General properties of the ENZYMES

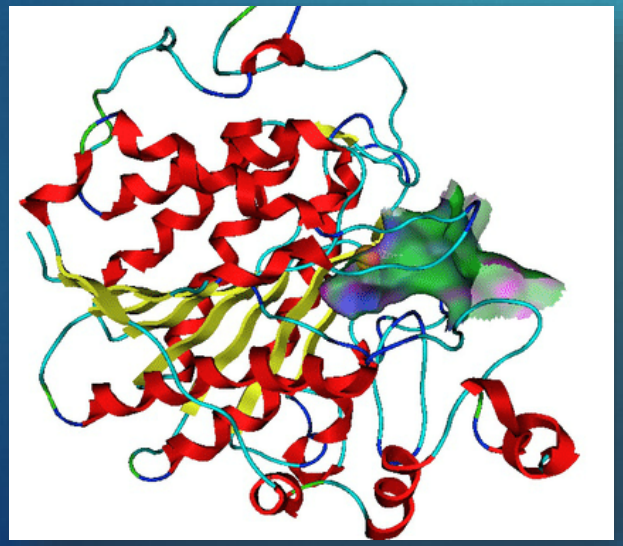
The aim:

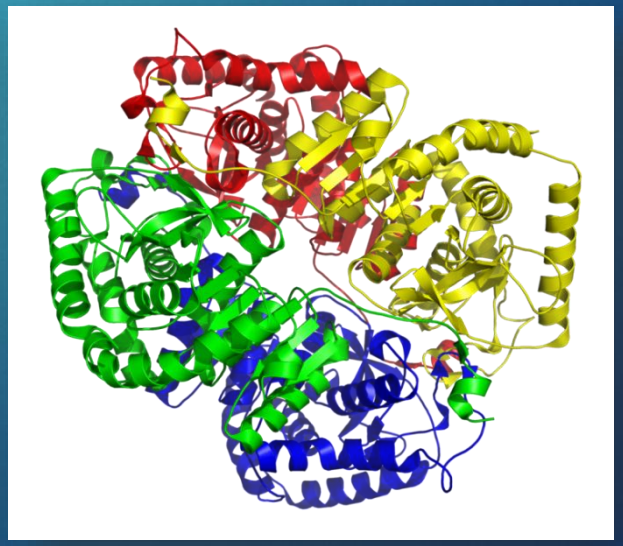
To determine the effect of the environmental conditions (temp. and pH) on the velocity of enzymatic catalysis using amylase as model

Enzyme

Enzyme - a protein acts as biological catalyst for specific chemical reactions.

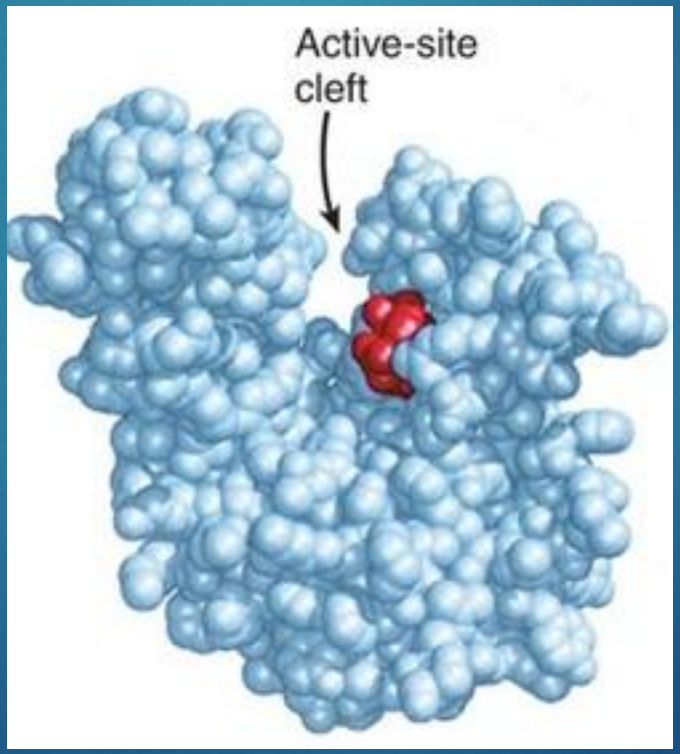
Enzyme, like any protein molecule, has 3D structure. The region that contains catalytic residues (around 3–4 amino acids with polar side chains) is known as the **active site.**





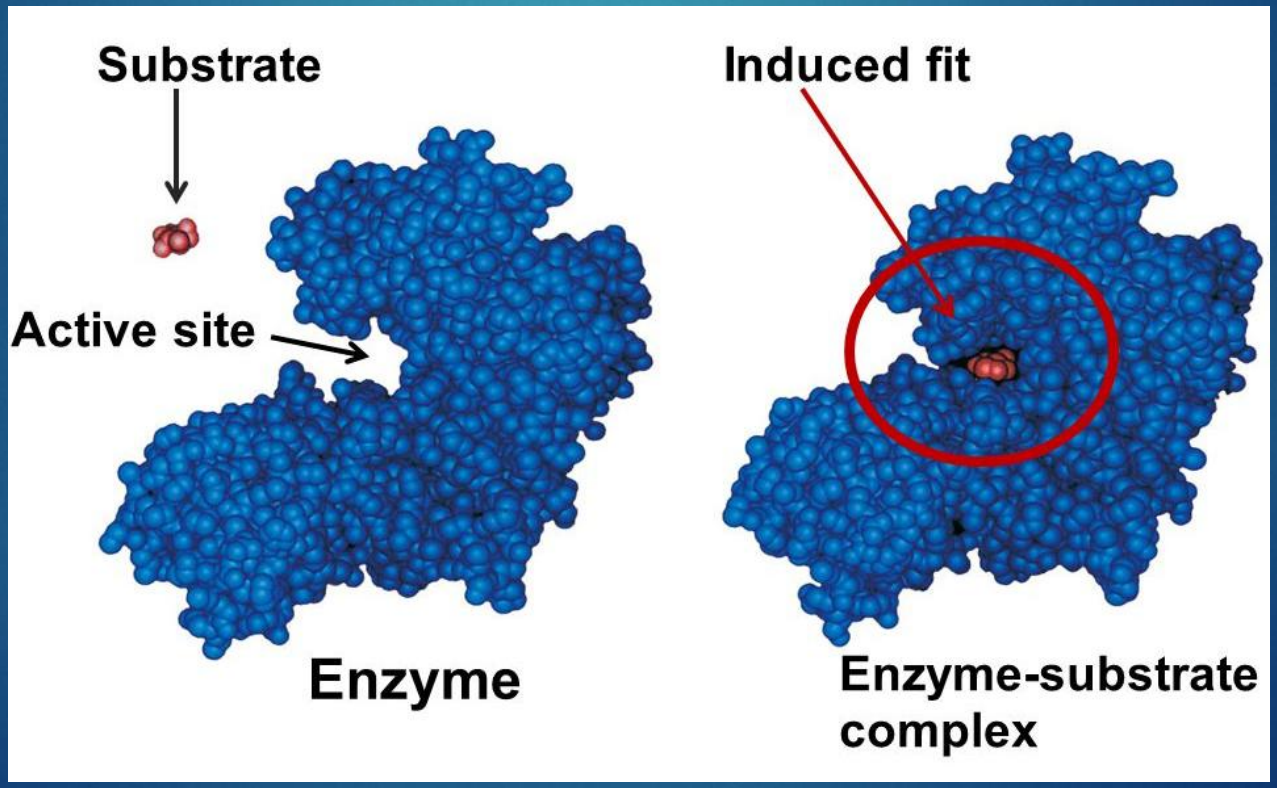
Enzyme

Active site - a cleft which the specific set of reactants (called substrates) bind to, and then, they are converted into specific products.

