Spectrophotometry

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

Nr.1, Nr.2, Nr.3, Nr.4, Nr.5, Nr.6.

Calibration graph preparation in Table 1.

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 "Jenway" 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

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

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**

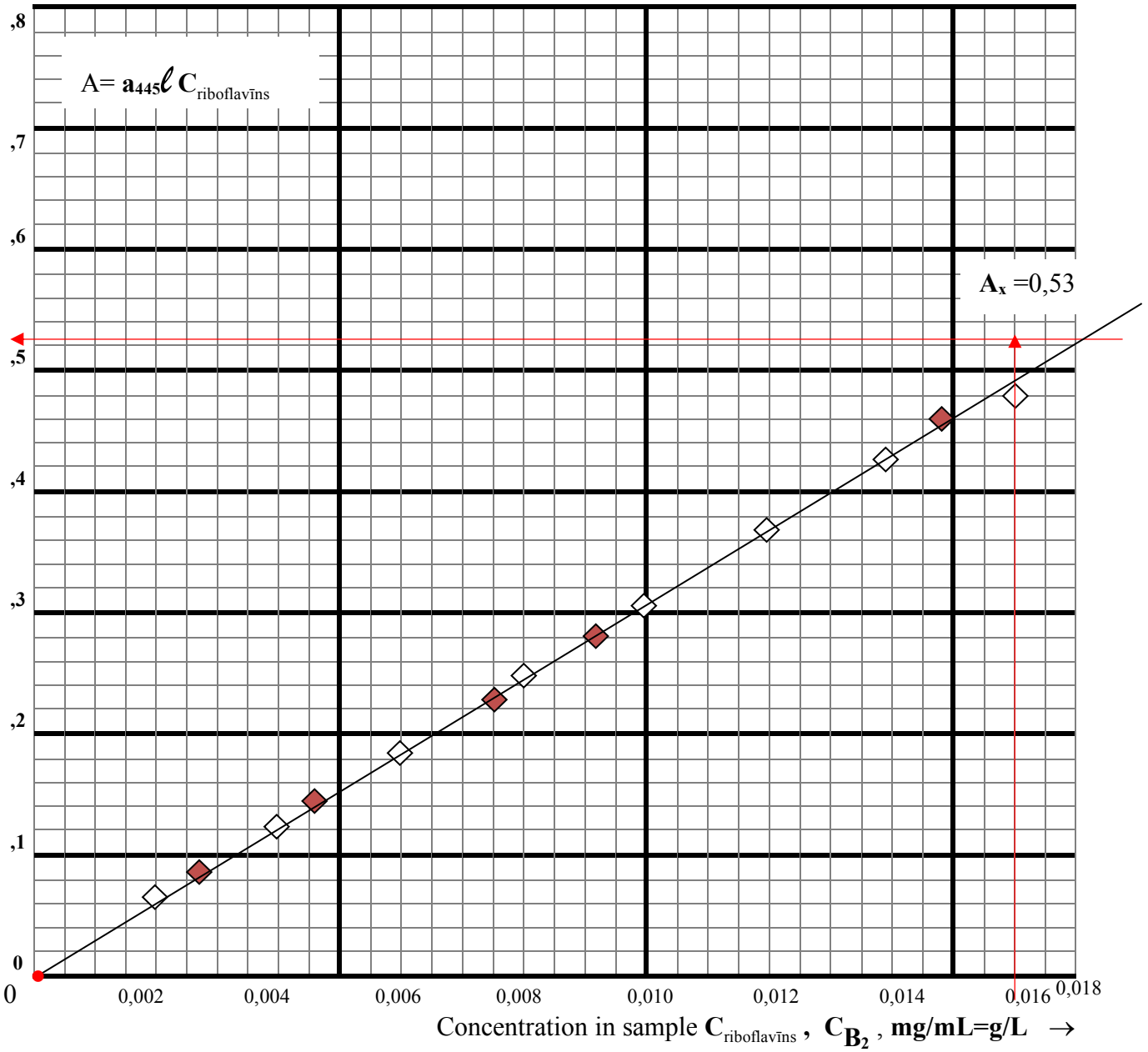
On spectrophotometer "JanyWay" measure the **sample** for analyze absorption **A_x** and calculate its concentration **C_x** using the graph (**mg/mL**).

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

Converting riboflavin content to miligram 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.

