fructofuranose – cyclic form
- circle the hemiacetal hydroxyl group

sucrose

What did you learn about sucrase from the experiments?

At which pH does it work best? Where in the human body would such pH naturally occur (compare with stomach, duodenum, small intestine, large intestine pH values)? At which temperature does it work best? Which substrate does it prefer?

Problems for discussion

1. **Sucrase in humans**

In human carbohydrate digestion occurs in the small intestine, specifically in the duodenum and jejunum. Hydrolysis is performed at the enterocyte membrane where the sucrase-isomaltase complex is located. Sucrase catalyses hydrolysis of sucrose into glucose and fructose. The isomaltase subunit hydrolyses mainly α-1,6 bonds (the branched parts of amylopectin). Without isomaltase activity, limit-dextrins would remain in the lumen and may be fermented by gut bacteria in the large intestine, causing bloating and diarrhoea.

• Compare the pH optimum of human sucrase with microbial sucrase.

2. **How to maintain cells in the laboratory**

For in-vitro cell culture we use media with adjusted pH appropriate to the cell type. For typical mammalian cells the medium is adjusted to pH ≈ 7.2–7.4.

• What must the culture medium contain to buffer pH changes?

• Compare pH in extracellular, intracellular and lysosomal compartments.

3. Identify a thermostable enzyme

In PCR (polymerase chain reaction) an enzyme is used to synthesise a new DNA strand from nucleotides. PCR proceeds in repeated cycles of three main steps:

Denaturation — double-stranded DNA is heated (~95 °C) to break hydrogen bonds and separate the strands.

Annealing — temperature is lowered (typically 50–65 °C) to allow primers to hybridise to target sequences.

Extension — at an optimal temperature (e.g. ~72 °C), the enzyme recognises the 3′ end of the primer and incorporates dNTPs to synthesise the complementary strand.

• Identify the enzyme used in PCR and explain the principle of the reaction and the terms thermolability, thermostability and denaturation.