Aris Kaksis Riga Stradin's University 2022 aris.kaksis@rsu.lv

Spectrophotometry

TASK: Determine by spectrophotometer "JanyWay" the concentration of riboflavin vitamin B_2 and appreciate sensitivity calculating molar absorbtion coefficient:

Nr.1, Nr.2, Nr.3, Nr.4, Nr.5, Nr.6., Nr.7, Nr.8, Nr.9, Nr.10, Nr.11,Nr.12., Nr.13, Nr.14.

Calibration graph preparation history.

1. Standard solution of riboflavin $C_{riboflavin}$=0.04 mg/mL have been prepared in 8 test tubes 10 mL standard solution with distilled water.

2. Calculated concentrations in 8 tubes are fix results in table: $C_{B2}=\frac{C_{Riboflavin} \bullet V_{B2}}{10mL}$.

3. Choose on spectrophotometer "JanyWay" wave length λ= 445 nm and measure absorption **A_x=log(Io/I)** of chosen **samples** Nr.__. Use calibration graph from table and read unknown concentration of the **sample C_x**.

Table of results

Solution Nr.	***blank***	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***
V_{B2} (mL) C_{B2}=0.04	0.00	0.50	1.00	1.50	2.00	2.50	3.00	3.50	4.00
mL $V_{ūdens}$	10.00	9.50	9.00	8.50	8.00	7.50	7.00	6.50	6.00
Absorption **A=log(Io/I)**	**0.000**	**0.063**	**0.121**	**0.184**	**0.247**	**0.302**	**0.368**	**0.426**	**0.482**
C_{B2},mg/mL	0,000	0,002	0,004	0,006	0,008	0,010	0,012	0,014	0,016

Calculate the molarity of **B_2** solution **$C_M=C_x/M_{B2}$** and molar absorption factor **a** $M^{-1}cm^{-1}$, if glass cell thickness size is measured. Use the ruler and check the glass cell thickness! **l**=1..... cm or no?

$C_M=C_x/M_{B2}$ =.....g/L/.....g/mol=..... M^-

a_{445} =**A_x**/**C_M**/ $\ell = \frac{A_x}{C_M \cdot l}$, $M^{-1}cm^{-1}$=----------=..... $M^{-1}cm^{-1}$

Concentration of **sample** : **C_x =…… mg/mL= g/L**

Graph for calibration line **A = a·C l** drawn through zero **0** value . **A = C=0**

