Michaelis-Menten kinetics on the enzyme urease

Aims of the experiment

- To carry out an enzymatic experiment.
- To recognise the connection between reaction rate and concentration.
- To understand the relationship between conductivity and concentration.
- To construct Michaelis-Menten and Lineweaver-Burk diagrams.
- To evaluate the Michaelis constant.

Principles

Enzymes belong to the substance family of proteins. Proteins are macromolecules which consist of chains of amino acids. 23 proteinogenic amino acids are known so far in living cells.

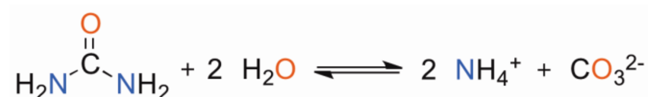
Enzymes regulate many chemical processes in cells through biocatalysis. In simplified terms, an enzymatic reaction can be shown as follows:

$$E + S \rightleftharpoons ES \rightleftharpoons P + E$$

An enzyme E binds a substrate S and so changes its conformation. The enzyme substrate complex ES is formed, which in most cases stabilises the transition state between substrate and product. The activation energy is therefore lowered but without a shift in the equilibrium of the reaction. The consequence is that the rate of the reaction is increased. The enzyme substrate complex breaks down into product P and the unchanged enzyme E. All these steps are equilibrium equations.

The simplified reaction equation serves as a basis for kinetic investigations according to the model of Leonor Michaelis and Maud Menten. It was proposed in 1913, is still valid and is also able to describe the kinetics of the enzyme urease.

The enzyme urease catalyses the decomposition of urea into ammonium and carbonate ions according to the following reaction equation:



$$H_2N{-}C({=}O){-}NH_2 + 2\ H_2O \rightleftharpoons 2\ NH_4^+ + CO_3^{2-}$$

Kinetic parameters of enzymes can be determined using Michaelis-Menten kinetics. The rate of a chemical reaction states how many particles are converted per unit of time. In catalytic reactions such as the decomposition of urea, the reaction rate is independent of the concentration of the reactants in most cases. The reaction rate is constant. Therefore, this is a case of a zero-order reaction. The reaction rate is, however, dependent on the initial substrate concentration.

In this experiment, the reaction rate for the decomposition of urease does not need to be measured directly. Only the time-dependent change in the conductivity is measured. This is a measure of the reaction rate, as the non-conducting urea decomposes to ammonium and carbonate ions through urease. These ions conduct the electrical current. The increase

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

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in conductivity is therefore proportional to the reaction rate.

According to Michealis-Menten, if the reaction rate is plotted against the initial substrate concentration, a saturation curve is obtained. The maximum reaction rate v_{max} can also be determined from this. Michaelis and Menten introduced the enzyme-specific constant K_M. This constant K_M, named after Michaelis and Menten, indicates for a certain enzyme the initial substrate concentration at which the enzyme works at half of the maximum rate v_{max}.

$$K_M = \frac{1}{2} v_{max}$$

The saturation curve of the Michaelis-Menten kinetics can be described by the following equation:

$$v = v_{max} \cdot \frac{[S]}{K_M + [S]}$$

Where v_0 is the initial rate, v_{max} the maximum rate of the reaction with substrate saturation, [S] the substrate concentration at the start of the reaction and K_M the Michaelis-Menten constant.

In this experiment, the Michaelis-Menten constant is to be determined for the decomposition of urea through urease.

Risk assessment

The chemicals used are in general non-hazardous.