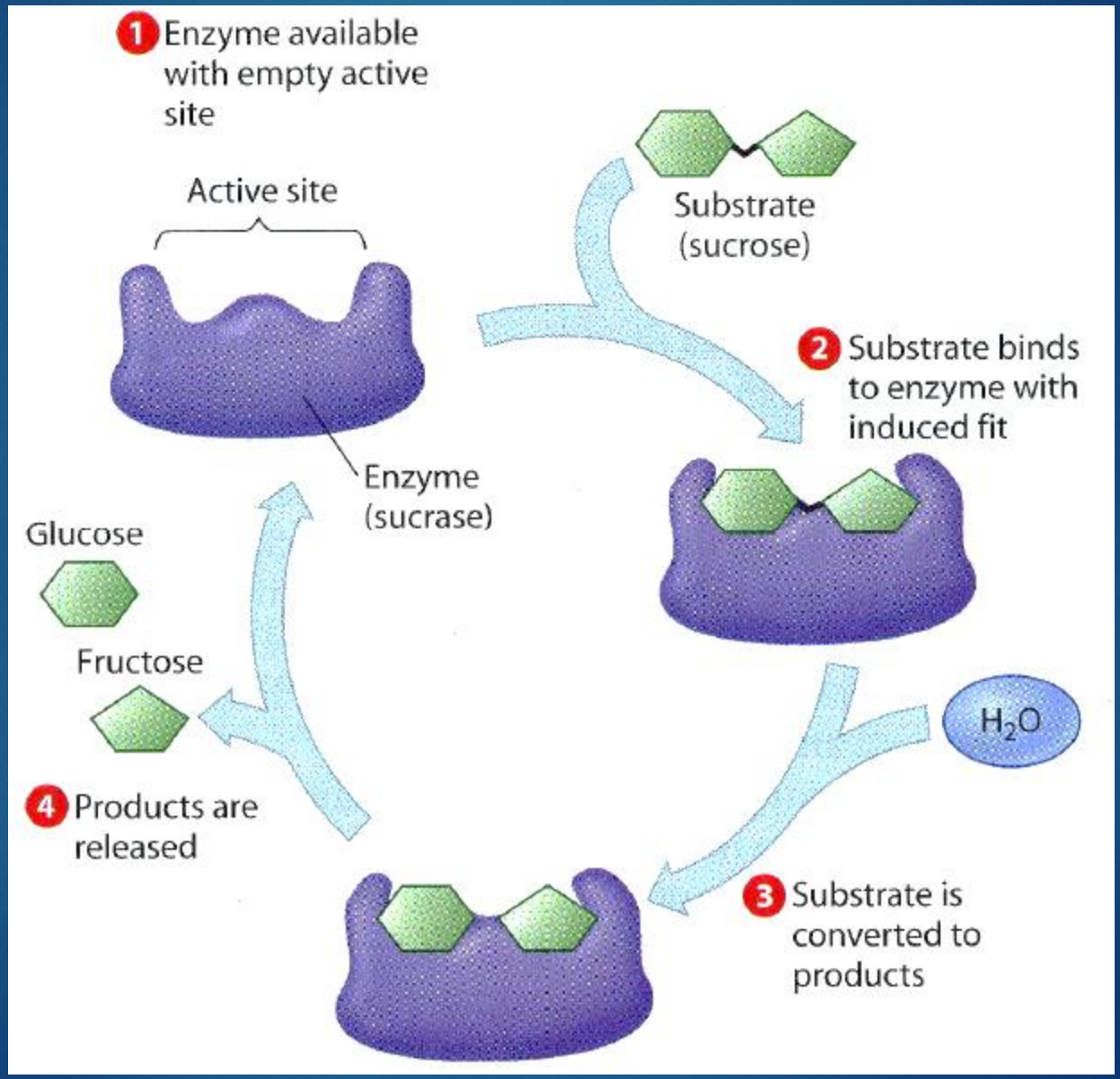


Enzyme

When a substrate binds to the enzyme's active site, it induces a conformational change in the active site to better accommodate the substrate.



Enzymatic catalysis



Enzyme classification

An official commission of the International Union of Biochemistry and Molecular Biology (IUBMB) has classified enzymes in the following six categories, based on **the type of catalyzed reaction**:

1. Oxidoreductases	catalyze dehydrogenation or other oxidation and reduction reactions; one substrate is oxidixed, another is reduced
2. Transferases	transfer certain groups from a substrate to another
3. Hydrolases	catalyze the hydrolytic cleavage of C-C, C-O, C-N bonds
4. Lyases	catalyze non-hydrolytic cleavage of different bonds by elimination
5. Isomerase	catalyzing rearrangement reactions
6. Ligases / Synthetases	catalyze condensation with simultaneous cleavage of ATP and related reactions
7. Translocases	catalyze the movement of ions or molecules across membranes or their separation within membranes

Enzyme nomenclature

Common names for enzymes begin with some description of its action and end with the *–ase* suffix, eg.:

lactate dehydrogenase (EC 1.1.1.27.)

The commision (IUBMB) has also developed a numerical system for classifying enzymes (to avoid errors occurring during translation into other languages).

The names begin with EC (for Enzyme Commission) and end with four numbers, separated by decimal points, that describe the enzyme. The code is unique for each enzyme.

Enzyme nomenclature

EC **1**.1.1.27.

1st number – **class** tells us which of the six major enzyme classes the enzyme belongs to. In this case, the 1, designates the enzyme as an oxidoreductase

EC 1.**1**.1.27.

2nd number - **subclass** indicates what group the enzyme acts on (CH-OH; dehydrogenation)

EC 1.1.1.27.

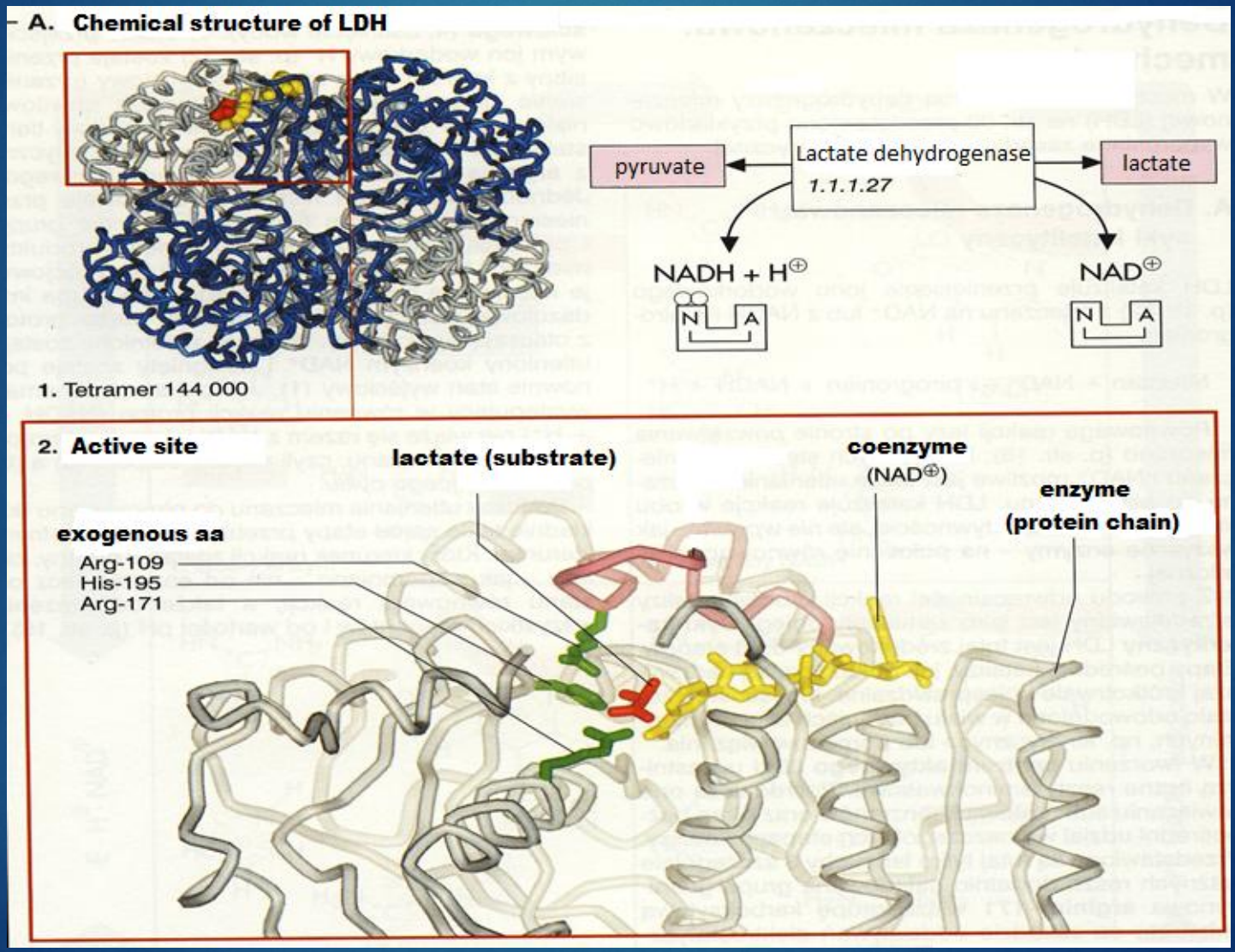
EC 1.1.**1**.27.