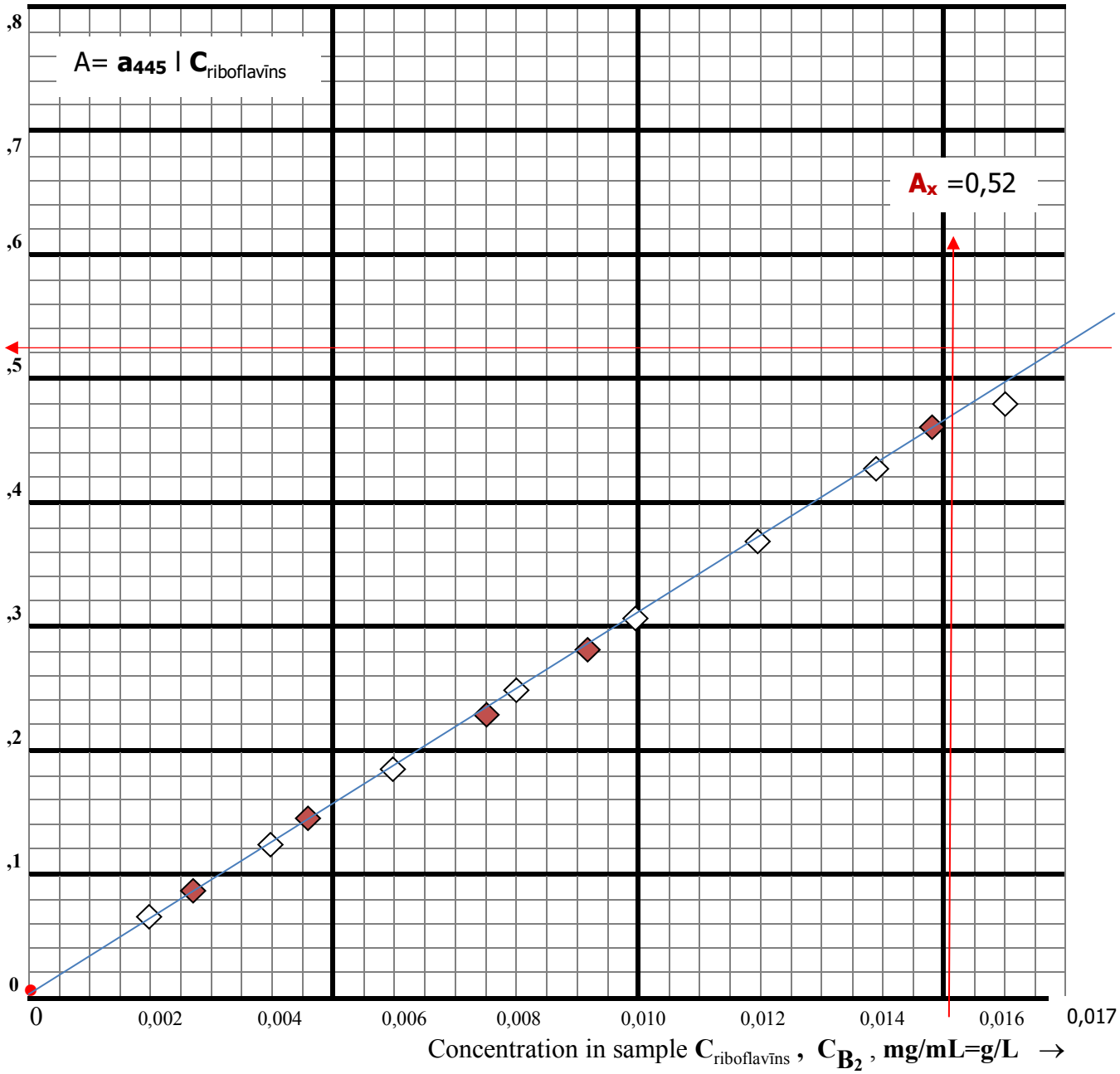
On spectrophotometer "JanyWay" measure the **sample** for analyze absorption **A_x** and calculate its concentration **C_x** using the graph (**mg/mL**).

Give the calculated results for riboflavin content mg%, which shows

mg%= mg/100mL•100%=.....mg%

The molar mass of oxidized form riboflavin (B_2 vitamin) is **M_{B2} = 454.35** g/mol.

↑A= a_{445}•C• ℓ =log(Io/I) Place for reading data from calibration graph **A** - C_{B_2}



Use calibration graph strait line through zero point for each sample concentration in units of mass concentration $\gamma = C_{riboflavīns}$, mg/mL=g/L!

1) Determine measured samples mass concentration, using calibration strait line!

*3. table.*Measured sample solutions concentrations

Sample Nr.	**1**	**2**	**3**	**4**	**5**
Absorption A_x	**0,147**	**0,452**	**0,086**	**0,228**	**0,280**
Concentration γ, mg/mL	0,0046	0,014	0,00275	0,0075	0,00925

Calibration graph strait line **A = a·C l** mathematically through zero point , as both are **A=C=0.**

On "JanyWay" spectrophotometer measure **sample** absorption A_x and use the graph for its concentration C_x (**mg/mL**) evaluation.

Converting riboflavin content to milligram percents mg%, what shows amount on 100 mL samples mg%= mg/100mL•100%=.....mg%

Mol mass oxidized riboflavin B_2 vitamin form is M_{B2} **= 454.35** g/mol.

