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A 36

Michaelis-Menten kinetics
Hydrolysis of urea

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1.7

Bitte wieder vorlegen
10.03.17
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please do the following in the next version:-

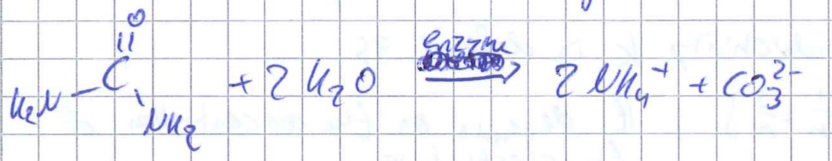
1)- plot again the Lineweaver-Burk plot with the right values of $\frac{1}{V_0}$.

2)- show me how do you calculate $\frac{1}{V_0}$ (the calculation procedure with numbers)

3)- Re-calculate again V_{max} and K_M regarding your new plot and discuss again the results.

Objective

The kinetics of the decomposition of urea with urease should be investigated according to the following reaction.



$$H_2N-\overset{\overset{O}{\|}}{C}-NH_2 + 2\,H_2O \xrightarrow{\text{enzyme}} 2\,NH_4^+ + CO_3^{2-}$$

basics

Michaelis-Menten kinetics

For the above reaction, urease is the enzyme which cleaves urea. It acts as a biocatalyst.

To show the reaction rate of this enzymatic reaction, the Michaelis-Menten mechanism can be used.

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$ E: enzyme S: substrate P: products

The temporal change of the enzyme-substrate complex is described as follows

$$\frac{d[ES]}{dt} = k_1[E][S] - k_{-1}[ES] - k_2[ES]$$

initial enzyme concentration

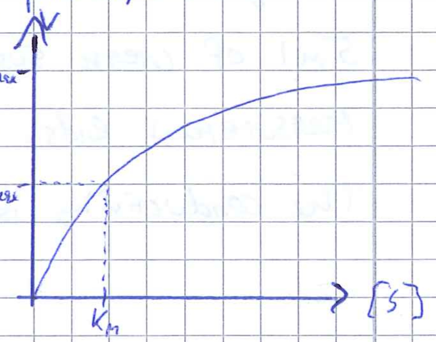
also $v = \frac{d[P]}{dt} = k_2[ES]$ and $[E] + [ES] = [E]_0$ mass balance

We now apply Bodenstein's steady-state principle

$$\frac{d[ES]}{dt} = k_1[E][S] - k_{-1}[ES] - k_2[ES] = 0$$

By rearranging and insertion we get

$$v = \frac{d[P]}{dt} = \frac{k_2[E]_0[S]}{[S] + K_m} \qquad K_m = \frac{k_{-1} + k_2}{k_1}$$



If $[S] \gg K_m$: $[S] + K_m \approx [S]$

$$\frac{d[P]}{dt} = k_2[E]_0$$

If $[S] \ll K_m$: ~~$v_0 = \left(\frac{d[P]}{dt}\right)_{t=0} = \frac{k_2[E]_0[S]}{[S]_0 + K_m}$~~ $\frac{d[P]}{dt} = k_2\frac{[E]_0[S]}{K_m}$

Since $[S]_0$ is known, following equation applies:

$$v_0 = \left(\frac{d[P]}{dt}\right)_{t=0} = \frac{k_2[E]_0[S]}{[S]_0 + K_m}$$

With the Lineweaver-Burk plot: $\frac{1}{v_0} = \frac{1}{v_{max}} + \frac{1}{[S]_0}\frac{K_m}{v_{max}}$; $v_{max} = k_2[E]_0$

With this equation, K_m and v_{max} can be determined.

conductivity of electrolytes

One defines specific resistance ρ which is charactristic of an electrolytic conductor: $\rho = R \frac{A}{l}$

l: electrode distance
A: surface of the electrode

The specific conductivity k is defined as

$k = \frac{1}{\rho} = \frac{l}{RA}$ $\left[\frac{1}{\Omega m} = \frac{S}{m}\right]$; It depends on the concentration of the electrolyte.

