

Laboratory Courses in Physical Chemistry
for students the study courses chemical engineering
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Elaboration of the experiment 9.2

Michaelis Menten Mechanism

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1 Problem

In this experiment, we should eximinate the enzymatic hydrolysis of ures and determinate the Michaelis-Menten constant K_M with the aid of conductivity.

2 Theory

The principal features of many enzyme-catalysed reaction are as follows:

- For a given initial concentration of substrate, $[S]_0$, the initial rate of the product formation is proportional to the total concentration of enzyme $[E]_0$.
- For a given $[E]_0$ and low values of $[S]_0$, the rate of product formation is proportional to $[S]_0$.
- For a given $[E]_0$ and high values of $[S]_0$, the rate of product formation becomes independent of $[S]_0$, reaching a maximumvalue known as maximun velocity.

The Michaelis-Menten mechanism accounts for these features. According to this mechanism, an enzyme-substrate complexed is formed in the fist step and either the substrate is released unchanged or after modification to form products.



E=enzyme S=substrate P=product [ES]=enzyme-substrate complex

The rate of product formation according to this mechanism is

$$v = k_2 [ES] \quad (2)$$

We can obtain the concentration of the enzyme-substrate complex by invoking the steady-state approximation and writing

$$[ES] = \left(\frac{k_1}{k_{-1} + k_2} \right) \quad (3)$$

$$k_1 ([E] - [ES]) [S] - k_{-1} [ES] - k_2 [ES] = 0 \quad (4)$$

After shaping the equation, we get:

$$K_M = \frac{[S] ([E] [ES])}{[ES]} = \frac{k_{-1} + k_2}{k_1} \quad (5)$$

K_M is called Michaelis constante. Now, the concentration of [ES] in steady-state approximation is

$$[ES] = \frac{[E] [S]}{K_M + [S]} \quad (6)$$

So the velocity of an enzymatic reaction is classified as

$$v = -\frac{d[S]}{dt} = k_2 [ES] = k_2 \cdot \frac{[E] [S]}{K_M + [S]} \quad (7)$$

With equation (2) we get

$$v = \frac{v_{max} \cdot [S]}{K_M + [S]} \quad (8)$$

In case of $v = \frac{1}{2} \cdot v_{max}$ we gain an important relation

$$\frac{v_{max}}{2} = \frac{v_{max} \cdot [S]}{K_M + [S]} \quad (9)$$

$$\frac{1}{2} = \frac{[S]}{K_M + [S]} \quad (10)$$

$$\Rightarrow K_M = [S] \quad (11)$$

The value of K_M depends on pH value and temperature.

For the experimental definition of K_M one uses the *Lineweaver-Burk plot*

$$\frac{1}{v} = \frac{K_M}{v_{max}} \frac{1}{[S]} + \frac{1}{v_{max}} \quad (12)$$

$1/v$ is plotted against $1/[S]$ and should yield a straight line with slope K_M/v_{max} , a y-intercept at $1/v_{max}$ and an x-intercept at $-1/K_M$.

3 Experiment

3.1 Procedure

The conductance is increased by developing ions from the reaction of urea and its enzyme urease. So the velocity is measured with the aid of conductivity.

We prepared the solutions with different concentrations of urea before the measurement started to make sure they are fresh.

1. 0.4% urea solution (parent solution): 1 g urea and in 250 mL of dest. water
2. 0.2% urea solution: Thinning 100 mL parent solution in 100 mL dest. water
3. 0.1% urea solution: Thinning 100 mL of 0.2% solution in 100 mL dest. water
4. 0.05% urea solution: Thinning 100 mL of 0.1% solution in 100 mL dest. water
5. 0.025% urea solution: Thinning 100 mL of 0.05% solution in 100 mL dest. water
6. 0.0125% urea solution: Thinning 100 mL of 0.25% solution in 100 mL dest. water

After preparing the different solutions, we started to pipette 100 mL of the 0,0125% solution in a flask, then added 50 μ L urease with a gun and measured the conductivity for ten minutes. We did the same with the other five solutions.