3rd number - **sub-subclass** indicates what is the acceptor of hydrogen atoms (NAD)

EC 1.1.1.**27**.

4th number - **number within the sub-subclass**

Structure and function LDH



The effects of external factors on the velocity of enzymatic catalysis

Several factors have influence on:

- the distribution of charges in a protein molecule
- the ability to create hydrophobic interactions, hydrogen bonds and van der Waals forces

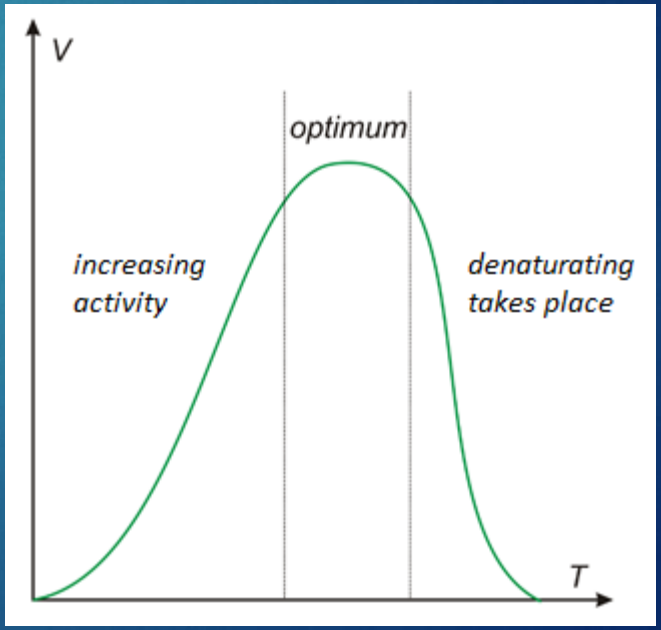
These factors are as follows:

- the presence of **mono-** and **bivalent ions**
- **pH** of the solution
- **temperature**

The effect of the TEMPERATURE

- Like most chemical reactions, the rate of an enzyme-catalyzed reaction increases as the temperature is raised.
- A ten degree Celsius (10°C) rise in temperature increases the activity of most enzymes 2-3 times.
- However, most animal enzymes rapidly become denatured at temperatures above 40°C.

- The tolerance of the various enzymes against the temperature is different.
- For enzymes of animal origin body temperature **37°C** is optimal.



The effect of pH

The velocity of an enzymatic reaction depends on the H^+ ion concentration.

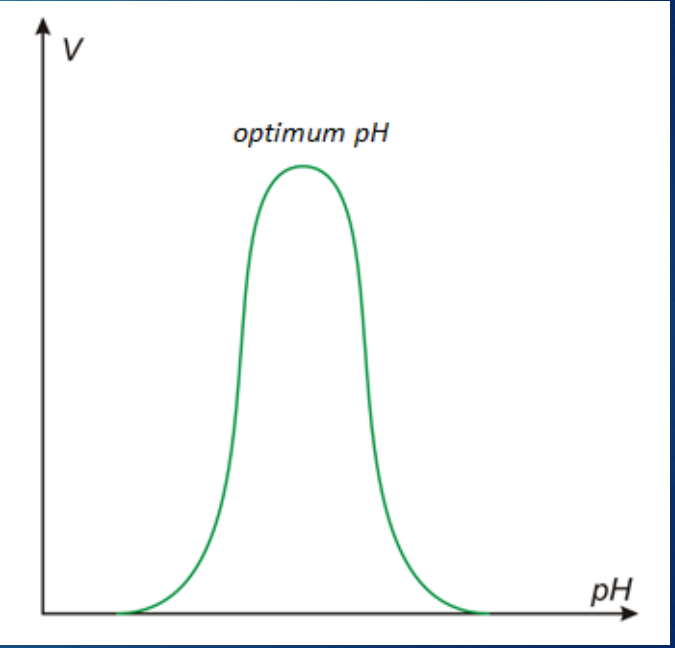
The most favorable pH value - the point where the enzyme is most active - is known as the **optimum pH** (unique for each enzyme).

Changes in the pH value (↑ and ↓) alter:

- the chain conformation of the enzyme protein, or
- the electric charge of either the substrate or the enzyme

and by this the binding of substrate.

These effects result in the decrease of the speed of the reaction.



The effect of ions

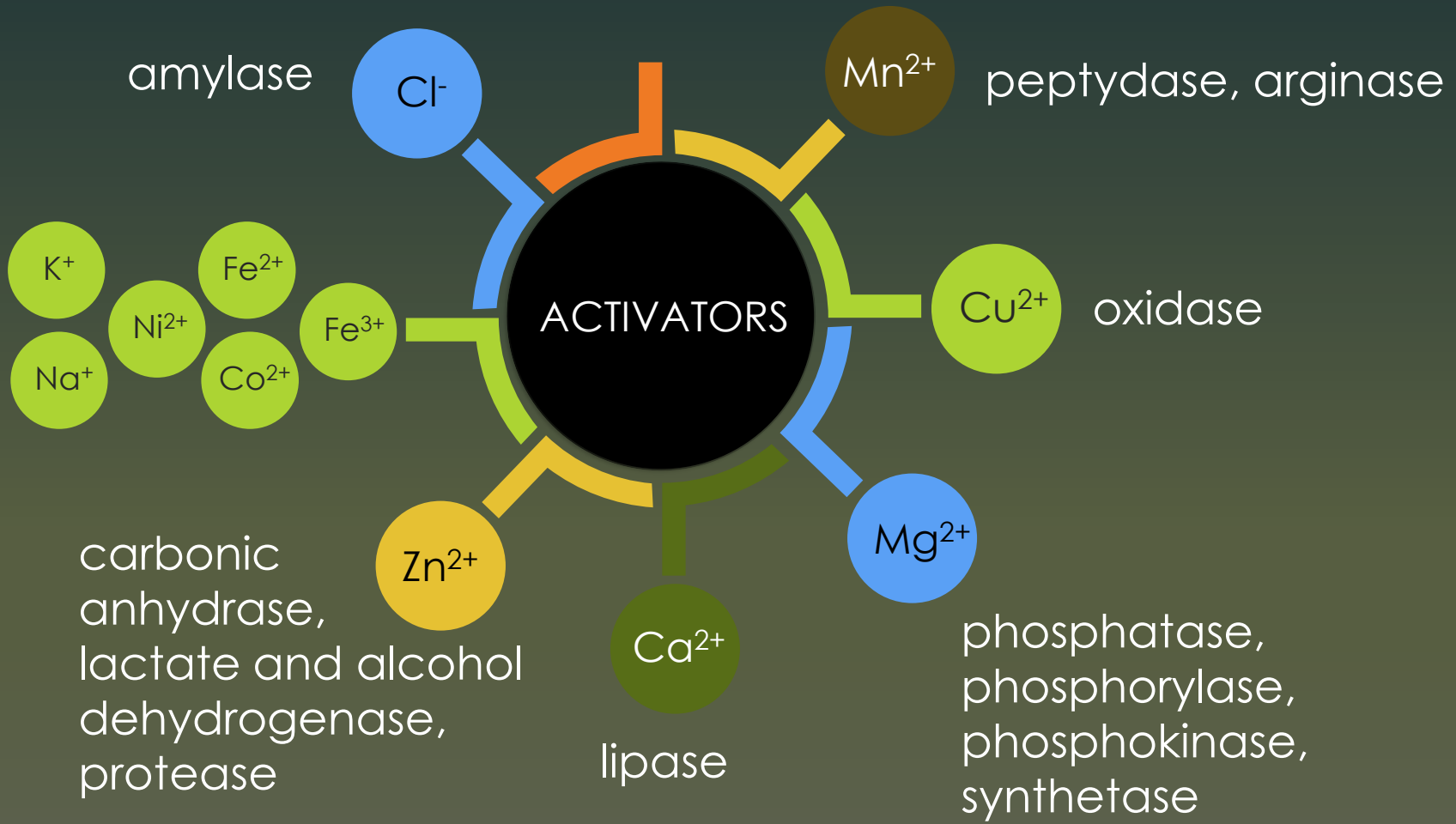
Activators increase the velocity of enzymatic reactions. The mechanism of this activation is yet unclear. It is supposed that the activators may loosen the bonds of the substrate.

