

## Enzyme kinetics: Splitting urea with urease

### Aims of the experiment

- To get to know an experiment using an enzyme
- To investigate an enzyme reaction that occurs in nature
- To recognise the connection between ion concentration and conductivity
- To understand the way in which catalysts work
- To get an insight into biochemical processes

### Principles

Enzymes belong to the class of substances known as proteins and consist of amino acids. There are 20 different amino acids in nature from which many different proteins can be constructed. Enzymes are one group of proteins. These are biocatalysts that, for example, catalyse metabolic pathways. An enzyme does not bind covalently to the substrate to be converted. It forms an enzyme-substrate complex which leads to a conformational change in the protein. This again stabilises the transition state of the reaction to be catalysed. The desired product is then formed when the enzyme is split off. The enzyme exits from the reaction unchanged and is then able to convert further substrates. The general reaction of enzymes is therefore:



Where E is the enzyme, S is the substrate, ES the enzyme-substrate complex and P the desired product.

In catalytic processes, the reaction rate is always increased but without a shift in the reaction equilibrium. This is possible

because enzymes as catalysts lower the activation energy of a reaction. In this way, more molecules are present in the transition state, which leads to a more rapid formation of the product. In most cases the enzyme stabilises the transition state, whereby the energy for this is lowered. The product can therefore be formed more quickly as the energy peak (activation energy) that must be overcome for the reaction is lowered.

However, the increase in the reaction rate through enzymes can be suppressed by certain inhibitors. This does play an important role in nature, as so many biochemical processes are regulated. It is therefore possible that all reactions can be switched on and off, also in human cells.

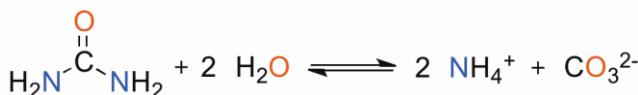
There are two types of inhibition, reversible and irreversible inhibition. With reversible inhibition, the inhibitor can be released again by the enzyme, but not with the latter. In this case, the enzyme loses its catalytic properties. Reversible inhibition can act in two ways. The inhibitor can bind the enzyme instead of the substrate (competitive inhibition). The enzyme is then occupied and no longer free for the substrate.



Fig. 1: Set-up of the experiment for conductivity measurement.

The inhibitor and the substrate compete for the binding sites. An inhibitor can, however, bind to a different site on the enzyme (non-competitive inhibition) and in this way change the conformation of the protein such that the reaction cannot be catalysed, or can only be catalysed a little.

The enzyme urease is to be investigated in this experiment. This enzyme occurs frequently in nature in plants. Here it catalyses the reaction in an aqueous medium of urea to ammonia and carbon dioxide, or to ammonium and carbonate ions (see Fig. 2).



**Fig. 2:** Decomposition of urea to ammonium and carbonate ions catalysed by the enzyme urease.

In the course of the reaction, the number of ions in the solution is increased. These conduct electric current and therefore cause an increase in the conductivity. The course of the reaction can therefore easily be followed and recorded using a conductivity sensor. This is followed by a simple kinetic evaluation of the reaction (determination of the reaction rate and the order of reaction).

In addition, the formation of product will be irreversibly inhibited during the course of the experiment using copper sulfate, as an example. The enzyme will be deactivated and the reaction comes to a standstill.

## Risk assessment

The chemicals used are in general non-hazardous. However, when working with copper sulfate, contact with the skin and eyes should be avoided.