In the urea solution, the conductivity is derived from that of the water. If $k > 7 \frac{\mu S}{cm}$, the solution must be reset due to impurities. Due to urease, ammonium carbonat is formed, which then increases the conductivity.

Experimental procedure

Prepare a urease suspension with $\beta = 2\ g/l$ as well as 50ml urea solutions with the following concentrations:

$\left[\frac{mol}{l}\right]$ $2 \cdot 10^{-3}$; $2{,}2 \cdot 10^{-3}$; $2{,}5 \cdot 10^{-3}$; $2{,}8 \cdot 10^{-3}$; $3{,}4 \cdot 10^{-3}$; $4 \cdot 10^{-3}$; $5 \cdot 10^{-3}$; $8 \cdot 10^{-3}$; $2 \cdot 10^{-2}$; $5 \cdot 10^{-2}$

Also prepare $(NH_4)_2CO_3$ solutions with followin concentrations:

$\left(\frac{mol}{l}\right)$ $5 \cdot 10^{-4}$; $5 \cdot 10^{-3}$; $1 \cdot 10^{-2}$; $1{,}5 \cdot 10^{-2}$; $2 \cdot 10^{-2}$

The room temperature warm urea solution is stirred with a magnetic stirrer. After the start of the measurement 5ml of urease suspension are added. After 3 minutes the measurement ends.

The conductivity is also measured from the $(NH_4)_2CO_3$ solutions.

Source: description of experiment

data analysis

Graph 1 shows the linear fit with the resulting slope $m_{cal} = 138407{,}4 \pm 10887{,}3 \frac{\mu S}{s\,ms}$ of the calibration measurement with ammonium carbonate.

Graph 2 shows all the measured conductivities of each concentration. The linear fit includes just the times between 20 and 140 s because this is the time when the actual reaction happens, in the first 20 seconds the urease was added to the urea-solution.

To get the single initial reaction rate v_0 each slope of the linear regressions of graph 2 was multiplied with m_{cal}: $v_0 = m_{cal} \cdot m_{urea}$ (listed in table 1).

~~Th~~ The next task was to draw a Lineweaver-burk plot. As a preparation we had to calculate $\frac{1}{v_0}$ as you can see in table 1 and also determine $\frac{1}{[S_0]}$ because of the function that had to be plotted: $\frac{1}{v_0} = \frac{1}{v_{max}} + \frac{K_M}{v_{max}} \cdot \frac{1}{[S_0]}$

5ml urease solution was added every time to the 50ml urea-solution so that the concentration of urea changed and had to be taken into account. (see in table 1 column 6)

$V_1 = 50ml$, $V_2 = 50ml + 5ml$, $c_1 = c_{urea}$ (from 1st column)

Graph 3 shows the resulting Lineweaver-burk-plot with which we can determine v_{max} (from the y-intercept $\frac{1}{v_{max}}$) and K_M (from the slope $m \cdot v_{max}$) with a linear regression.

In the last diagram we already added the errorbars $\Delta \frac{1}{v_0}$ and $\Delta \frac{1}{S_0}$ which are also listed in table 1. The errors were calculated as follows:

- $v_0 = m_{cal} \cdot m_{urea} \Rightarrow \frac{1}{v_0} = \frac{1}{m_{cal} \cdot m_{urea}} \Rightarrow \Delta \frac{1}{v_0} = \left|\frac{\partial \frac{1}{v_0}}{\partial m_{cal}}\right| \Delta m_{cal} + \left|\frac{\partial \frac{1}{v_0}}{\partial m_{urea}}\right| \Delta m_{urea}$

$$= \left|\frac{\Delta m_{cal}}{m_{cal}^2 \cdot m_{urea}}\right| + \left|\frac{\Delta m_{urea}}{m_{cal} \cdot m_{urea}^2}\right|$$

with $\Delta m_{cal} = 10887{,}3 \frac{\mu S}{cm\,s}$, $m_{cal} = 138407{,}4 \frac{\mu S}{cm\,s}$ => table 1 8th column

- $c_{urea} = \frac{m_{urea}}{M_{urea} \cdot V_{H_2O}} \Rightarrow \Delta c_{urea} = \left|\frac{\partial c}{\partial m}\right| \Delta m + \left|\frac{\partial c}{\partial V}\right| \Delta V = \left|\frac{\Delta m}{M_{urea} V_{H_2O}}\right| + \left|\frac{m_{urea}\, \Delta V}{M_{urea}\, V_{H_2O}^2}\right|$

as M_{urea} was given in the description of the experiment ($60{,}06 \frac{g}{mol}$) thee is no error of M that can be calculated with.

