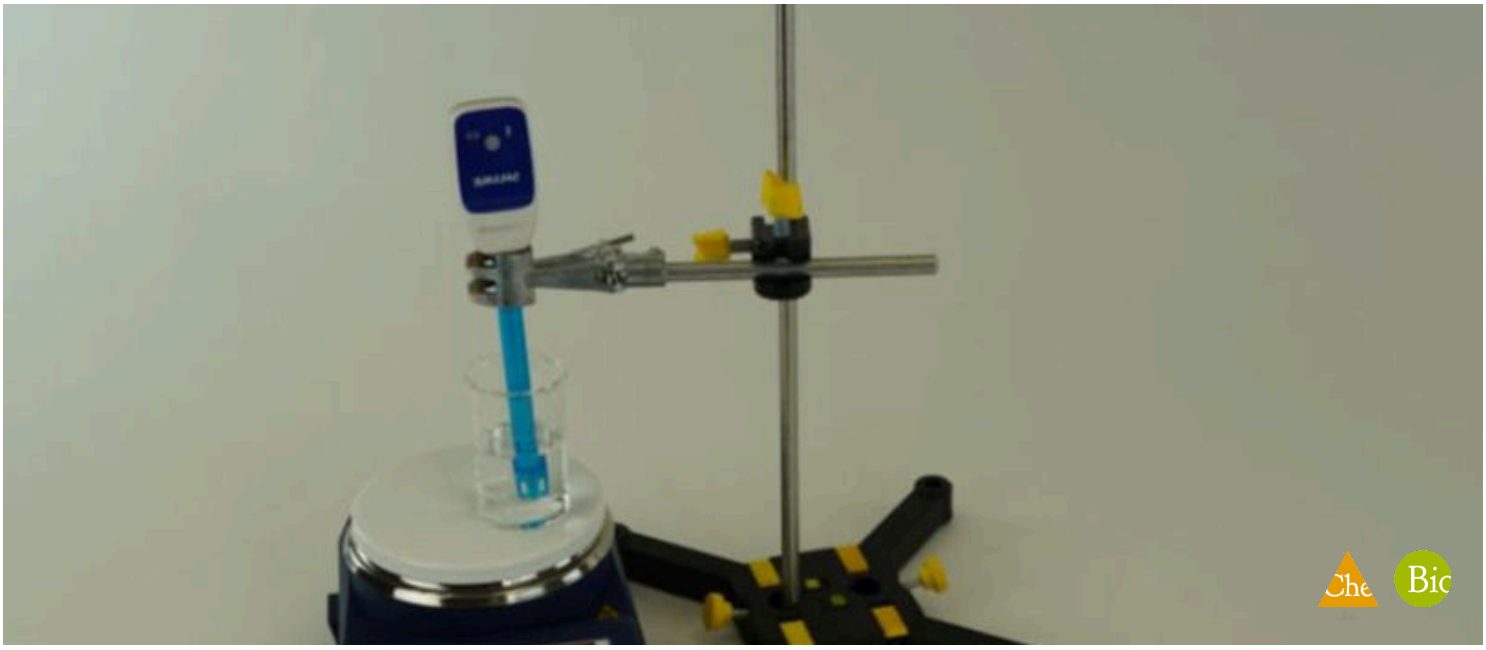
Determination of the Michaelis constant with CobraSMARTsense



Chemistry > Organic chemistry > Biochemistry

Biology > Biochemistry

Difficulty level	Group size	Preparation time	Execution time
medium	2	10 minutes	40 minutes

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General information

Application

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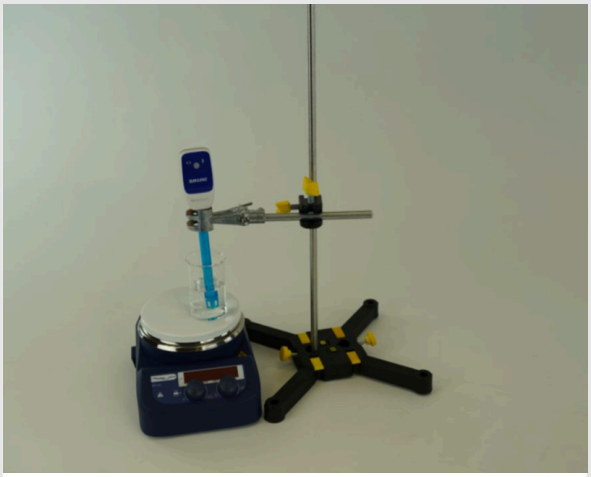

Experimental setup

The enzymatic hydrolysis of urea in aqueous solution yields carbon dioxide and ammonia. The ions of these compounds increase the conductivity of the solution. Conductivity measurements can be used to determine the rates of urea hydrolysis by the enzyme urease at different substrate concentrations. From these values, the Michaelis constant can be calculated.