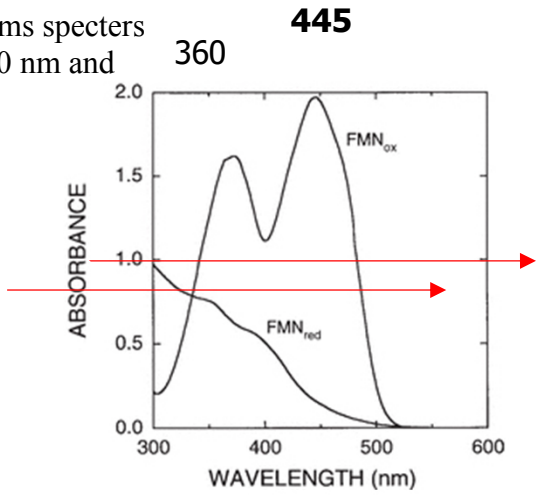
Calculate molarity of **B_2** vitamin solution $C_M=C_x/M_{B2}$ and molar absorption coefficient **a** $M^{-1}cm^{-1}$, if cuvette thickness measured. With ruler check the thickness! ℓ=1 cm !?

$$C_M=C_x/M_{B2} = 0.017\ ^{g}/L / \mathbf{454.35} = 3.74*10^{\wedge(-5)}\ (^{mol}/L)\ M$$

$$a_{445} = A_x/C_M/\ \ell = \frac{A_x}{C_M \cdot l},\ M^{-1}cm^{-1} = 0.53/3.74/10^{\wedge(-5)} = 14171\ M^{-1}cm^{-1}$$

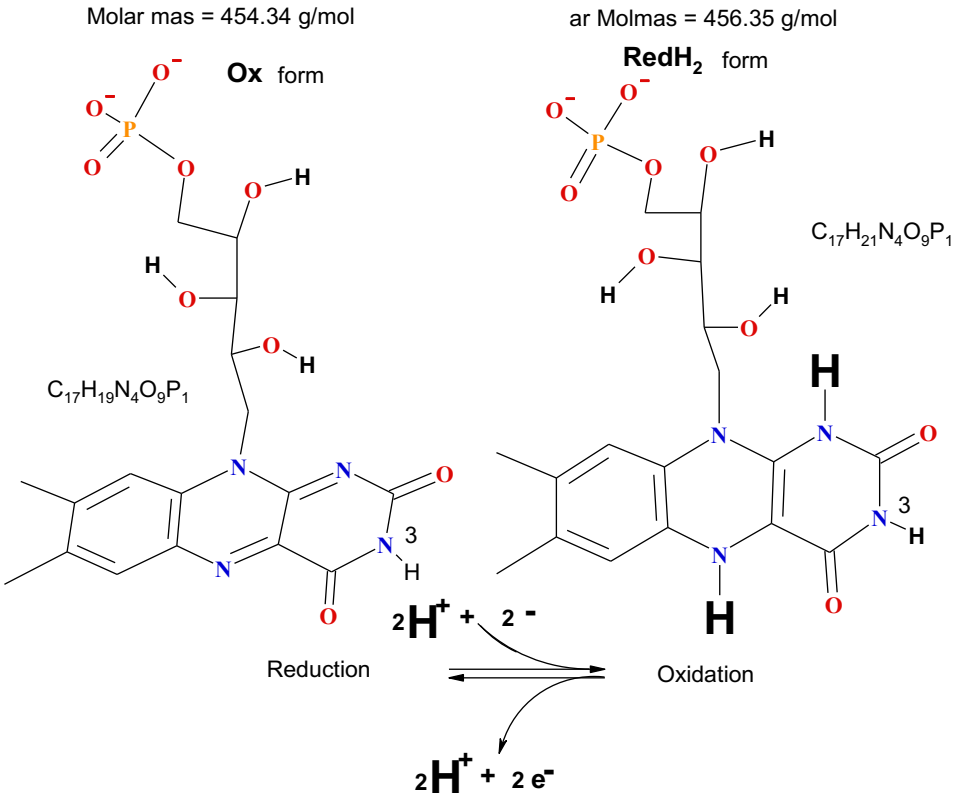
Absorption A_x give concentration: $C_M=A_x/a_{445}/l=0.53/14171/1= 3.74*10^{\wedge(-5)}$ $(^{mol}/L)$ M