$\Delta m_{urea} = 0{,}1 mg$, $m_{urea} = 0{,}6\,g$, $V_{H_2O} = 100\,ml$, $\Delta V = 0{,}5\,ml$

$\Rightarrow \Delta c_{urea} = 2{,}265 \cdot 10^{-3} \frac{mol}{l}$

- $[S_0] = \frac{c_{urea} \cdot V_{urea}}{V_2} \Rightarrow \frac{1}{[S_0]} = \frac{V_2}{c_{urea} \cdot V_{urea}} \Rightarrow \Delta \frac{1}{[S_0]} = \left|\frac{\partial \frac{1}{[S_0]}}{\partial V_2}\right| \Delta V_2 + \left|\frac{\partial \frac{1}{[S_0]}}{\partial V_{urea}}\right| \Delta V_{urea} + \left|\frac{\partial \frac{1}{[S_0]}}{\partial c_{urea}}\right| \Delta c_{urea}$

$$= \left|\frac{\Delta V_2}{c_{urea} \cdot V_{urea}}\right| + \left|\frac{V_2 \cdot \Delta V_{urea}}{c_{urea} \cdot V_{urea}^2}\right| + \left|\frac{V_2 \cdot \Delta c_{urea}}{V_{urea} \cdot c_{urea}^2}\right|$$

$c_{urea} = 0{,}1\,\frac{mol}{l}$, $\Delta c_{urea} = 2{,}265 \cdot 10^{-3}\,\frac{mol}{l}$, $\Delta V_{urea} = 0{,}05\,ml$, $\Delta V_2 = 0{,}55\,ml$

V_1 are the Volumes that were use to make the urea solutions

$\Rightarrow \Delta\frac{1}{[S_0]}$ see table 1 column 9

Calculate again with the new plot.

$\Rightarrow$ y-intercept of Graph 3 (linear fit) $\hat{=}$ $y_0 = (3{,}1 \cdot 10^{-6} \pm 8{,}8 \cdot 10^{-8})\,\frac{l \cdot s}{mol}$

$\Rightarrow \Delta V_{max} = \left|-\frac{1}{y_0^2}\right| \Delta y_0$ $\Rightarrow V_{max} = \frac{1}{y_0} = (325042{,}6 \pm 9287{,}7)\,\frac{mol}{l \cdot s}$

$\Rightarrow K_m = m\,(\text{slope}) \cdot V_{max}$, $\Delta K_m = |V_{max} \Delta m| + |m \Delta V_{max}|$

$\Rightarrow K_m = (2{,}89 \cdot 10^{-3} \pm 2{,}03 \cdot 10^{-4})\,\frac{mol}{l}$

discussion

Comparing K_m to the literature(1) ($K_m = (3{,}21 \pm 0{,}36) \cdot 10^{-3}\,\frac{mol}{l}$) the experiment worked very well. Sadly our measurment inaccurancy was too low that the literature value isn't in the range of tolerance.