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Other information (1/7)

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Prior knowledge

Students should be familiar with enzyme-substrate complexes, enzymatic urea hydrolysis and the principle of conductivity measurement.

Principle

Conductivity measurements are used to calculate the Michaelis constant.

Other information (2/7)

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Learning objective

Students should learn how to calculate the Michaelis constant by determining the conductivity (via hydrolysis of urea, which yields carbon dioxide and ammonia).

Tasks

The students are to determine the Michaelis constant from the measured values obtained in this experiment.

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Other information (3/7)

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Further information on the experiment

- To determine the Michaelis constants, the conductivity values at the time points 100 s and 200 s and their difference Δ Y are determined and recorded for all six measurements carried out.
- The mechanism of enzyme-catalysed reactions according to Michaelis-Menten starts from an enzyme-substrate complex ES, which is formed from enzyme E and substrate S in an upstream equilibrium reaction and decomposes to product P and unchanged enzyme E.

$$\mathrm{E} + \mathrm{S} \underset{k_1'}{\overset{\kappa_1}{\rightleftarrows}} \mathrm{ES} \xrightarrow{k_2} \mathrm{P} + \mathrm{E} \quad (1)$$

$$\frac{dc_{\mathrm{ES}}}{dt} = k_1 c_{\mathrm{E}} c_{\mathrm{S}} - k_1' c_{\mathrm{ES}} - k_2 c_{\mathrm{ES}} \approx 0 \quad (2)$$

According to the Bodenstein principle, the temporal change in the concentration of ES ≈ 0.

Other information (4/7)

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Converted according to the concentration of ES, the result is:

$$c_{\mathrm{ES}} = \frac{k_1 c_{\mathrm{E}} c_{\mathrm{S}}}{k_1' + k_2} \quad (3)$$

The free substrate concentration C_S can be equated to the total concentration of S, since only a small amount of enzyme is added. The total concentration of E, $C_{E,0}$ is equal to the sum of the concentration of free enzyme C_E and to enzyme-substrate complex C_{ES} :

$$c_{\mathrm{E},\,0} = c_{\mathrm{E}} + c_{\mathrm{ES}} \quad (4)$$

After converting and substituting equation (4) into equation (3), we obtain:

$$c_{\mathrm{ES}} = \frac{k_1 \cdot (c_{\mathrm{E},\,0} - c_{\mathrm{ES}}) c_{\mathrm{S}}}{k_1' + k_2} \quad (5)$$

Convert to C_{ES} delivers:

$$c_{\mathrm{ES}} = \frac{k_1 \cdot c_{\mathrm{E},\,0} \cdot c_{\mathrm{S}}}{k_1' + k_2 + k_1 c_{\mathrm{S}}} \quad (6)$$

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Other information (5/7)

For the product formation step, the time law is: $$\frac{dc_P}{dt} = k_2 c_{ES} \quad (7)$$

If one sets for C_{ES} the expression equation (6), one obtains: $$\frac{dc_p}{dt} = \frac{k_1\, k_2 c_{E,0} c_S}{k_1' + k_2 + k_1 c_S} \quad (8)$$

The quotient $\frac{k_1' + k_2}{k_1} = K_M$ (9) becomes the Michaelis constant, K_M summarised. Thus the law of time is: $$\frac{dc_p}{dt} = \frac{k_2\, c_{E,0}\, c_S}{K_M + c_S} \quad (10)$$

The speed of enzymolysis is thus linearly dependent on the enzyme concentration. The influence of the substrate concentration is more complicated. For the case $C_S > K_M$ the equation (10) simplifies to

$$\frac{dc_p}{dt} = k_2 c_{E,0} \quad (11)$$

Other information (6/7)

In this case the reaction is of zero order according to S and the enzymolysis has its maximum velocity with k_2 $C_{E,0}$. If $C_S = K_M$ half of the maximum speed is reached. The Michaelis constant thus corresponds to the substrate concentration at which the reaction proceeds at half maximum speed. In the case where there is only little substrate left, i.e. $C_S > K_M$, results in

$$\frac{dc_p}{dt} = \frac{k_2}{K_M} \cdot c_{E,0} c_S \quad (12)$$

i.e., the rate of formation of P is first order after E and S.
For evaluation, the average speeds of enzymolysis between 100 s and 200 s after the start are determined. For this purpose, the difference of the conductivity values after 100 and 200 seconds in each case is to be formed (Δ Y) and divided by 100 s. The conductivity values are then calculated. These velocities (in µS cm-1 s-1) are plotted against the urea concentration (in mmol l-1). The substrate concentration C_S (in mmol/l) is calculated according to the formula:

Cs=(W10000)/M (13) with **W** = concentration of the urea solution in % and **M** = molar mass of urea = 60.06 g/mol.