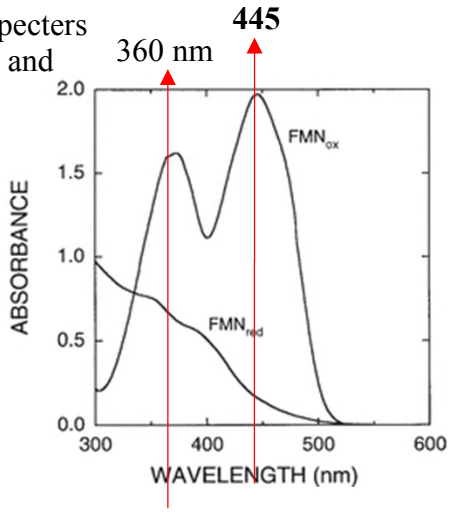
Calculate molarity of **B_2** vitamin solution $C_M=C_x/M_{B2}$ and molar absorption coefficient **a** $M^{-1}cm^{-1}$, if cuvette thickness measured. With ruler check the thickness! ℓ=1 cm !?

$$C_M=C_x/M_{B2} = 0.018\ ^{g}/L / \mathbf{454.35} = 3.96*10^{\wedge(-5)}\ (^{mol}/L)\ M$$

$$a_{445} = A_x/C_M/\ \ell = \frac{A_x}{C_M \cdot l},\ M^{-1}cm^{-1} = 0{,}53/3{,}96/10^{\wedge(-5)} = 13383{,}8\ M^{-1}cm^{-1}$$

Absorption A_x give concentration: $C_M=A_x/a_{445}/l=0.53/13383{,}8/1= 3.96*10^{\wedge(-5)}\ (^{mol}/L)\ M$

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.

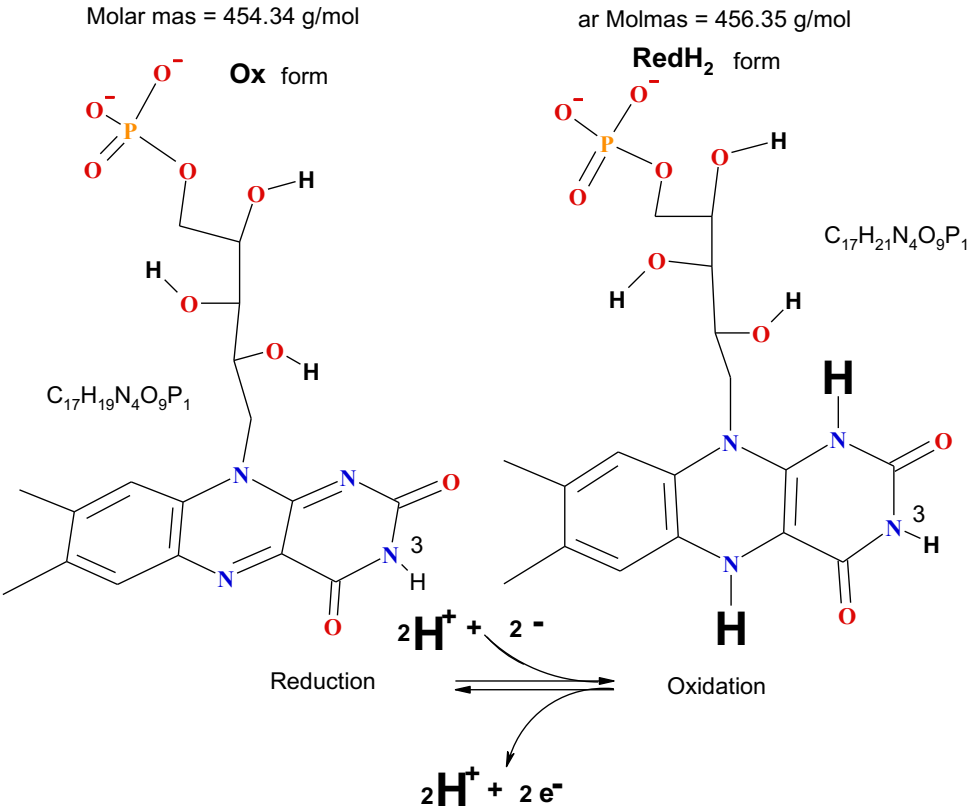


Proteins sometimes decrease pK_a=10.3 N(3)-H proton protolysis constant of free flavin and decrease molar absorption coefficient a_{445}=9200 $M^{-1}cm^{-1}$. For example in glycolate oxidase (4).

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

1a. Riboflavin B_2 vitamin is 2 H carrier in enzyme class E.1 **Oxidoreductases**.....

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

2. Light absorption maximums for riboflavin wave length ..360.....nm and..445.....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 is a_{445}= 13383,8 $M^{-1}cm^{-1}$.