I think you will have better values with the right plot :)

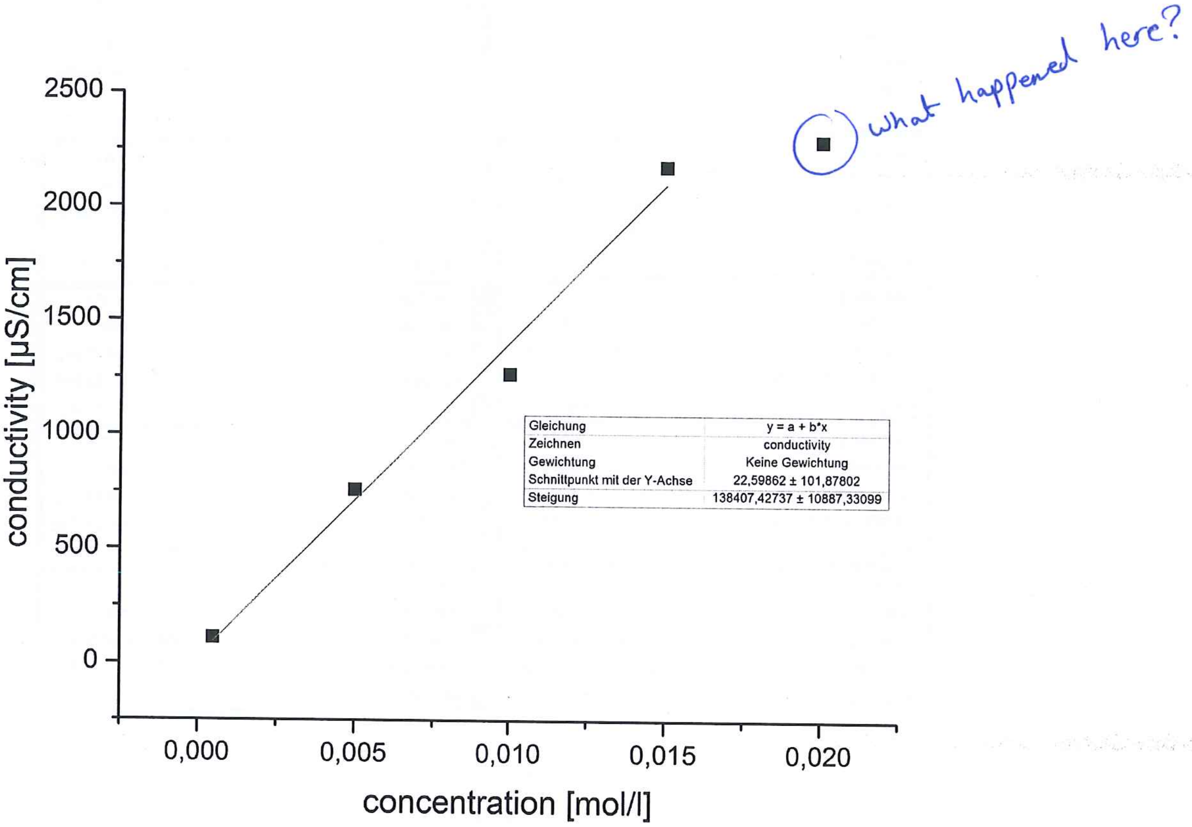
Despite from that it was right to leave out the last conductivity value of the calibration, because it didn't fit into the linear curve very well.

Maybe the errors of volumes and masses had to be set higher because some single values where inaccurate.