<b>Copper(II) sulfate pentahydrate</b>	
	<b>Hazard statements</b> H302 Harmful if swallowed. H319 Causes serious eye irritation. H315 Causes skin irritation. H410 Very toxic to aquatic life with long-lasting effects.
<b>Signal word:</b> <b>Caution</b>	<b>Precautionary statements</b> P273 Avoid release to the environment. P302+P352 IF ON SKIN: Wash with soap and water. P305+P351+P338 IF IN EYES: Rinse continuously with water for several minutes. Remove contact lenses if present and easy to do. Continue rinsing.
<b>Ammonium carbonate</b>	
	<b>Hazard statements</b> H302 Harmful if swallowed.
<b>Signal word:</b> <b>Caution</b>	

## Equipment and chemicals

1	Pocket-CASSY 2 Bluetooth.....	524 018
1	CASSY Lab 2 .....	524 220
1	Conductivity adapter S .....	524 0671
1	Conductivity sensor .....	529 670
1	Magnetic stirrer, mini .....	607 105
1	Saddle base .....	300 11
1	Stand rod 25 cm, 10 mm diam. ....	301 26
1	Bosshead S.....	301 09
1	Universal clamp, 0...80 mm.....	666 555
1	Volumetric flask, Boro 3.3, 100 mL.....	665 793
2	Beaker, Boro 3.3, 150 mL, hF .....	602 010
1	Graduated pipette, 10 mL.....	665 997
1	Pipetting ball (Peleus ball).....	666 003
2	Watch glass dish, 60 mm diam.....	664 153
2	Powder funnel, glass, 50 mm diam. ....	602 680
2	from: Test tube, 16 x 160 mm, set of 10.....	664 043
2	Rubber stopper, solid, 14-18 mm diam. ....	667 253
1	Test tube rack for 9 tubes, 18 mm diam.....	667 050
1	Micro double-ended spatula .....	666 961
1	Electronic balance 200 g: 0.01 g .....	667 7977
1	Ammonium carbonate, 100 g .....	670 3900
1	Urea, 100 g .....	672 1700
1	Urease (1 U/mg), 5 g .....	675 2810
1	Copper(II) sulfate pentahydrate, 100 g.....	672 9600
1	Water, pure, 1L.....	675 3400
additionally required		
1	PC with Windows XP, Vista, 7 or 8	
Also necessary for wireless measurement:		
1	Battery for Pocket-CASSY 2 Bluetooth .....	524 019
1	Bluetooth dongle .....	524 0031

## Set-up and preparation of the experiment

### Preparing the solutions

**0.1 molar ammonium carbonate solution:** For 100 mL of solution, weigh 0.96 g of ammonium carbonate onto a watch glass dish and wash it into the volumetric flask (100 mL) using a powder funnel. Fill the flask up to the 100 mL mark with water. Then dissolve the ammonium carbonate completely by shaking.

**0.1 molar urea solution:** To obtain a 0.1 molar solution, weigh 0.12 g of urea onto a watch glass dish and transfer it to a test tube using a powder funnel. Then add exactly 10 mL of distilled water to the test tube using a graduated pipette. It is advisable to wash the residual urea on the watch glass dish into the test tube. Then close the test tube with a stopper and shake it until the urea has completely dissolved.

**Urease solution:** Weigh out 0.1 g of urease and place it in a test tube. As previously with the urea solution, add 10 mL of distilled water and wash in the residues. Dissolve the solid material by shaking.

**Note:** The solution as prepared has an initial enzyme activity of 10,000 U/L. With the stated activity of urease of 1 U/mg, 10 mmol of the substrate can be converted per minute and litre. The solution should be prepared as shortly as possible before starting the experiment and kept in the refrigerator to maintain this activity. In solution and at room temperature, the urease is easily denatured and can therefore become inactive.

### Construction of the apparatus

An apparatus for conductivity measurement will be constructed from a magnetic stirrer, stand materials, a conductivity sensor and a conductivity adapter (see Fig. 1). The sensor will then be connected via the Pocket-CASSY to the computer.

