Spectrophotometry http://aris.gusc.lv/BioThermodynamics/LabSpectr16.pdf

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 $\mathbf{A_x=log(Io/I)}$ of chosen **samples** Nr.__. Use calibration graph from table and read unknown concentration of the **sample** $\mathbf{C_x}$.

Table of results

	1	2	3	4	5	6	7	8	9	**sample** X
V_{B2} (mL) C_{B2}=0.04	0.5	1.	1.5	2.	2.5	3.	3.5	4.	10	Nr._____
mL V_{water}	9.5	9.	8.5	8.	7.5	7.	6.5	6.	0	
C_{B2},mg/mL	0,002	0,004	0,006	0,008	0,010	0,012	0,014	0,016	0,036	measure $\mathbf{A_x}$
Absorption **A=log(Io/I)**	0,091	0,141	0,205	0,285	0,341	0,440	0,510	0,580	1,300	

Concentration of **sample** : $\mathbf{C_x}$ =…… **mg/mL= g/L**

Graph for calibration **Beer–Lambert–Bouguer**`s line $\mathbf{A = a \cdot C \ell}$ crosses zero value $\mathbf{A = C=0}$.

On spectrophotometer "Jenway" measure the **sample** for analyze absorption $\mathbf{A_x}$ and calculate its concentration $\mathbf{C_x}$ using the graph (**mg/mL**).

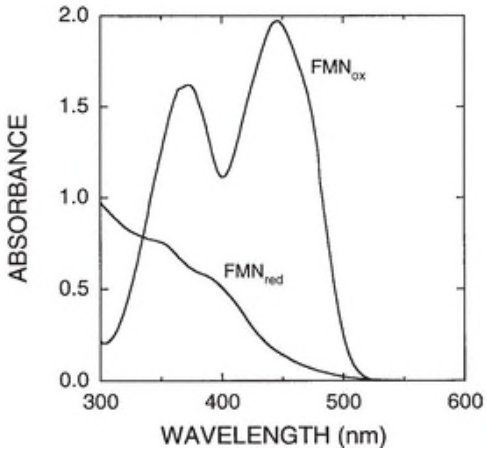
Give the calculated results for riboflavin content mg%, which shows mg%= mg/100mL•100%=.....mg%

3. The molar mass of oxidized form riboflavin (B_2 vitamin) is $\mathbf{M_{B2}}$ **= 454.35** g/mol. http://aris.gusc.lv/FlavinMonoNucleoB2vitamPO4.Tgf. Calculate the molarity of $\mathbf{B_2}$ solution $\mathbf{C_M=C_x/M_{B2}}$ 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?

$\mathbf{C_M=C_x/M_{B2}}$ =.....g/L/**454.35**.....g/mol=..... M^-

$$\mathbf{a} = \frac{\mathbf{A_x}}{\mathbf{C_M \cdot l}},\ M^{-1}cm^{-1}=----------=.....\ M^{-1}cm^{-1}$$

Fig. Riboflavin B_2 vitamin Ox and Red form specter.