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 607 105
1 Saddle base 300 11
1 Stand rod 25 cm, 10 mm diam. 301 26
1 Bosshead 0...16 mm 666 543
1 Universal clamp 0...80 mm 666 555
1 Volumetric flask Boro 3.3, 100 mL 665 793
5 Beaker, Boro 3.3, 150 mL, tall 602 010
5 Stirring magnets 666 851
1 Graduated pipette 10 mL 665 997
1 Pipetting ball (Peleus ball) 666 003
1 Measuring cylinder 100 mL, with plastic base 665 754
2 Watch glass dish, 6 mm diam. 664 153
2 Powder funnel Boro 3.3, 60 mm diam. 602 681
6 Test tube Fiolax, 16 x 160 mm 664 043
6 Rubber stopper, solid, 14...18 mm diam. 667 253
1 Test tube rack for 9 tubes,18 mm diam. 667 050
1 Double microspatula, stainless steel, 185 mm 666 961
1 Compact balance 200 g: 0.01 g 667 7977
1 Urea, 100 g 672 1700
1 Urease (1 U/mg), 5 g 675 2810
1 Water, pure, 1L 675 3400
1 Wash bottle, PE, 500 mL 661 243
Also required:
1 PC with Windows XP, 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

Dilution series of a 0.2 molar urea solution: In order to prepare a dilution series of urea solutions, first prepare a 0.2 of molar stock solution. Weigh 0.24 g of urea onto a watch glass dish and transfer it to a test tube using a powder funnel. Then add exactly 20 mL of distilled water to the test tube. It is advisable to wash the residual urea from the watch glass into the test tube. Close the test tube with a stopper and dissolve the solid urea by shaking.

Then produce a dilution series as follows: First place 10 mL of water into each of 4 test tubes. Draw up half (10 mL) of the 0.2 M stock solution with a pipette, add it to the first test tube filled with 10ml of water and shake well. Draw up again half (10 mL) of the resulting solution and add it to the next test tube filled with water. Repeat this two more times to finally produce solutions with the concentrations 0.2; 0.1; 0.05; 0.025 mol/L.

Urease solution: For the urease solution, weigh 0.5 g of urease onto a watch glass dish and place it into a test tube. Add 50 mL of distilled water to the urease. Completely dissolve the solid 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.

Set-up of the apparatus

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

Performing the experiment

1. Load CASSY Lab settings.
2. Fill a 150 mL beaker with 80 mL of water and add 10 mL of urea solution and a stirring magnet. In this way, the urea solution is diluted 1:10. The urea concentrations being investigated will therefore be 0.02; 0.01; 0.005 and 0.0025 mol/L.
3. Immerse the conductivity sensor to a depth of about 2 cm into the urea solution and switch on the stirrer.
4. Start the measurement. After about 10 seconds, add 10 mL of the urease solution to the aqueous urea solution. The measurement can be terminated after 2 – 3 minutes.
5. Repeat points 2 and 3 for every single concentration. In each case it is best to use a fresh beaker and magnetic stirrer flea.

Observation

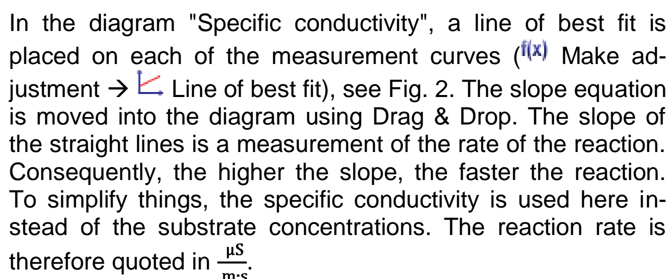
In the reaction, the urea is converted into the electrically conductive products NH_4^+ and CO_3^{2-}, which increase the current flow.

In the diagram "Specific conductivity", the values for the specific conductivity are plotted against time (see Fig. 2). The specific conductivity increases almost linearly after a brief starting phase. The greater the initial substrate concentration, the more quickly the conductivity increases.

Evaluation

The evaluation is performed in CASSY Lab. Three diagrams have been prepared for this.

Determination of the relative reaction rates



In the diagram "Specific conductivity", a line of best fit is placed on each of the measurement curves (f(x) Make adjustment → Line of best fit), see Fig. 2. The slope equation is moved into the diagram using Drag & Drop. The slope of the straight lines is a measurement of the rate of the reaction. Consequently, the higher the slope, the faster the reaction. To simplify things, the specific conductivity is used here instead of the substrate concentrations. The reaction rate is therefore quoted in $\frac{\mu S}{m \cdot s}$.