## Performing the experiment

### 1. Load CASSY Lab settings.

2. In order to determine the concentration of ammonium carbonate at every point in time, a reference value will be recorded for a 0.1 mol/L ammonium carbonate solution. For this, wash a beaker and the conductivity sensor with 30 mL of ammonium carbonate solution. Discard the washing solution and pour the remainder of the ammonium carbonate solution into the beaker. Place a stirring magnet into the beaker and switch on the magnetic stirrer. Then immerse the conductivity sensor into the liquid to a depth of at least 2 cm.

3. Read off the measured value. In the settings under "Parameter" for "Conductivity of ammonium carbonate", enter the value read (12,430  $\mu\text{S}/\text{cm}$ ) instead of the default value. This value would correspond with the complete hydrolysis of the 0.1 molar urea solution to ammonium and carbonate ions.

4. Fill the second beaker (150 mL) with 80 mL of water and add 10 mL of urea solution and a stirring magnet. Then immerse the conductivity sensor and switch on the stirrer.

5. Start the measurement. After about 10 seconds, add all 10 mL of the urease solution to the aqueous urea solution. The urea solution now has a concentration of 0.01 mol/L.

6. To inhibit the reaction, add some crystals of copper sulfate after about 3 minutes.

*Note: Be sure not to add too much copper sulfate, as otherwise the conductivity would be increased too much through the copper sulfate ions.*

7. Finish the measurements after a further 2 minutes.

## Observation

The specific conductivity increases after addition of the urease. When the copper sulfate is added after a few minutes, it increases again slightly through the ions from the copper sulfate. After a few seconds, the increase slows down until the conductivity remains almost constant.

## Evaluation

Several diagrams have been prepared in CASSY Lab in the CASSY example for the evaluation.

In the diagram "Specific conductivity", the values for the specific conductivity are plotted against time (see Fig. 3).

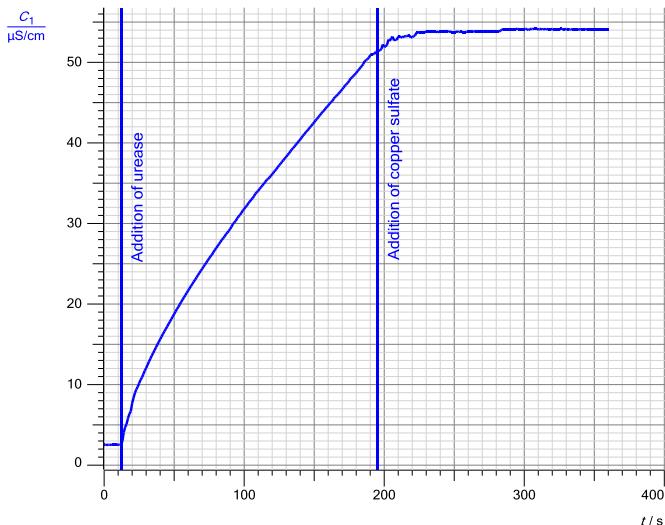


Fig. 3: Plot of the specific conductivity of the ammonium carbonate solution against time.

The conductivity can be converted to a urea concentration with the help of the calibration. This is demonstrated in the

diagram "Urea concentration" (see Fig. 4). It is calculated automatically as follows:

$$[\text{NH}_2\text{CONH}_2] = \frac{\kappa_1 - c_{A1}}{\kappa_1 - \kappa_0} \cdot 100 \text{ mmol/L}$$

Here,  $\kappa_1$  is the reference value of the 0.1 molar ammonium carbonate solution from the calibration.  $c_{A1}$  is the measured value for the conductivity at each point in time and  $\kappa_0$  the conductivity of the solution at the start of the reaction.

After adding the urease, a linear fall in the urea concentration can be seen in the diagram. When copper sulfate is added, the urea concentration no longer falls, but remains almost constant.