Figure. Riboflavin B_2 vitamin oxidized and reduced forms specters
Molar absorption coefficients **a** a_{360}=10500 $M^{-1}cm^{-1}$ at λ=360 nm and a_{445}=**15499** $M^{-1}cm^{-1}$ at λ=**445** nm.
Proteins sometimes decrease pK_a=10.3 N(3)-H proton protolysis constant of free flavin and decrease molar absorption coefficient a_{445}=9200 $M^{-1}cm^{-1}$. For example in glycolate oxidase (4).
Proteins cause bound flavin protolytic deprotonation N(3)-H => $N(3)^-+H^+$. Latin *flavus* – yellow.



FMN Flavin mono nucleotides riboflavin-5'-phosphate B_2 vitamin

http://aris.gusc.lv/FlavinMonoNucleoB2vitamPO4.Tgf.



FMN+2 H oxidized form **Ox +2 H** = Reduced form **$RedH_2$ $FMNH_2$**
Oxidized form bind two hydrogen atoms **+2 H** turns to reduced form **$RedH_2$** ,
Both and **$RedH_2$** forms are water soluble two hydrogen atoms **H** and **H** carrier which transfer atoms together with two electrons as sum $2H^+ + 2e^-$.
Biochemical **oxidation** – **reduction** reactions progressing with water soluble two electron equivalents transfer.
Biochemistry call vitamins about enzyme cofactors.
Small molecules for transfer call about cofactors. Vitamin B2 is cofactor for oxidoreductases.
Cofactors - vitamins will study in course of Biochemistry.

Conclusions

1. Riboflavin B_2 vitamin is 2 H carrier in ENZYME class E....

1b. Reduction is: hydrogen addition..... 1c. Oxidation is hydrogen removing.....

2. Light absorption for riboflavin are at wavelength .....nm and.....nm

3. Ratio falling light intensity **Io** over throughout going light **I** as logarithm .....
absorption measure is A_x=.....

4. Light absorption calculates as logarithmic expression $A_x = \log(Io/I)$= .....

5. Riboflavin concentration in the sample **Nr**..... exhibits absorption A_x=.....

6. Beer-Bugeers-Lambert's Law $A_x = a_{445} \cdot C \cdot \ell = \log(Io/I)$ according light absorption A_x is

proportional to vitamin B_2 concentration C_x=.....g/L

Riboflavin Vitamin B_2 molar concentration is C_M=.....mol/L.

7. Calculated molar absorption coefficient at λ=445 nm a_{445}=14171 .....

Reaction **velocity** proportional on compounds concentration

Task: Observe the influence of $H_2S_2O_3$ sodium thio-sulfuric acid concentration $C_{H_2S_2O_3}$ to rate of reaction: $H_2S_2O_3 \Rightarrow \underline{S}\downarrow + H_2O + SO_2\uparrow$, (1)

$$\vec{v} = \frac{\overrightarrow{\Delta[S]}}{\Delta t} \quad , \quad (2)$$

$$\vec{v} = \vec{k} * C_{H_2S_2O_3} \quad (3)$$

$H_2S_2O_3$ arises due to reaction: $Na_2SN_2O_3 + H_2SO_4 \Rightarrow H_2S_2O_3 + Na_2SO_4$, (4)

where $H_2S_2O_3$ is week acid electrolyte $\alpha \to 0$ that decays according reaction (1).

Description of work: Fill in three identical test tubes (§ 1, § 2, § 3, § 4, § 5) 0.5 N $Na_2S_2O_3$ sodium thio-sulfate solutions plus distilled water (pure H_2O) with in different concentrations of $Na_2S_2O_3$;

2.) In other five test tubes 2 mL 1.5 N H_2SO_4 sulfuric acid.