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Other information (7/7)

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Since it is difficult to determine the concentration corresponding to the half-maximum speed, i.e. K_MTo read off the data directly, the Lineweaver-Burk order is used.

With the reaction speed v=dc_P/dt and in reciprocal representation is obtained from equation (10):

$$\frac{1}{v} = \frac{1}{k_2} + \frac{K_M}{k_2} \cdot \frac{1}{c_S} \quad (14)$$

The plot of 1/v against $1/C_S$ (cf. Fig.5) provides k_2(to the power of -1) as an ordinate intercept (1/cs=0) and K_M/k_2 as the rise of a straight line. First, the ordinate intercept and the slope of the straight line are determined. This slope is then divided by the ordinate intercept to obtain the Michaelis constant. The calculation gives a value of 4.86 x 10-3 mol/l for the Michaelis constant of urease.

A small K_M-value means a high affinity of the enzyme to its substrate.

Urease was the first enzyme to be presented in crystalline form (Sumner, 1926). In contrast to the allosteric enzymes, it belongs to the "normal" enzymes that satisfy the Michaelis-Menten mechanism.

Safety instructions

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- The general instructions for safe experimentation in science lessons apply to this experiment.

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Theory

Urease catalyses the enzymatic hydrolysis of urea in water, yielding carbon dioxide and water. With the help of the SMARTsense Conductivity Sensor, the conductivity of the resulting solution can be measured. This makes it possible to follow how the ions of the compound increase the conductivity.

The Michaelis constant is the substrate concentration at which half the maximum speed of an enzyme is reached.

Since the rates of urea hydrolysis at different substrate concentrations can be measured via the conductivity measurement, the Michaelis constant can be calculated from these values.

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Equipment

Position	Material	Item No.	Quantity
1	Cobra SMARTsense Conductivity - Sensor for measuring conductivity in liquids 0 ... 20000 µS/cm / 0 ... 100°C (Bluetooth)	12922-00	1
2	Separator for magnetic bars	35680-03	1
3	Magnetic stirrer without heating, 3 ltr., 230 V	35761-99	1
4	Magnetic stirring bar 30 mm, cylindrical	46299-02	1
5	Compact Balance, OHAUS TA 302, 300 g / 0.01 g	49241-93	1
6	Retort stand, h = 750 mm	37694-00	1
7	Boss head	02043-00	1
8	Universal clamp	37715-01	1
9	Beaker, Borosilicate, tall form, 100 ml	46026-00	1
10	Beaker, 250 ml, plastic (PP)	36013-01	1
11	Erlenmeyer flask, stopper bed, 100 mlSB 19	MAU-EK17082002	6
12	Rubber stopper, d=22/17 mm, without hole	39255-00	6
13	Volumetric pipette, 20 ml	36579-00	1
14	Volumetric pipette, 50 ml	36581-00	1
15	Pipettor	36592-00	1
16	Micro-l syringe, 100 micro-l	02606-00	1
17	Microspoon, steel	33393-00	1
18	Wash bottle, plastic, 500 ml	33931-00	1
19	Urea, 250 g	30086-25	1
20	Urease soln.in 50% glycerol,10ml	31924-03	1
21	Water, distilled 5 l	31246-81	1
22	measureAPP - the free measurement software for all devices and operating systems	14581-61	1

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Additional equipment

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Position	Art. No.	Designation
1		Mobile device (smartphone / tablet)
2	14581-61	measureAPP

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Set-up & Procedure

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Set-up (1/3)

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To measure conductivity, you need the Cobra SMARTsense Conductivity and the measureAPP. Check whether "Bluetooth" is activated on your device (tablet, smartphone) (the app can be downloaded free of charge from the App Store - QR codes below). Now open the measureAPP on your device.

measureAPP for

Android operating systems

measureAPP for

iOS operating systems

measureAPP for

Tablets and PCs with Windows 10

Set-up (2/3)