Most common activators: cations

- Anions have little effect on enzyme activity, except **Cl^-, acting as an amylase activator**
- Heavy metal cations (Pb^{2+} , Hg^{2+}) have **inhibitory effects.**

The effect of ions

Most common activators: microelements



Salivary α-amylase (EC 3.2.1.1)

Amylase belongs to the 3rd class of enzymes (Hydrolases).

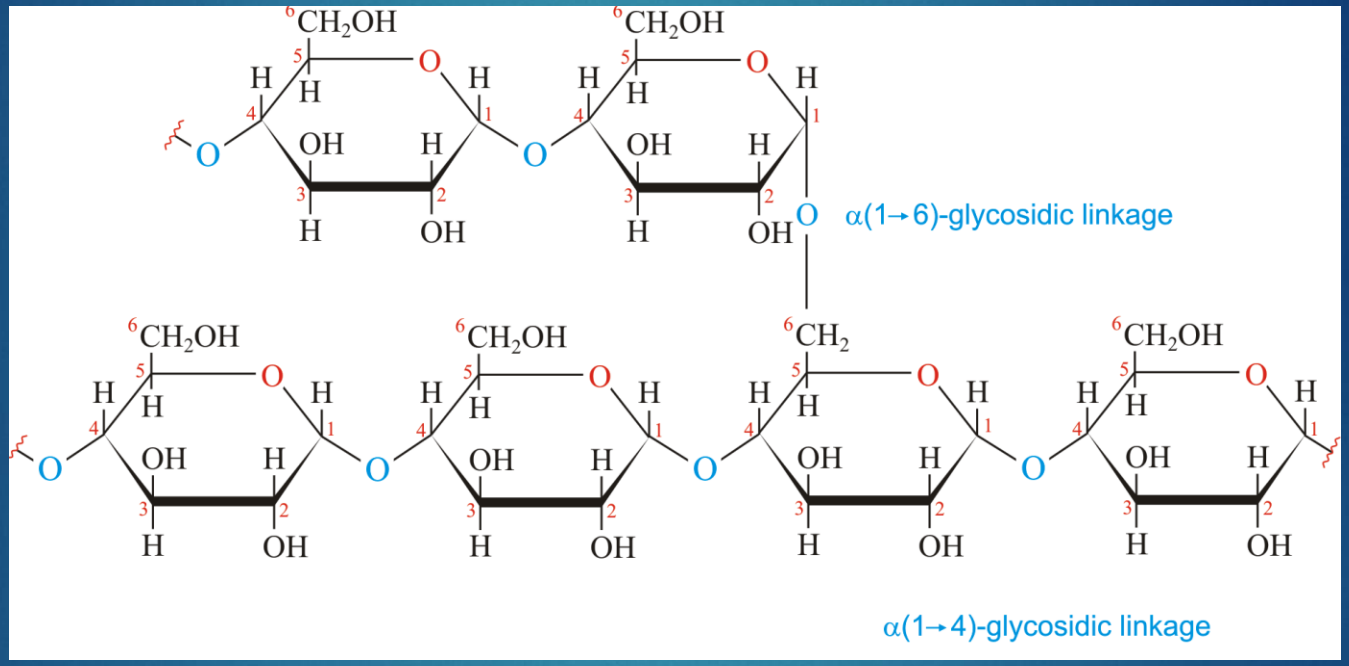
Reaction:

- it cleaves **(1→4)-α-D-glucosidic linkages** in polysaccharides (starch and glycogen) and products of their gradual hydrolysis,

Products:

- oligosaccharides containing 6-7 glucose units (dextrins),
- trisaccharides
- maltose, isomaltose

Salivary α-amylase (EC 3.2.1.1)



A fragment of a starch molecule, which consists of a straight chain (amylose) and a branched chain (amylopectin).

Practical uses of amylase

- Breadmaking

Amylases break down complex sugars (starch in flour), into simple sugars. Yeast then feeds on these sugars and converts them into ethanol and CO_2 – this causes the bread to rise

- Fermentation

Yeast ingests sugars and excretes EtOH

- Medical application

Amylase is used in pancreatic enzyme replacement therapy (PERT) - to help in the breakdown of saccharides into simple sugars

Methods for determining the activity of various enzymes

Measuring of the activity of an enzyme can be performed by measuring:

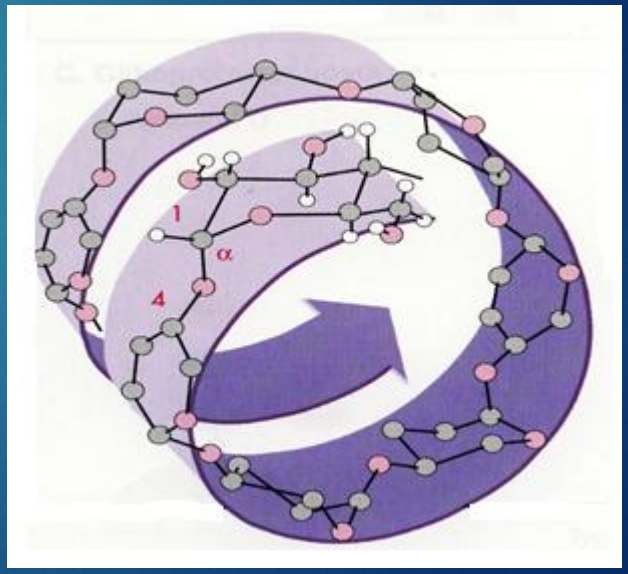
1) the decrease of the substrate concentration
2) the increase of the new product concentration

1. Reaction of starch digestion

Iodine test

- The non-hydrolysed starch gives a positive result in the test with iodine solution (blue color). Blue color indicates the presence of undigested starch.

The shorter intermediary products of the starch degradation show blue-brownish (amylodextrin), red (erythrodextrin) or no colour (achrodextrin) with I_2 solution.



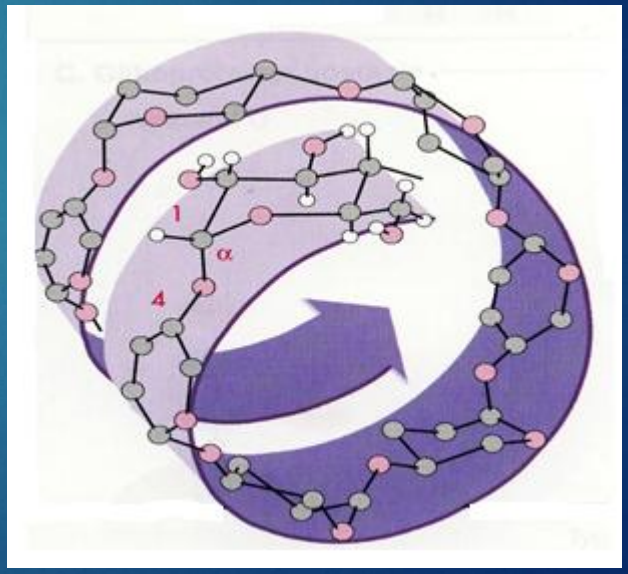
1. Reaction of starch digestion

Iodine test

The iodine-starch color change occurs when iodine molecules enter the helical structure of amylose. In this apolar (hydrophobic) environment, their electron structure changes, shifting light absorption and producing a blue color.

Amylose gives a blue color with iodine, amylopectin gives a purple color.

The reaction takes place in an acidic environment*.

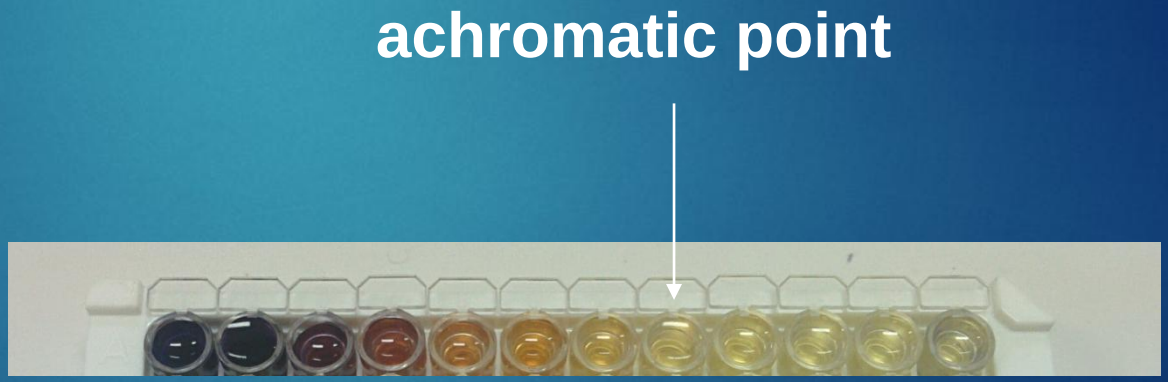


*It does not take place in an alkaline environment, because then iodine reacts with the base.