Literature sources : - description of experiment

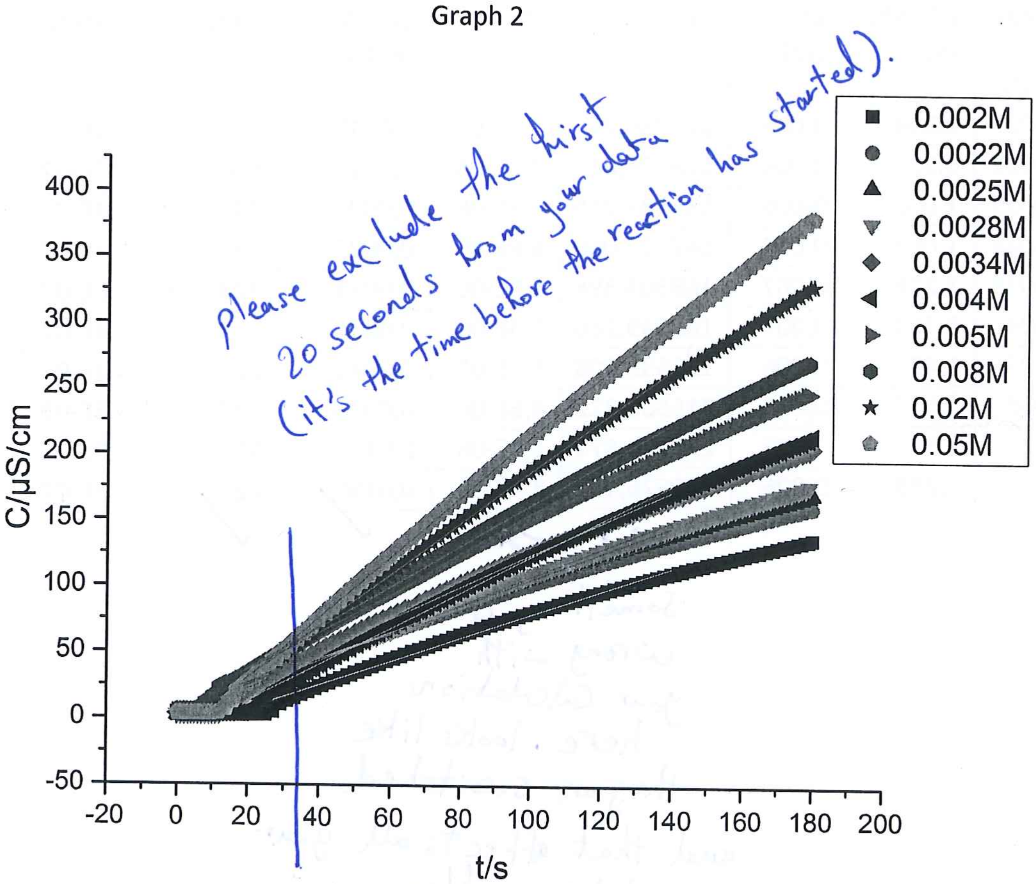
(1) - M. Fidaleo, R. Lavecchia "Kinetic Study of Enzymatic Urea Hydrolysis in pH-Range 4-9", Chemica and Biochemical Engineering Quartely, December 2003, p. 317

Graph 1



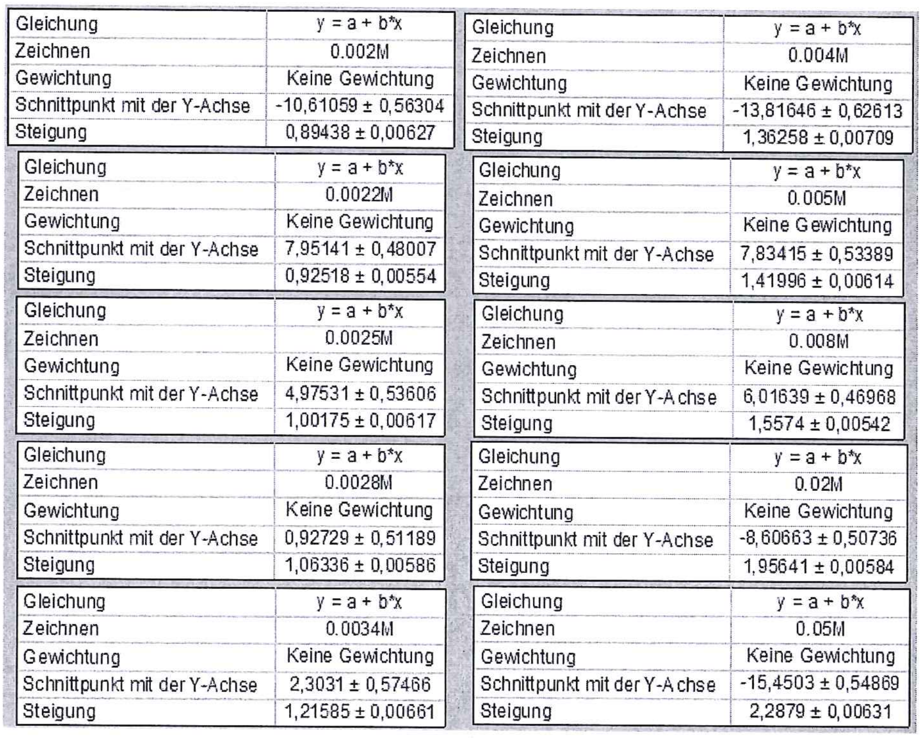
what happened here?