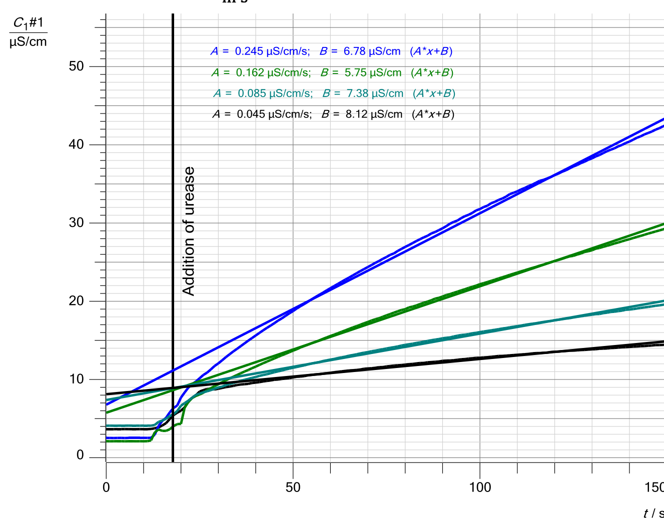


Fig. 2: Recording of the conductivity over time with various substrate concentrations. Blue is: 0.02, green: 0.01, turquoise: 0.005 and black 0.0025 mol/L of urea.

Michaelis-Menten kinetics

In the diagram "Michaelis-Menten", the substrate concentration is plotted against the calculated slopes (see Fig. 3). For this, enter into the relevant table (see Table 1) for the x value the concentration, and for the y value the slope of the corresponding line of best fit.

Tab. 1: Reaction rate *v* of the urease for the substrate concentrations S.

S $\frac{mol}{l}$	v $\frac{\mu S}{m \cdot s}$
0.0200	0.254
0.0100	0.162
0.0050	0.085
0.0025	0.045

The reaction rate reaches a saturation point with a high substrate concentration. This saturation point represents the maximum rate v_{max}. The Michaelis-Menten constant K_M can then be determined graphically, that is, the substrate concentration at half the maximum rate.

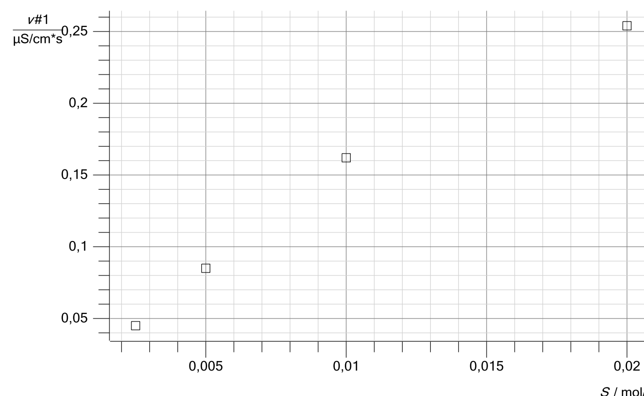


Fig. 3: Michaelis-Menten diagram for urease. The reaction rate *v* is plotted against the substrate concentration S.

The Michaelis-Menten constant can still be determined even if saturation is not reached. The Lineweaver-Burk plot is used for this.

Plot according to Lineweaver-Burk

The plot according to Lineweaver-Burk has also been prepared in CASSY Lab in diagram form (see Fig. 4). The saturation curve is shown here as a straight line. This is achieved by a double-reciprocal transformation of the Michaelis-Menten equation (see above) to produce a straight line equation.