1. Reaction of starch digestion

Iodine test

The time at which color is no longer visible, indicating the disappearance of iodine-starch complex, is defined as **achromatic point**.



2. Reaction of starch digestion

Tests for reducing sugars

In general, the following reactions are used for the detection of the reducing aldehyde groups:

- Benedict's test
- Fehling's test
- Trommer's test
- Tollen's test

These tests allow to show the presence of monosaccharides or maltose - the end products of starch hydrolysis.

Principles of enzyme activity determination in tested samples

1. Prepare a solution in which the reaction will take place (incubation mixture) and which most commonly consists of:

- a buffer of optimum pH
- an appropriate substrate

2. Insert the incubation mixture into a water bath to ensure a constant, desired temperature - usually 37°C

3. Add an enzyme solution. The reaction starts when the enzyme is mixed with the substrate. The reaction time is counted from that moment: time „0” (not from the insertion of the incubation mixture into the water bath!)

**Sometimes the enzyme can be added to the buffer solution first. Then, the addition of substrate starts the reaction.*

Practical part

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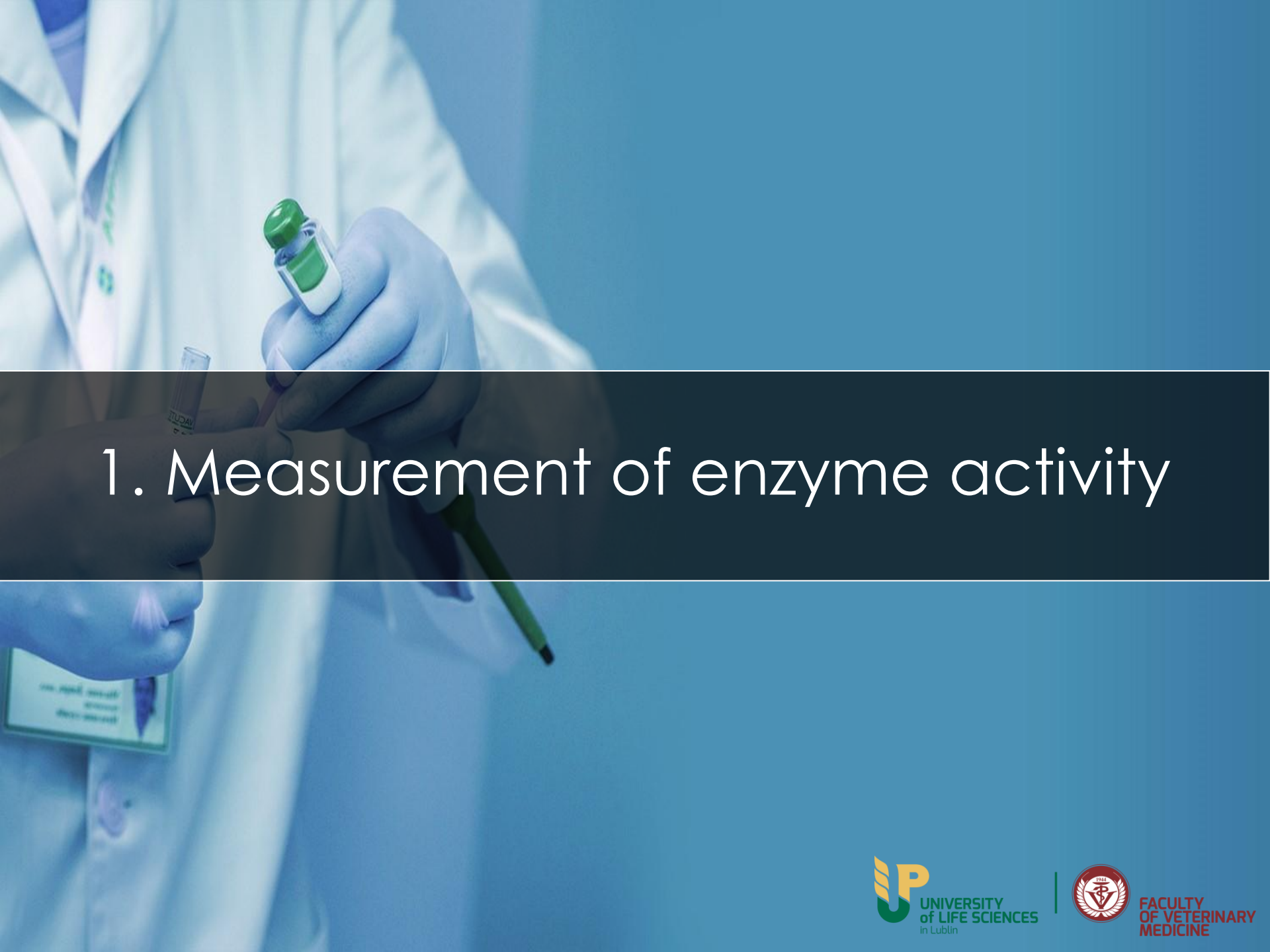

1. Measurement of enzyme activity

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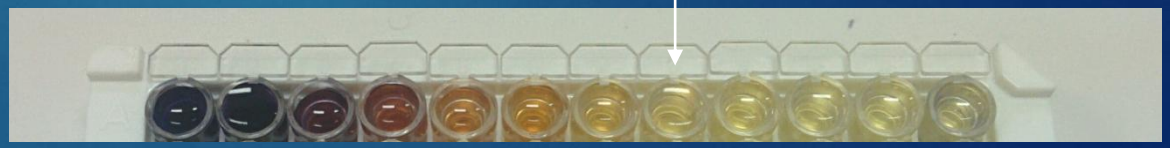
Task 1. Procedure for determining the achromatic point

The aim: to measure the enzyme (amylase from saliva) activity

I stage: We should establish optimal concentration of saliva (source of the enzyme) to obtain the achromatic point within 3-15 minutes

Achromatic point - the time at which the products from the hydrolysis of starch are released and the color of iodine-starch complex is no longer visible

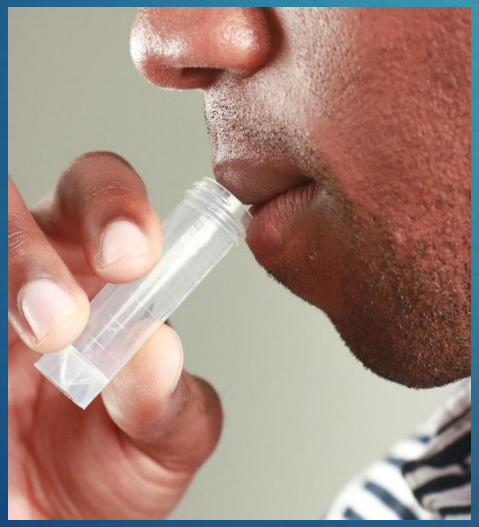
achromatic point



Task 1. Procedure for determining the achromatic point

Preparation of enzyme solution:

1. Collect about 5 ml of saliva in clean beaker (or test tube).
2. Add 1 ml of saliva to the graduated cylinder, dilute it with distilled 100x (1:99) and mix.



collection

dilution

Task 1. Procedure for determining the achromatic point

Procedure:

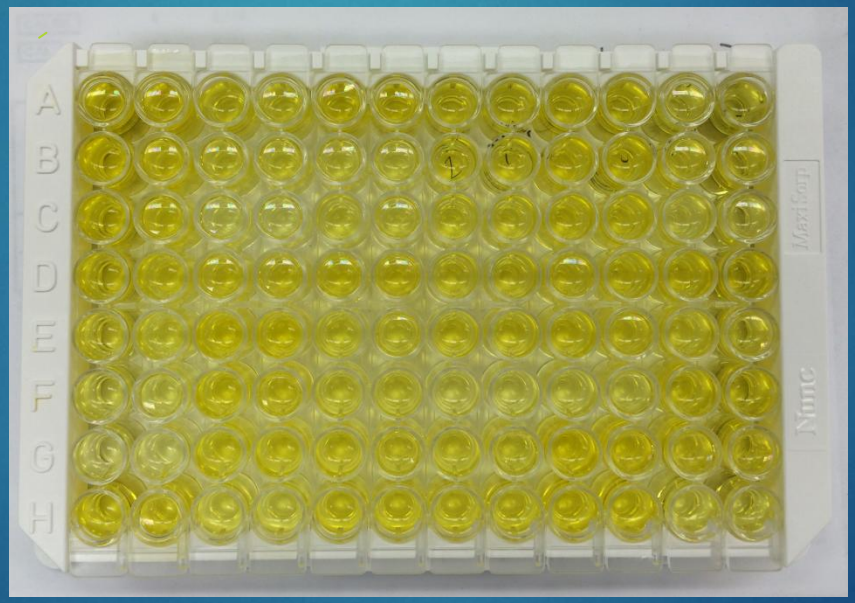
1. Add 5 cm^3 of starch solution, 2 cm^3 of NaCl and 2 cm^3 of phosphate buffer solution (pH=6.6) to a test tube and mix (incubation mixture).
2. Incubate it for 5 minutes in water bath (37°C).

Incubation mixture (substrate +buffer)

Task 1. Procedure for determining the achromatic point

Procedure:

3. Add 3 drops of iodine solution (I_2 in KI; 0,001 mol/dm^3) and 1 drop of HCl to 15 wells of the plate.



Task 1. Procedure for determining the achromatic point

Procedure:

4. Add 1 cm^3 of prepared enzyme solution (diluted saliva).

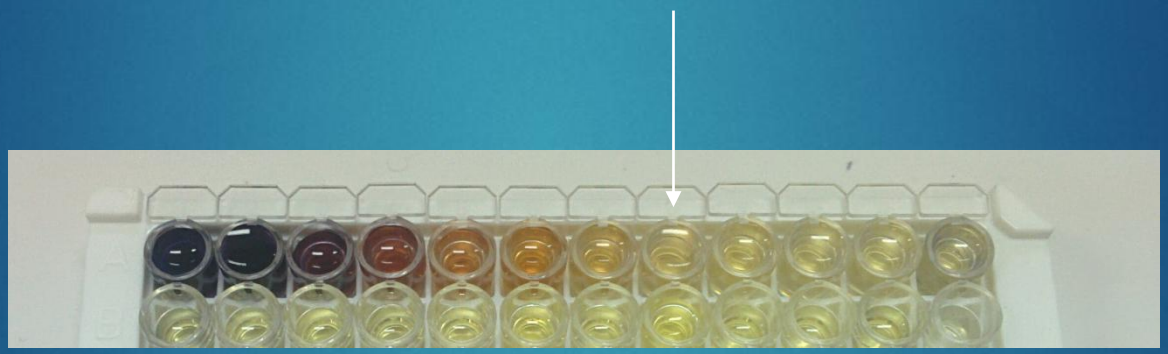
   -> This is the zero time.

5. After 1 minute transfer 0.2 cm^3 of incubation mixture to the first well containing the iodine solution.

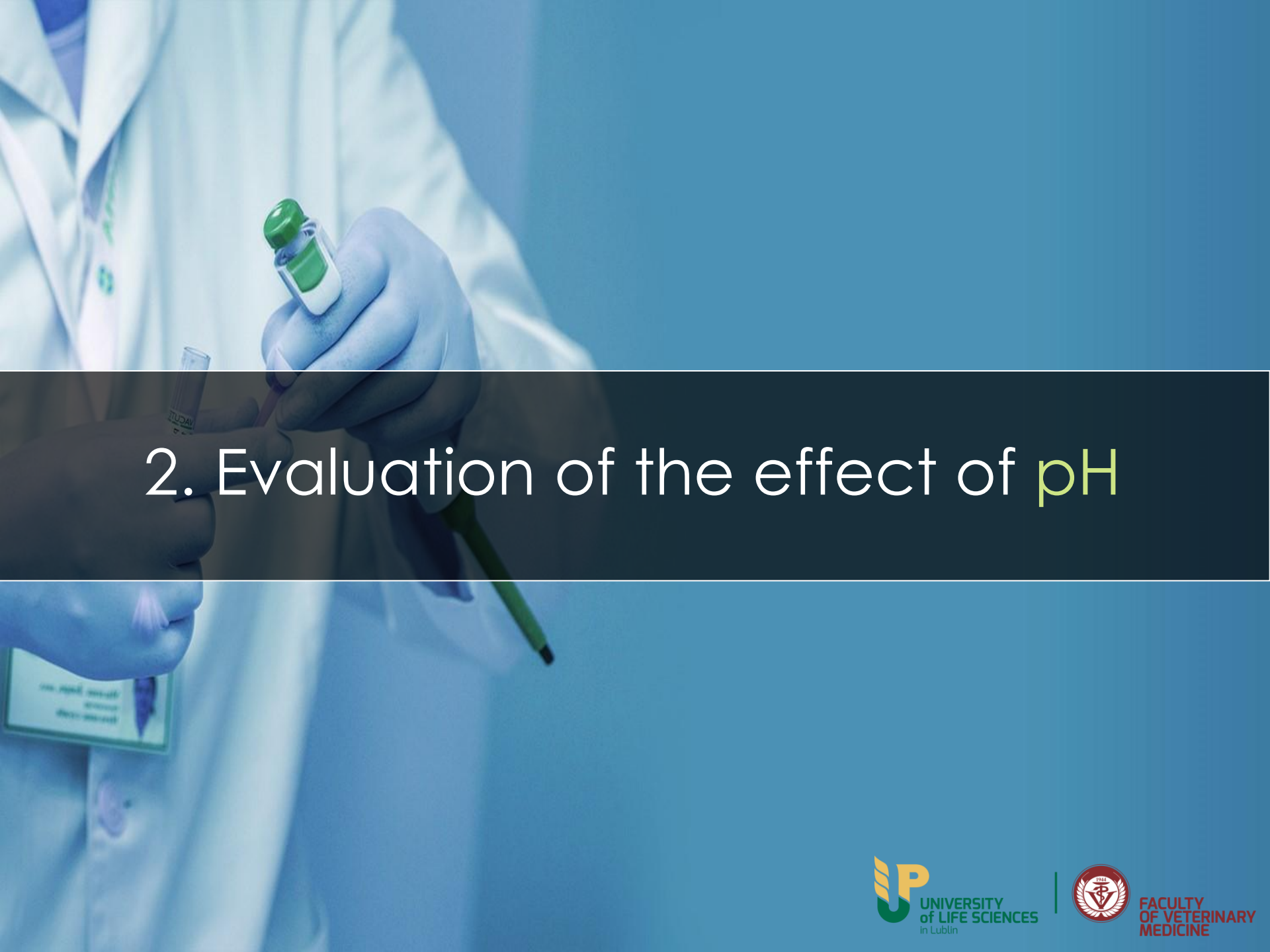
6. From this point, continue this procedure at **one minute intervals** until the achromatic point is reached. This well will have the same color as iodine without starch (yellow).

Task 1. Procedure for determining the achromatic point

achromatic point



In case of too short time (less than 3 minutes) or too long (more than 15 minutes), repeat the test with **another dilution of saliva**.