In § 1 test tube mix $Na_2S_2O_3$ with 2 mL 1.5 N H_2SO_4 and switch on timer!

├──────── I ────────┤--- II -----|Table of DATA $Na_2S_2O_3+H_2O+H_2SO_4$

Salt Nr.	$Na_2S_2O_3$ *0,5 $^{mol}/_{L}$* volume, mL	H_2O **mL**	Create solution $Na_2S_2O_3$ concentration, $^{mol}/_{L}$	**Time t,** seconds	**velocity,** $\vec{v}$ **=1/t, s^{-1}**	Time for concentration, $tC=\tau^{1}/_{2}\ln(C^{o}/C_M)/\ln 2$;
1.	**10**	**0**	10*0,5/12=0,417	**19**	1/19=0,0526	sākotnējais tC^{o}=0 s
2.	**8**	**2**	8*0,5/12=0,333	**24**	1/24=0,04167	1,814062
3.	**6**	**4**	6*0,5/12=0,25	**33**	1/33=.0,0303	4,126011
4.	**4**	**6**	4*0,5/12=0,167	**55**	1/55=0,01818	7,379777
5.	**2**	**8**	2*0,5/36=0,0833	**114**	1/114=0,00877	12,98901

Write this result of reaction time t on table for test tubes (§ 1, § 2, § 3, § 4, § 5)!

Calculate $\vec{v}$ =1/t sec^{-1} reaction rate from data ((§ 1, § 2, § 3, § 4, § 5) in table!

Draw the graphic of velocity $\vec{v}$, sec^{-1} dependence on concentration $C_{H_2S_2O_3}$

On graph calculate constant $\vec{k}$ in ratio $\vec{v}$, s^{-1} over concentration $C_{H_2S_2O_3}$=0,5 M:

Velocity $\vec{v} = \vec{k}\ C_{H_2S_2O_3}$.; velocity constant: $\vec{k} = \vec{v}\ /C_{H_2S_2O_3}$=0,062/0,5=0,124 s^{-1}

Half life time: $\tau^{1}/_{2}=\frac{\ln 2}{\vec{k}}$ =0,693/0,124=5,59 s; $t_{1/10}=\frac{\ln 10}{\vec{k}}$ =2,3/0,124 =18,6 s;