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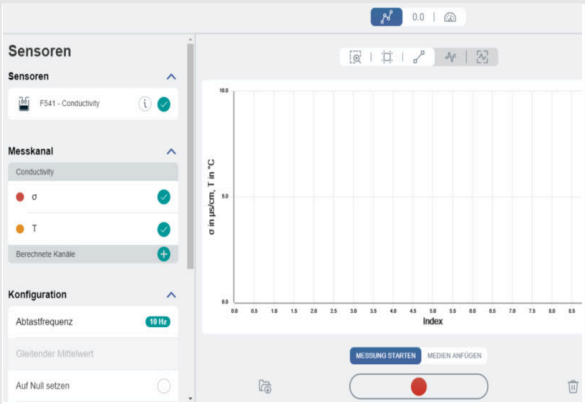

User interface measureApp

in the Windows 10 version

- Switch on the SMARTsense Conductivity Sensor by pressing and holding the power button.
- Connect the sensor in the measureAPP under the item "Measure" to the device as shown in the figure on the left.
- The SMARTSense Conductivity Sensor is now displayed in the app.

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Set-up (2/3)

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Solutions with different concentrations of urea are required for the experiment. These must be freshly prepared before the start of the experiment:

- 0.4% urea solution (urea stock solution): Weigh 0.40 g urea into a 100 ml conical flask and dissolve it in 99.6 g distilled water.
- 0.2% urea solution: By pipetting 50 ml of the 0.4% urea solution with the 50 ml volumetric pipette into a 100 ml conical flask and adding 50 ml distilled water.
- 0.1% urea solution: By pipetting 50 ml of the 0.2% urea solution with the 50 ml volumetric pipette into a 100 ml conical flask and adding 50 ml distilled water.
- 0.05% urea solution: By pipetting 50 ml of the 0.1% urea solution with the 50 ml volumetric pipette into a 100 ml conical flask and adding 50 ml distilled water.

Set-up (2/3)

- 0.025% urea solution: By pipetting 50 ml of the 0.05% urea solution with the 50 ml volumetric pipette into a 100 ml conical flask and adding 50 ml of distilled water.
- 0.0125% urea solution: By pipetting 50 ml of the 0.025% urea solution with the 50 ml volumetric pipette into a 100 ml conical flask and adding 50 ml distilled water.

Note: The urease solution should always be stored in the refrigerator!

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Set-up (3/3)

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- Set up the equipment as shown in the experimental set-up illustration.
- Attach the universal clamp with the double socket to the stand rod of the Bunsen tripod.
- Fix the SMARTsense Conductivity Sensor with the universal clamp.

Procedure (1/2)

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- Add 40 ml of the 0.0125% urea solution (lowest concentration first) and a magnetic stirring rod to a 100 ml beaker by pipetting twice with the 20 ml volumetric pipette.
- Place the beaker on the magnetic stirrer and immerse the conductivity probe in the solution.
- Set the stirrer to a medium stirring speed. (Caution: The magnetic stirring rod must not hit the conductivity probe!).
- With the microlitre syringe, 50 µl of the urease solution is added and the measurement is started without delay by clicking the start button.
- The time course of the reaction can be followed visually on the monitor.
- After finishing the measurement, save the data for further data processing.

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Procedure (2/2)

- In this way, the measurements are carried out with all six prepared urea solutions (in ascending order).
- For the individual measurements, the beaker is removed from the magnetic stirrer in each case and the magnetic stirring rod is taken out of the solution with the removal rod.
- The magnetic stir bar is rinsed thoroughly with distilled water, dried briefly with a paper towel and placed in the next solution.
- The conductivity probe must also be rinsed thoroughly with distilled water after each test.

Report

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Task 1

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Drag the words into the correct places.

The enzymatic hydrolysis of urea in aqueous solution yields _____ and ammonia. The _____ of these compounds increase the _____ of the solution. Conductivity measurements can be used to determine the rates of urea hydrolysis by the enzyme _____ at different substrate concentrations. The _____ can be calculated from these values.

- Michaelis constant
- conductivity
- ions
- carbon dioxide
- urease

Check

Task 2

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What does a small value of the Michaelis constant mean?

- A small Michaelis constant value means a low affinity of the enzyme to its substrate.
- A small Michaelis constant value means that the enzyme cannot do anything in the substrate and another enzyme must be chosen.
- A small Michaelis constant value means a high affinity of the enzyme to its substrate.
- A small Michaelis constant value has no significance for the enzyme-substrate relationship.

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Task 3

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Choose the correct statements.

- ○ The Michaelis constant indicates the substrate concentration at which the maximum speed of an enzyme is reached.
- ○ The Michaelis constant is always 50.
- ○ The Michaelis constant is the substrate concentration at which half the maximum speed of an enzyme is reached.

Check

Slide	Score / Total
Slide 23: Michaelmas constant	0/5
Slide 24: Meaning Michaelmas constant	0/2
Slide 25: Statements on the Michaelis constant	0/1

Total 0/8

Solutions Repeat

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