2. Evaluation of the effect of pH

Task 2. Evaluation of the effect of pH on the amylase activity

Procedure:

1. Prepare 3 test tubes with 5 cm^3 of starch solution and 2 cm^3 of NaCl.

2. Then, add 2 cm^3 of phosphate buffer of

- pH=6.6 into the 1st tube
- pH=5.0 into the 2nd tube
- pH=8.0 into the 3rd one

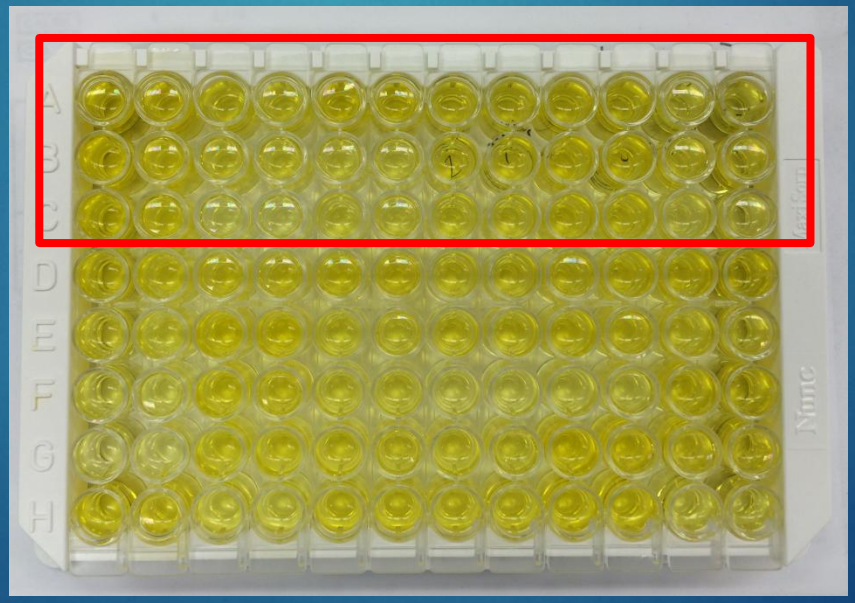
Incubate them for 5 minutes in water bath (37°C).

Incubation mixtures (substrate +buffer)

Task 2. Evaluation of the effect of pH on the amylase activity

Procedure:

3. Add 3 drops of iodine solution (I_2 in KI; 0,001 mol/dm^3) and 1 drop of HCl to the wells of the plate creating 3 rows.

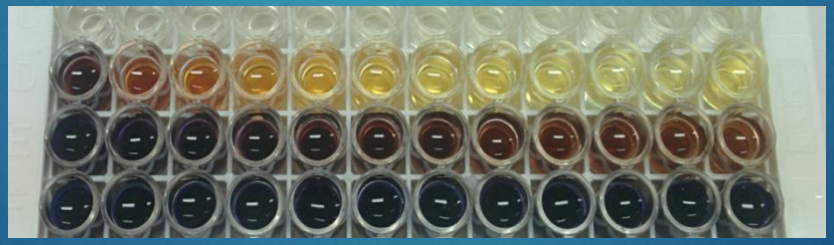


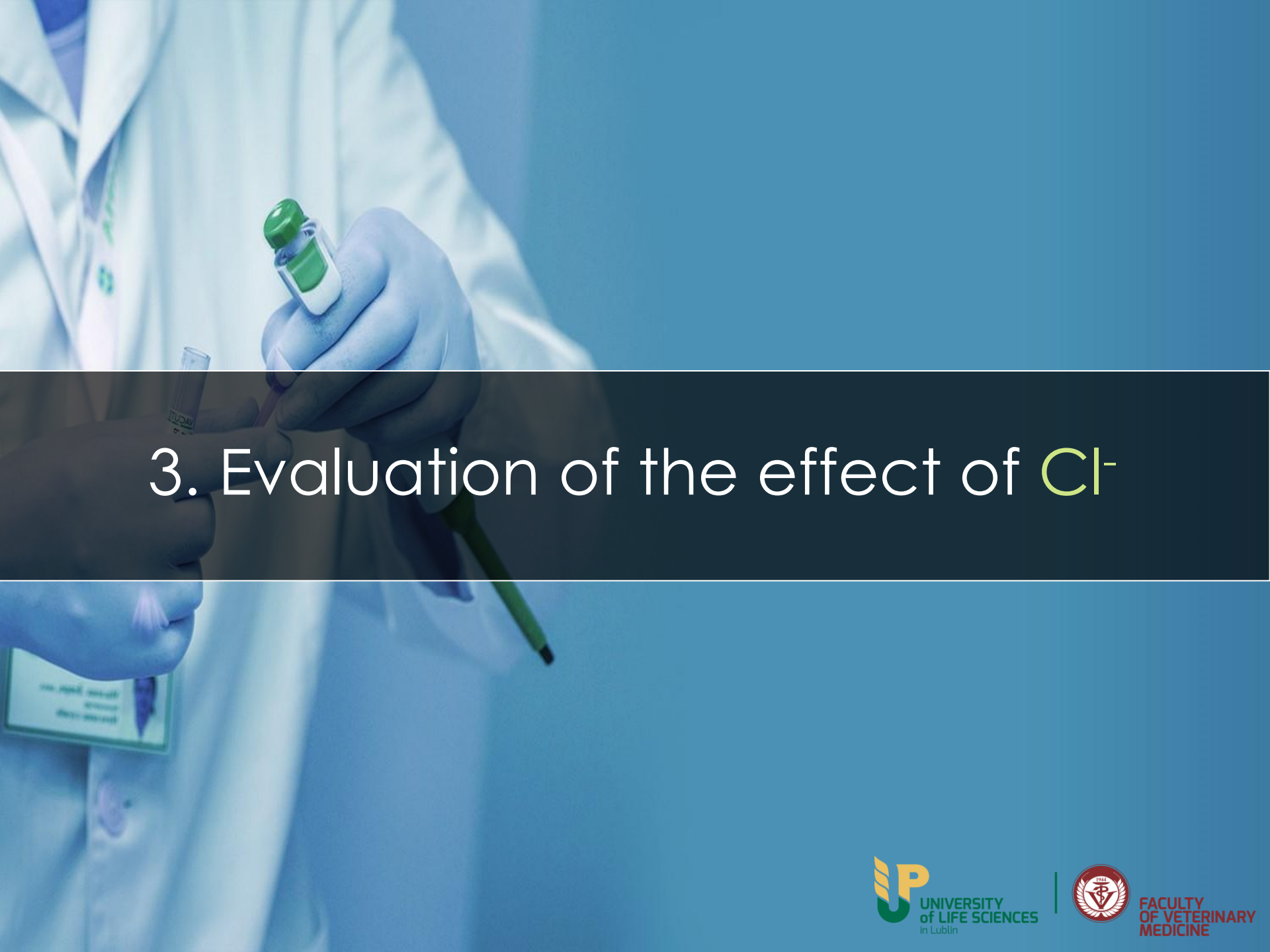
Task 2. Evaluation of the effect of pH on the amylase activity

Procedure:

5. After 5 minute incubation add 1 cm^3 of prepared enzyme solution to each tube.
6. Every one minute transfer 2 drops of incubation mixture to the following wells. Note achromatic points for each tube.

pH = 6.6
pH = 5.0
pH = 8.o





3. Evaluation of the effect of Cl^-

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Task 3. Evaluation of the effect of chloride ions on the amylase activity

Procedure:

1. Prepare 2 test tubes with 5 cm^3 of starch solution and 2 cm^3 of phosphate buffer of optimal pH (the result of Task 2).

2. Then, add 2 cm^3 of

- 1% NaCl into the 1st tube
- distilled water into the 2nd tube

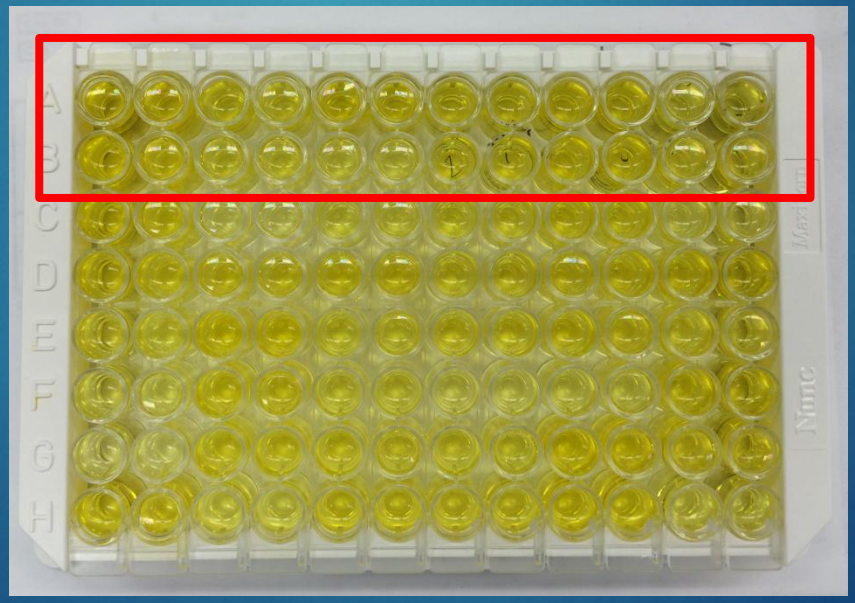
Incubate them for 5 minutes in water bath (37°C).