Graph 2



please exclude the first 20 seconds from your data (it's the time before the reaction has started).

Linear regressions of graph 2



Gleichung	y = a + b*x
Zeichnen	0.002M
Gewichtung	Keine Gewichtung
Schnittpunkt mit der Y-Achse	-10,61059 ± 0,56304
Steigung	0,89438 ± 0,00627

Gleichung	y = a + b*x
Zeichnen	0.0022M
Gewichtung	Keine Gewichtung
Schnittpunkt mit der Y-Achse	7,95141 ± 0,48007
Steigung	0,92518 ± 0,00554

Gleichung	y = a + b*x
Zeichnen	0.0025M
Gewichtung	Keine Gewichtung
Schnittpunkt mit der Y-Achse	4,97531 ± 0,53606
Steigung	1,00175 ± 0,00617

Gleichung	y = a + b*x
Zeichnen	0.0028M
Gewichtung	Keine Gewichtung
Schnittpunkt mit der Y-Achse	0,92729 ± 0,51189
Steigung	1,06336 ± 0,00586

Gleichung	y = a + b*x
Zeichnen	0.0034M
Gewichtung	Keine Gewichtung
Schnittpunkt mit der Y-Achse	2,3031 ± 0,57466
Steigung	1,21585 ± 0,00661

Gleichung	y = a + b*x
Zeichnen	0.004M
Gewichtung	Keine Gewichtung
Schnittpunkt mit der Y-Achse	-13,81646 ± 0,62613
Steigung	1,36258 ± 0,00709

Gleichung	y = a + b*x
Zeichnen	0.005M
Gewichtung	Keine Gewichtung
Schnittpunkt mit der Y-Achse	7,83415 ± 0,53389
Steigung	1,41996 ± 0,00614

Gleichung	y = a + b*x
Zeichnen	0.008M
Gewichtung	Keine Gewichtung
Schnittpunkt mit der Y-Achse	6,01639 ± 0,46968
Steigung	1,5574 ± 0,00542

Gleichung	y = a + b*x
Zeichnen	0.02M
Gewichtung	Keine Gewichtung
Schnittpunkt mit der Y-Achse	-8,60663 ± 0,50736
Steigung	1,95641 ± 0,00584