$$\frac{1}{v} = \frac{K_M}{v_{max}} \cdot \frac{1}{[S]} + \frac{1}{v_{max}}$$

$$y = a \cdot x + b$$

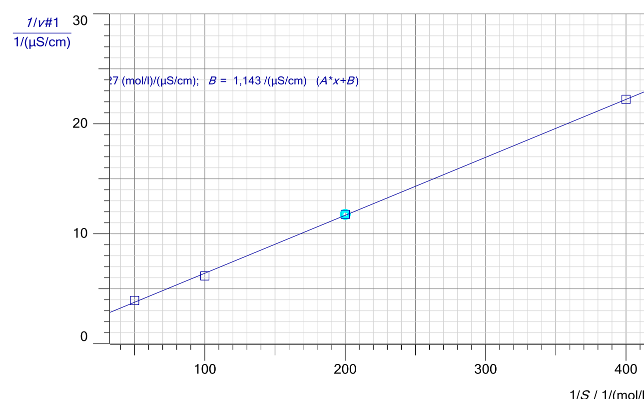


Fig. 4: Lineweaver-Burk diagram.

The y values (reciprocal of the reaction rate) can now be plotted against the x values (reciprocal of the substrate concentration) (see Table 2). By forming the reciprocal, the maximum reaction rate can be determined from the y-intercept, and the Michaelis-Menten constant from the x-intercept.

Tab. 2: Converted values for the plot according to Lineweaver-Burk.

S $\frac{mol}{l}$	$\frac{1}{S}$ $\frac{l}{mol}$	v $\frac{\mu S}{m \cdot s}$	$\frac{1}{v}$ $\frac{cm \cdot s}{\mu S}$
0.0200	50	0.254	3.937
0.0100	100	0.162	6.173
0.0050	200	0.085	11.765
0.0025	400	0.045	22.222

Results

Determination of the relative reaction rates

A line of best fit is placed on each of the graphs in the "Specific conductivity" diagram, and the linear equation is dragged into the diagram using Drag & Drop (see Fig. 2). The value of the slope is then entered into the table of values for the "Michaelis-Menten" diagram (see Table 1). By halving the substrate concentration, the reaction rate also approximately halves.

Michaelis-Menten kinetics

The Michaelis-Menten diagram can also be created from the table (see Fig. 3). Here, the reaction rate is plotted against the various concentrations. The four measured concentrations do not reach saturation, as too few measured values are used. For this reason, an estimation of the maximum rate according to Michaelis and Menten is not possible. For this reason, a diagram according to Lineweaver and Burk is created.

Plot according to Lineweaver-Burk

With the Lineweaver-Burk diagram (see Fig. 4), a double-reciprocal plot is made. The points lie on a straight line. A line of best fit is placed through the points and the slope equation dragged into the diagram using Drag & Drop. Now it is possible to read off the reciprocals of the maximum reaction rate on the y-intercept and of the Michaelis-Menten constant on the x-intercept.

From the values that have been read off, the following values for the maximum reaction rate v_{max} and the Michaelis-Menten constant K_M can thus be determined.

$$\frac{1}{v} = \frac{K_M}{v_{max}} \cdot \frac{1}{[S]} + \frac{1}{v_{max}}$$

$$\frac{1}{\mathrm{v_{max}}} = 1{,}1\ \frac{\mathrm{cm \cdot s}}{\mathrm{\mu S}}$$

$$\mathrm{v_{max}} = 0{,}9\ \frac{\mathrm{\mu S}}{\mathrm{cm \cdot s}}$$

$$-\frac{1}{K_M} = -20\,\frac{l}{\mathrm{mol}}$$

$$K_M = 0{,}05\ \frac{\mathrm{mol}}{\mathrm{l}} = 50\ \frac{\mathrm{mmol}}{\mathrm{l}}$$

The literature values of the Michaelis-Menten constant for urease lie between 0.12 $\frac{\mathrm{mmol}}{\mathrm{l}}$ and 130 $\frac{\mathrm{mmol}}{\mathrm{l}}$, depending on the organism. Therefore the K_M value of 50 $\frac{\mathrm{mmol}}{\mathrm{l}}$ is within the range of the literature values.

Cleaning and disposal

The solutions can be disposed of in the laboratory drain.

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