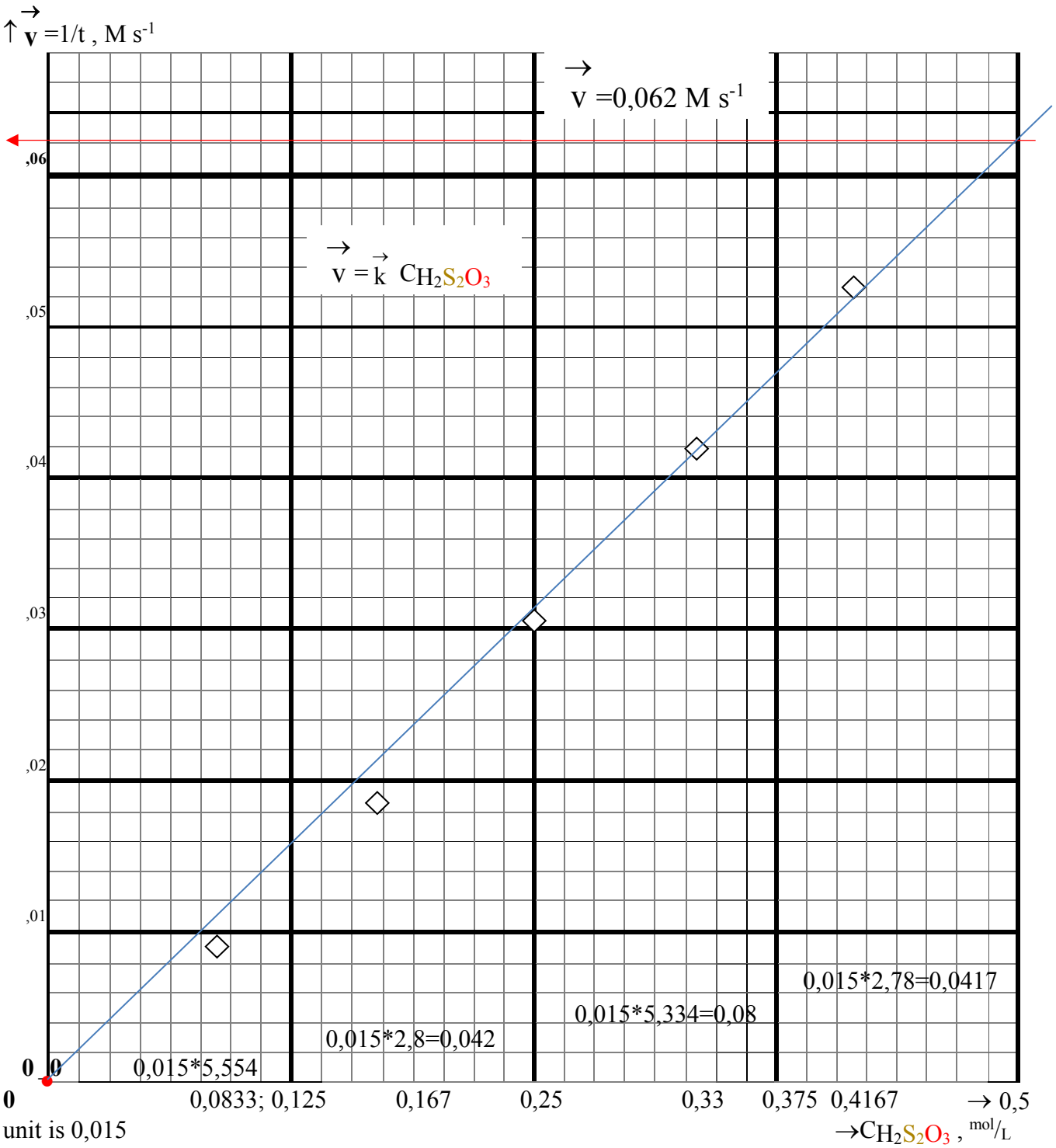
Secinājumi

a) Active mass law! Velocity $\vec{v}$ is proportional to reactant concentration $C_{H_2S_2O_3}$.

b) Velocity constant physical meaning $\vec{k}$ =0,124 s^{-1} is velocity if concentration equal to C=1 M one!

c) Half life time $\tau 1/2$=5,59 seconds are during compounds amount decreases per half 1/2 !

d) Time $t_{1/10}$=18,6 in seconds during thio sulfuric acid concentration $C_{H_2S_2O_3}$ decreases 10 times?



Conclusions questions

a) Active mass law! Velocity $\vec{v}$ is proportional to reactant concentration $C_{H_2S_2O_3}$.

b) Velocity constant physical meaning $\vec{k}$ =0,124 s^{-1} is velocity if concentration equal to C=1 M one!

c) Half life time $\tau_{1/2}$=5,59 seconds are during compounds amount decreases per half 1/2 !

d) Time $t_{1/10}$=18,6 in seconds during thio sulfuric acid concentration $C_{H_2S_2O_3}$ decreases 10 times?

Catalysis

Diverse catalysis in each of two test tubes pore approximately 1 mL H_2O_2 solution.
First test tube add in spoon of oxide $Fe(+3)_2O_3$. Secon test tube in add spoon of oxide Mn(+2)O.
1) Protolysis H_2O_2 deprotonate to anions $2H_2O_2+2H_2O \rightarrow H_3O^+ + HOO^- + {}^-OOH + H_3O^+$ pKa= 11,75.
2) Deprotonate negative anions $HOO^- >< {}^-OOH$ collision energy E_a=79000 $^J/_{mol}$ disproportion ,
oxidize colliding oxygen atoms to O_{2aqua} and second atoms reduce to 2 OH^- ions, which
3) neutralized to water $2OH^- + 2H_3O^+ => 2H_2O + 2H_2O$.