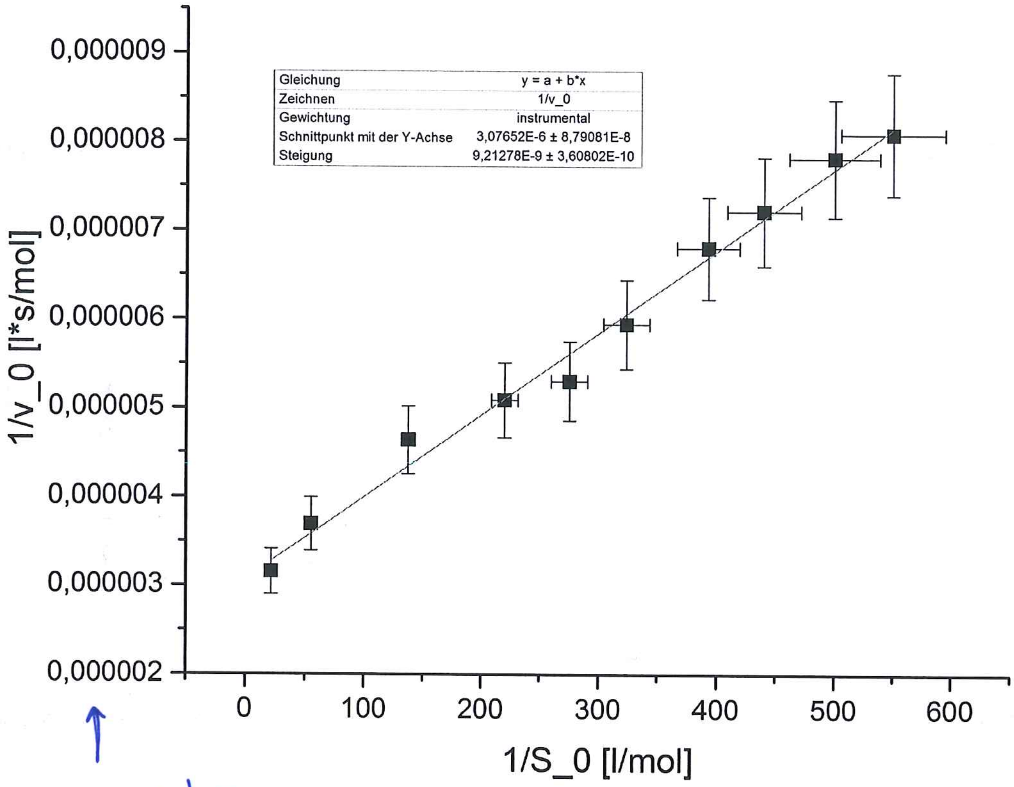
Gleichung	y = a + b*x
Zeichnen	0.05M
Gewichtung	Keine Gewichtung
Schnittpunkt mit der Y-Achse	-15,4503 ± 0,54869
Steigung	2,2879 ± 0,00631

Table 1

C (urea) in mol/l	m in µS/(s*cm)	Δm in µS/(s*cm)	v_0 in mol/(l*s)	$1/v_0$ in l*s/mol	c1V1/V2= c2= [S_0] in mol/l	$1/S_0$ in l/mol	$\Delta 1/v_0$ in l*s/mol	$\Delta 1/S_0$ in l/mol
0,002	0,894	0,006	123736,216	8,1E-06	0,0018	550	6,9E-07	44,905
0,0022	0,925	0,006	128026,845	7,8E-06	0,0020	500	6,7E-07	38,550
0,0025	1,002	0,006	138684,215	7,2E-06	0,0023	440	6,1E-07	31,524
0,0028	1,063	0,006	147127,066	6,8E-06	0,0025	392,857	5,7E-07	26,463
0,0034	1,216	0,007	168303,398	5,9E-06	0,0031	323,529	5,0E-07	19,754
0,004	1,363	0,007	188649,286	5,3E-06	0,0036	275	4,4E-07	15,577
0,005	1,42	0,006	196538,508	5,1E-06	0,0045	220	4,2E-07	11,362
0,008	1,557	0,005	215500,322	4,6E-06	0,0073	137,5	3,8E-07	6,070
0,02	1,956	0,006	270724,874	3,7E-06	0,0182	55	3,0E-07	2,015
0,05	2,288	0,006	316676,131	3,2E-06	0,0455 ✓	22 ✓	2,6E-07	0,740

Something is wrong with your calculations here. looks like they are switched and that affects all your calculations after.

Graph 3



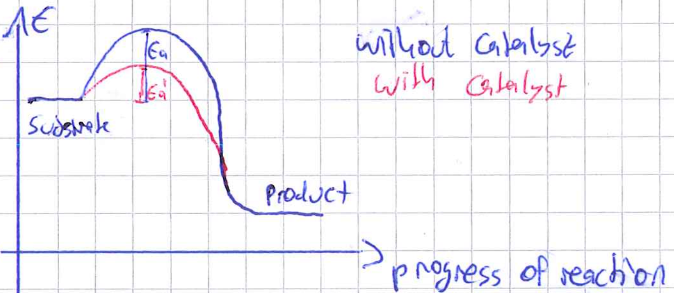
↑

Wrong values

Additional questions

1. Concentration - time profile $E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$

2.



✓

A catalyst lowers the activation energy. This increases the reaction rate and reactions which previously did not proceed or only very slowly can proceed with a catalyst. The catalysts are not used up during the reaction. An example for a catalyst would be V_2O_5 in the contact process for the production of H_2SO_4.