We did the measurement for the 0,1% (0,203 g in 200 mL) and 0,05% (0,100 g in 200 mL) solutions twice to get more accuracy for the Liwearer-Burk-Plot.

During this experiment, it is very important to work accurately because of the low concentrations.

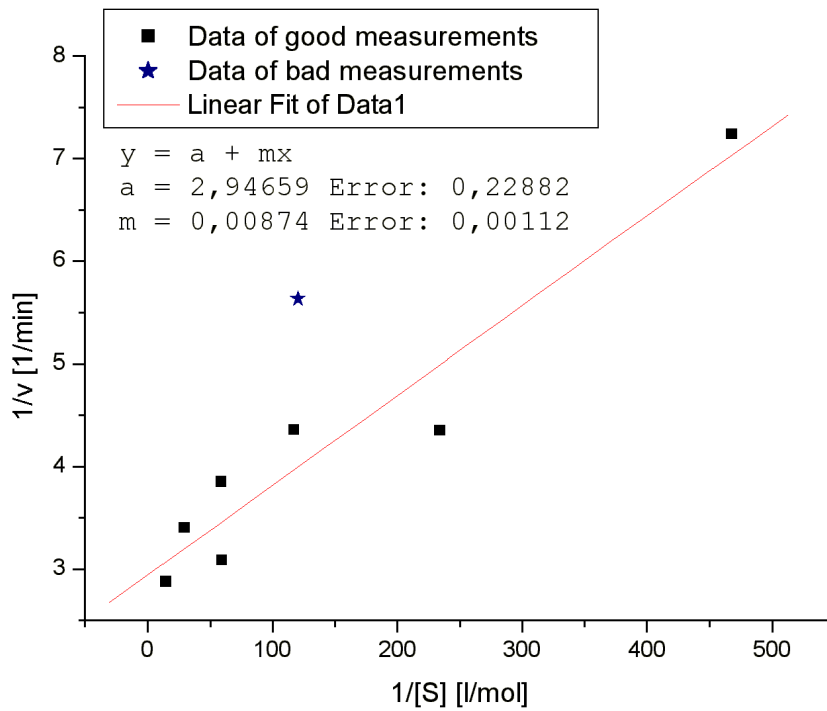
3.2 Evaluation

solution [%]	c [mol/L]	Δx [s]	Δy [$\mu\text{S}/\text{cm}$]	v [$\mu\text{S}/(\text{cm} \cdot \text{s})$]	$1/v$ [$\text{cm} \cdot \text{s}/\mu\text{S}$]	$1/c$ [L/mol]
0,4	0,068464868	327	113,60	0,34740	2,878521127	14,60603
0,2	0,034232434	361	106,00	0,29363	3,405660377	29,21206
0,1	0,017116217	339	87,90	0,25929	3,85665529	58,42412
	0,016899767	298	96,50	0,32383	3,088082902	59,17241
0,05	0,008558109	348	79,80	0,22931	4,360902256	116,84825
	0,008325008	343	60,90	0,17755	5,632183908	120,12000
0,025	0,004279054	328	75,30	0,22957	4,355909695	233,69650
0,0125	0,002139527	352	48,60	0,13807	7,242798354	467,39300

Table 1: Data for the velocity of urease hydrolysis

We will not consider one measurement of the 0,05 solution because it differs too much from the other values.

With these values we can create a *Linwearer-Burk-Plot* by plotting the inverse of the velocity (the rate constant) of the reaction $1/v$ against the inverse of the urea concentration $1/[S] = 1/c$.