Incubation mixtures (substrate +buffer)

Task 3. Evaluation of the effect of chloride ions on the amylase activity

Procedure:

3. Add 3 drops of iodine solution (I_2 in KI; 0,001 mol/dm^3) and 1 drop of HCl to the wells of the plate creating 2 rows.

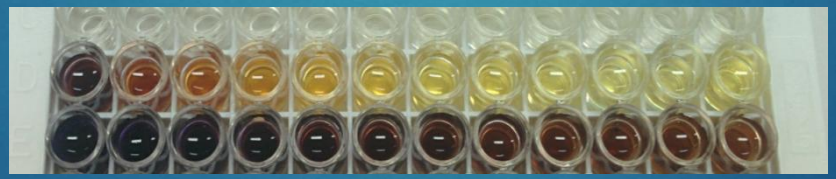


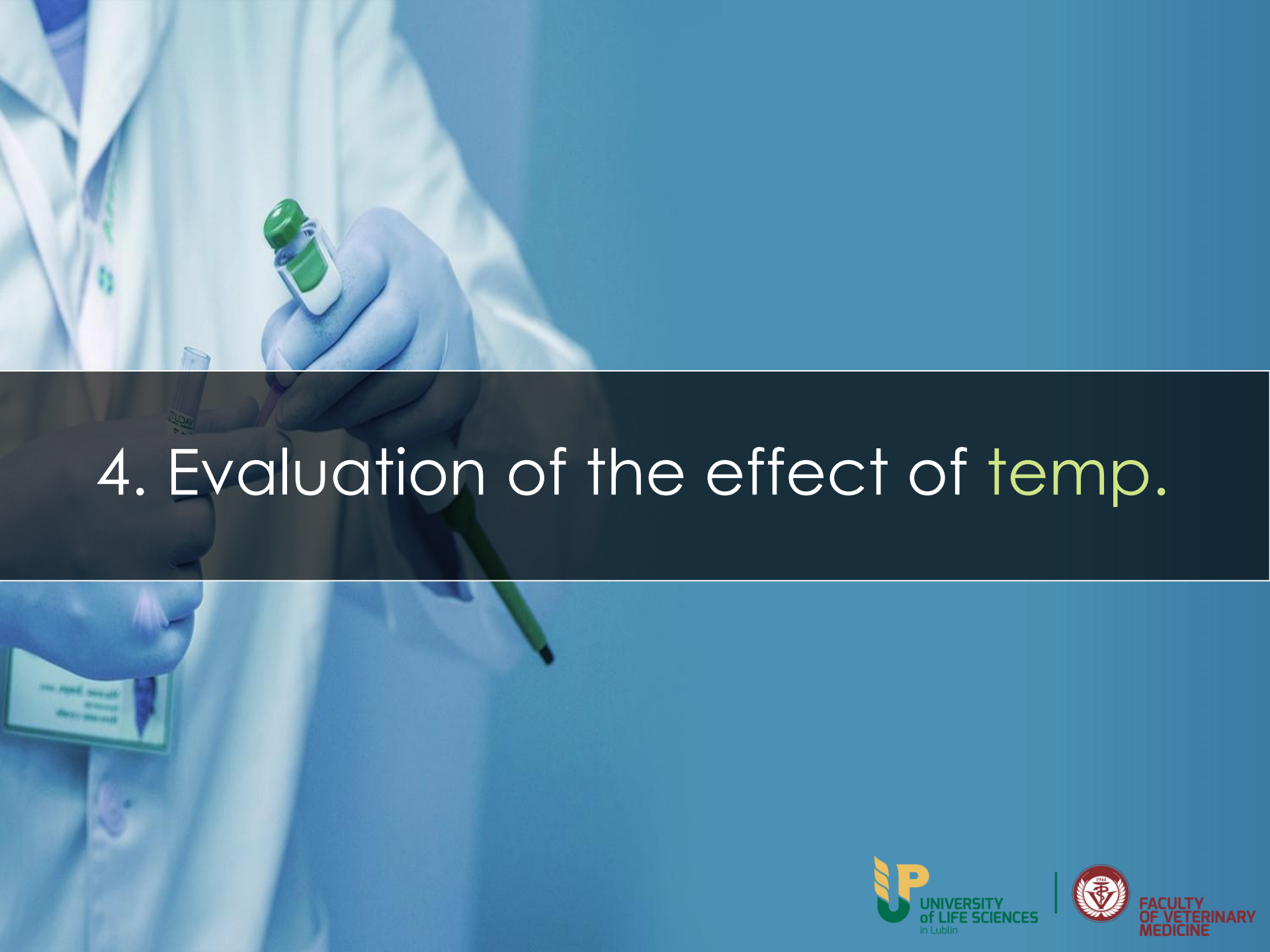
Task 3. Evaluation of the effect of chloride ions on the amylase activity

Procedure:

4. Add 1 cm^3 of prepared enzyme solution to each of 2 tubes (2 diffenet incubation mixtures).
5. Every one minute transfer 2 drops of incubation mixtures to the following wells.
6. Note color changes and achromatic points for each tube.

presence of Cl^-
lack of Cl^-





4. Evaluation of the effect of temp.

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Task 4. Evaluation of the effect of the temperature on the amylase activity

Procedure:

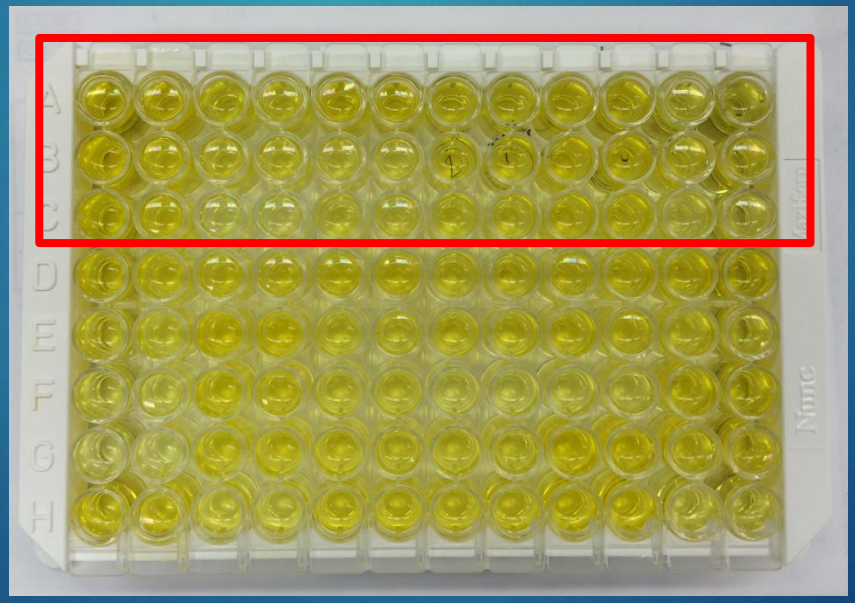
1. Prepare 3 test tubes with 5 cm^3 of starch solution, 2 cm^3 of 1% NaCl and 2 cm^3 of phosphate buffer of optimal pH (the result of Task 2).
2. Incubate the tubes as folllows:

- 1st tube in water bath (37°C)
- 2nd tube at RT
- 3rd tube on ice

Task 4. Evaluation of the effect of the temperature on the amylase activity

Procedure:

3. Add 3 drops of iodine solution (I_2 in KI; 0,001 mol/dm^3) and 1 drop of HCl to the wells of the plate creating 3 rows.

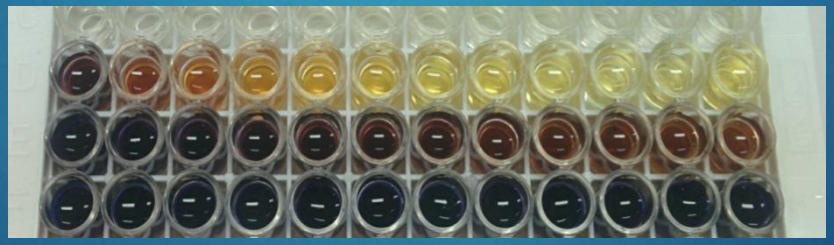


Task 4. Evaluation of the effect of the temperature on the amylase activity

Procedure:

4. After 5 minute incubation add 1 cm^3 of prepared enzyme solution to each tube.
5. Every one minute transfer 2 drops of incubation mixture to the following wells. Note achromatic points for each tube.

37˚C
RT
ice



Let’s do it !

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