Urea $10^{-1} M \cdot V_1 = 50ml \cdot c_{ges}$ $\Leftrightarrow V_1 = \frac{50ml}{0{,}1 \frac{mol}{l}} \cdot c_{ges}$

$= 0{,}5 \cdot c_{ges}$

								inaccurate ↓	
C	$2 \cdot 10^{-3}$	$2{,}2 \cdot 10^{-3}$	$2{,}5 \cdot 10^{-3}$	$2{,}8 \cdot 10^{-3}$	$3{,}4 \cdot 10^{-3}$	$4 \cdot 10^{-3}$	$5 \cdot 10^{-3}$	$8 \cdot 10^{-3}$	$2 \cdot 10^{-2}$	$5 \cdot 10^{-2}$
V	1	1,1	1,25	1,4	1,7	2	2,5	4	10	25

$(NH_4)_2CO_3$

$5 \cdot 10^{-4}$	$5 \cdot 10^{-3}$	$1 \cdot 10^{-2}$	$1{,}5 \cdot 10^{-2}$	$2 \cdot 10^{-2}$	C
0,25	2,5	5	7,5	10	V

0,6 g urea / 100 ml
0,2 g urease / 100 ml

0,484 g $(NH_4)_2CO_3$

$\Delta M = 0{,}1 mg$

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Corrections

To calculate v_0 you have to divide m_{urea} ~~from~~ by $m_{calibration}$: $v_0 = \frac{m_{urea}}{m_{cal}}$

=> calculation of $\frac{1}{v_0} = \frac{m_{cal}}{m_{urea}}$: for example the slope of the calibration ($m_{cal} = 138407{,}4 \pm 10887{,}3 \frac{\mu S \cdot l}{cm \cdot mol}$) is divided by the slope of the urea-concentration $c_1 = 0{,}002 \frac{mol}{l}$

$$\Rightarrow \frac{1}{v_0} = \frac{138407{,}4 \frac{\mu S \cdot l}{cm \cdot mol}}{0{,}894 \frac{\mu S}{cm \cdot s}} = 154818{,}12 \frac{l \cdot s}{mol}$$ (listed in first row of the table behind)

the other results are calculated equivalent.

=> error of $\frac{1}{v_0} = \Delta \frac{1}{v_0} = \frac{m_{cal}}{m_{urea}^2} \Delta m_{urea} + \frac{\Delta m_c}{m_{urea}}$ (listed in third column of the table behind)

These results were plotted in the new graph 4

=> y-intercept of graph 4: $(58335{,}68 \pm 1634{,}02) \frac{l \cdot s}{mol} = y_0$

$\Rightarrow \Delta v_{max} = \left| -\frac{1}{y_0^2} \right| \Delta y_0$ => $v_{max} = \frac{1}{y_0} = (1{,}697 \cdot 10^{-5} \pm 4{,}848 \cdot 10^{-7}) \frac{mol}{l \cdot s}$

$K_m = m(\text{slope}) \cdot v_{max}$, $\Delta K_m = |v_{max} \cdot \Delta m| + |m \, \Delta v_{max}|$, with $m = 176{,}486 \pm 6{,}912$

$\Rightarrow K_m = (2{,}995 \cdot 10^{-3} \pm 2{,}029 \cdot 10^{-4}) \frac{mol}{l}$

=> now v_{max} looks more realistic

Ok. This looks more reasonable now!

Correction

New results of v_0, $1/v_0$ and its error

V_0 in mol/(l*s)	$1/v_0$ in l*s/mol	$\Delta 1/v_0$ in l*s/mol
6,46E-06	154818,12	13217,27
6,68E-06	149629,62	12740,66
7,24E-06	138131,14	11692,73
7,68E-06	130204,52	10977,01
8,79E-06	113821,88	9608,621
9,85E-06	101546,15	8509,283
1,03E-05	97470	8078,979
1,12E-05	88893,642	7277,969
1,41E-05	70760,429	5783,176
1,65E-05	60492,745	4917,083

new graph 4