1), 2), 3) : $2H_2O_2+2H_2O \rightarrow H_3O^+ + HOO^- >< OOH + H_3O^+ \rightarrow O_{2aqua} + 2H_2O + 2H_2O + Q$;

1) Reaction start by protolysis , than 2) collision disproportion OO atoms and 3) finally neutralized.

$$2H_2O_2+2H_2O \rightarrow H_3O^+ + HOO^- >< {}^-OOH + H_3O^+ \rightarrow O_{2aqua} + 2H_2O + 2H_2O + Q_{exothermic};$$

Negative ion collision with positive +2 $HOO^- >$Mn(+2)O$< {}^-OOH$ activation energy is E_a=790 $^J/_{mol}$

$2H_2O_2+2H_2O+MnO \rightarrow H_3O^+ + HOO^- >$Mn(+2)O$< {}^-OOH + H_3O^+ \rightarrow O_{2aqua} + 2H_2O + 2H_2O + Q + MnO$

Catalase negative and positive ions collision $HOO^- > Fe^{3+}$ activation energy E_a=29 $^J/_{mol}$ decreases:

$H_2O_2 + H_2O_2 + Fe^{3+} \rightarrow \quad H^+ + HOO^- > Fe^{3+} < {}^-OOH + H^+ \rightarrow \quad O_{2aqua} + H_2O + H_2O + Q \qquad + Fe^{3+}$.

Protolysis activate active transition state complex **oxygene**+ **water**+ **heat**+ CATALYst

Negative ions collision with positive iron Fe^{3+} ion create life resources 30 million times faster.

Describe observations and reaction equation. State, which catalyst effective disproportionate hydrogen peroxide H_2O_2. Determine, are given catalysts homogenous or heterogenic.

2. Autocatalyst (self-catalyst)

Each of two test tubes pore approximately 2 mL potasium permanganate $KMnO_4$ solution.
Both test tubes add approximately 1 mL sulfuric acid H_2SO_4 solution.
First test tube add spoon solid mangase(II) sulfate $MnSO_4$.
Both test tubes add approximately 1 mL oxalic acid $H_2C_2O_4$ solution.

Describe observations. Remark, in which test tube $KMnO_4$ collor disappears first.

Complete oxidation-reduction equation.

Izskaidrojiet mangāna(II) jonu Mn^{2+} lomu reakcijā!

$KMnO_4 + H_2C_2O_4 + H_2SO_4 \rightarrow$

Oxidant:	$MnO_4^- + 5e^- + 8\ H^+ \rightarrow Mn^{2+} + 4H_2O$
Reductant	$H_2C_2O_4 - 2e^- \rightarrow 2CO_2 + 2\ H^+$
Oxidants:	$2MnO_4^- + 10e^- + 16\ H^+ \rightarrow 2Mn^{2+} + 8H_2O$
Reductant	$5H_2C_2O_4 - 10e^- \rightarrow 10CO_2 + 10\ H^+$
$2MnO_4^- + 16H^+ + 5H_2C_2O_4 \rightarrow 2Mn^{2+} + 8H_2O + 10\ H^+ + 10CO_2$	
$2MnO_4^- + 6H^+ + 5H_2C_2O_4 \rightarrow 2Mn^{2+} + 8H_2O + 10CO_2$	
$2KMnO_4 + 5H_2C_2O_4 + 3H_2SO_4 \rightarrow 2\ MnSO_4 + 10\ CO_2 + 8\ H_2O + K_2SO_4$	

Conclusions

1. Heterogeneous catalyst serves into reaction with surface area active centers.
are involved, .therefore velocity of reaction is proportional to value surface area S. .....
2. Auto catalysis $KMnO_4 + H_2C_2O_4 + H_2SO_4$ catalyses reaction product ion Mn^{2+}.....