The maximum velocity v_{max} is the inverse value of the intercept point with the ordinate (y-axis): So we got for v_{max} :

$$v_{max} = \frac{1}{a} = (2,94659\%)^{-1} \frac{\text{cm} \cdot \text{s}}{\mu\text{S}} = 0,33938 \frac{\mu\text{S}}{\text{cm} \cdot \text{s}}$$

The slope of the line is $m = \frac{\Delta y}{\Delta x} = \frac{K_M}{v_{max}}$. It follows:

$$\begin{aligned} K_M &= m \cdot v_{max} = 0,00874 \frac{\text{mol} \cdot \text{cm} \cdot \text{s}}{\mu\text{S} \cdot \text{L}} \cdot 0,3394 \frac{\mu\text{S}}{\text{cm} \cdot \text{s}} \\ &= 2,96614 \cdot 10^{-3} \frac{\text{mol}}{\text{L}} \end{aligned}$$

The error is determined graphically, the error for each value is very small (see error calculation), so it is safe to ignore it and we just take care of the error by linear regression. So we got a final result for the Michaelis-Menten constant of urease of:

$$K_M = 2,96 \pm 1,12 \cdot 10^{-3} \frac{\text{mol}}{\text{L}}$$

4 Error calculation

The apparatuses had the following errors:

- Weighing machine: $\Delta m \pm 0,001 \text{ g}$
- Pipette 100 ml (used for both water and urea): $\Delta V \pm 0,05 \text{ mL}$
- Measurement of the time: $\Delta t \pm 1 \text{ s}$
- Measurement of the conductivity: $\Delta \kappa = \pm 0,1 \mu\text{S}/\text{cm}$

For the error of the substrate concentration c of the 0,4 % solution we get by error propagation:

$$\frac{\Delta c_{0,4}}{c_{0,4}} = \sqrt{\left(\frac{\Delta m}{m}\right)^2 + \left(\frac{\Delta V}{V}\right)^2} \quad (13)$$

$$= \sqrt{\left(\frac{0,001 \text{ g}}{1,028 \text{ g}}\right)^2 + \left(\frac{0,2 \text{ mL}}{250 \text{ mL}}\right)^2} \quad (14)$$

$$= 0,1259\% \quad (15)$$

We assume a max. error of 0,2 mL for the volumina because we pipet 3 times to obtain 250 mL.

For the further solution we need to take account of the error of the concentration of the parent solutions and we get the following equation:

$$\frac{\Delta c_{n-1}}{c_{n-1}} = \sqrt{\left(\frac{\Delta c_n}{c_n}\right)^2 + \left(\frac{\Delta V}{V}\right)_{H_2O}^2 + \left(\frac{\Delta V_n}{V_n}\right)^2} \quad (16)$$

$$= \sqrt{\left(\frac{\Delta c_n}{c_n}\right)^2 + 2 \cdot \left(\frac{\Delta V}{V}\right)^2} \quad (17)$$

$$(18)$$

Furthermore the error of the rates v can be obtained by:

$$\frac{\Delta v}{v} = \sqrt{\left(\frac{\Delta t}{t}\right)^2 + \left(\frac{\Delta \kappa}{\kappa}\right)^2} \quad (19)$$

$$= \sqrt{\left(\frac{\Delta t}{\Delta x}\right)^2 + \left(\frac{\Delta \kappa}{\Delta y}\right)^2} \quad (20)$$

Finally we get the table 2 with the percentage errors of the ordinate and the abscissa.

solution [%]	abscissa	$\pm 1/c$ [L/mol]	ordinate	$\pm 1/v$ [cm · s/ μ S]
0,4	0,12595%	0,01840	0,31823%	0,00916
0,2	0,14444%	0,04219	0,29263%	0,00997
0,1	0,16082%	0,09396	0,31616%	0,01219
	0,49514%	0,29299	0,35121%	0,01085
0,05	0,17568%	0,20528	0,31349%	0,01367
	1,00124%	1,20269	0,33461%	0,01885
0,025	0,18937%	0,44256	0,33255%	0,01449
0,0125	0,20215%	0,94481	0,35078%	0,02541

Table 2: Error table