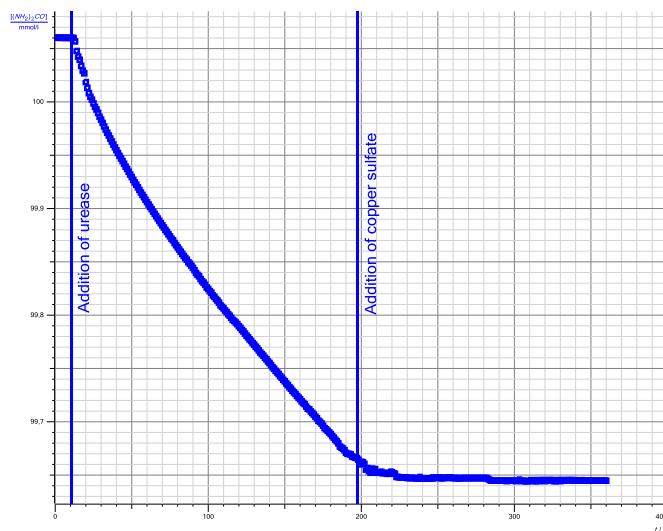


Fig. 4: Plot of urea concentration against time.

Finally, the automatically calculated reaction rate plotted against time is shown in the diagram "Reaction rate" (see Fig. 5). This is the change with time of the concentration of a starting material or a product (here: urea). If, therefore, the time derivation is formed by

$$r_{\text{H}_2\text{NCONH}_2} = -\frac{d[\text{H}_2\text{NCONH}_2]}{dt}$$

then the reaction rate at each point in time with respect to the educt urea can be calculated.

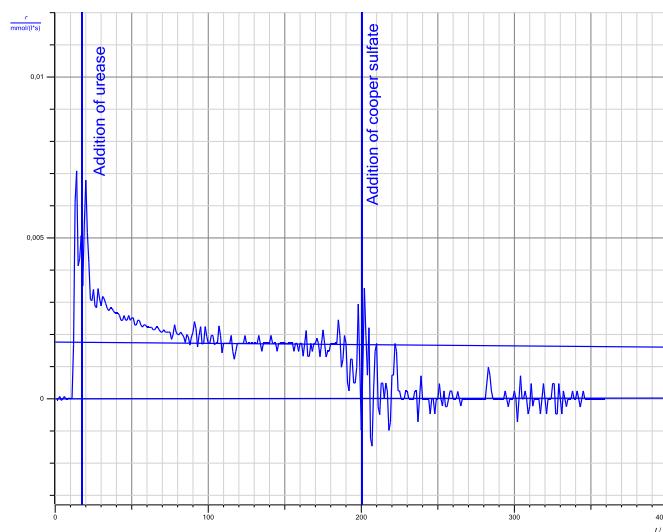


Fig. 5: Plot of reaction rate against time.

The reaction rate with respect to the urea concentration after adding the urease is almost constant. Therefore, this is a case of a zero-order reaction.

After adding the inhibitor, copper sulfate, an almost constant reaction rate of zero can be seen after a few seconds. The reaction has come to a standstill. To illustrate this, straight lines of best fit can be added, as shown in Fig. 5.

## Result

The enzyme urease catalyses the decomposition of urea to ammonium and carbonate ions in an aqueous medium. This results in an increase in conductivity. The conductivity can then be used to calculate the urea concentration at any point in time. It is thus apparent that this falls linearly during the course of the reaction. Therefore, it is a case here of a zero-order reaction. Such reactions are independent of the concentration of the substances involved. Many catalytic reactions behave in this way. The reason is that the rate-

determining steps take place at the catalyst, which is present in very small amounts compared with the reactants.

When the inhibitor copper sulfate is added, the reaction comes to a halt. The enzyme is denatured by the copper sulfate, as copper forms stable complexes with amino acids. These cause conformational changes in the urease which deactivate the enzyme.

## Cleaning and disposal

The solutions without copper sulfate can be poured into the laboratory drain. The solutions containing copper sulfate must be disposed of in the container for heavy metals without halogens. To reduce the volume, this solution can be allowed to dry or can be evaporated off beforehand.