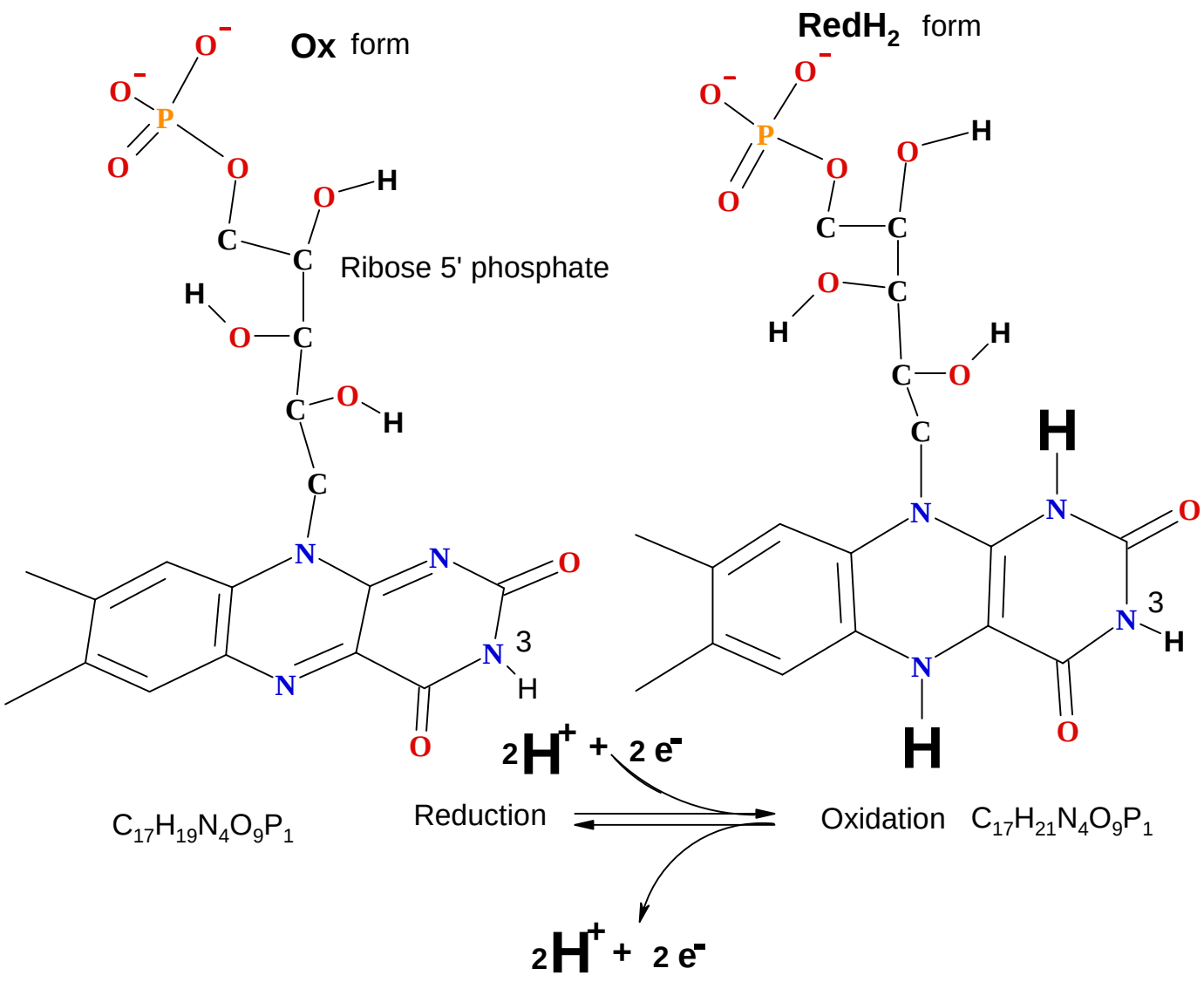
Molar absorption coefficient at λ=360 nm is **a** = 10500 $M^{-1}cm^{-1}$ (3) and at λ=445 nm is **a** = 15499 $M^{-1}cm^{-1}$ (3).

In some cases the proteins lower the pKa for the N(3)-H (in 10,3 for free flavin) promoting dissociation of proton and lower molar absorption coefficient **a** = 9200 $M^{-1}cm^{-1}$.

Latin *flavus* – yellow.

Biochemical electrons transfer molecules in **oxidation reduction** reactions - vitamins.
Two reducing equivalents transfer **H+H** carriers vitamins $\mathbf{B_2}$ and $\mathbf{B_3}$ in Biochemistry.
$\mathbf{B_2}$ and $\mathbf{B_3}$ vitamins are oxidizing - reducing cofactors collaborating with first class E.1 enzymes with **OxidoReductases**.
$\mathbf{B_2}$, $\mathbf{B_3}$ are water soluble molecules, which through water medium transfer two electrons $2\mathbf{e^-}$.

FMN - Flavin Mono Nucleotide, riboflavin-5'-phosphat, $\mathbf{B_2}$ vitamin



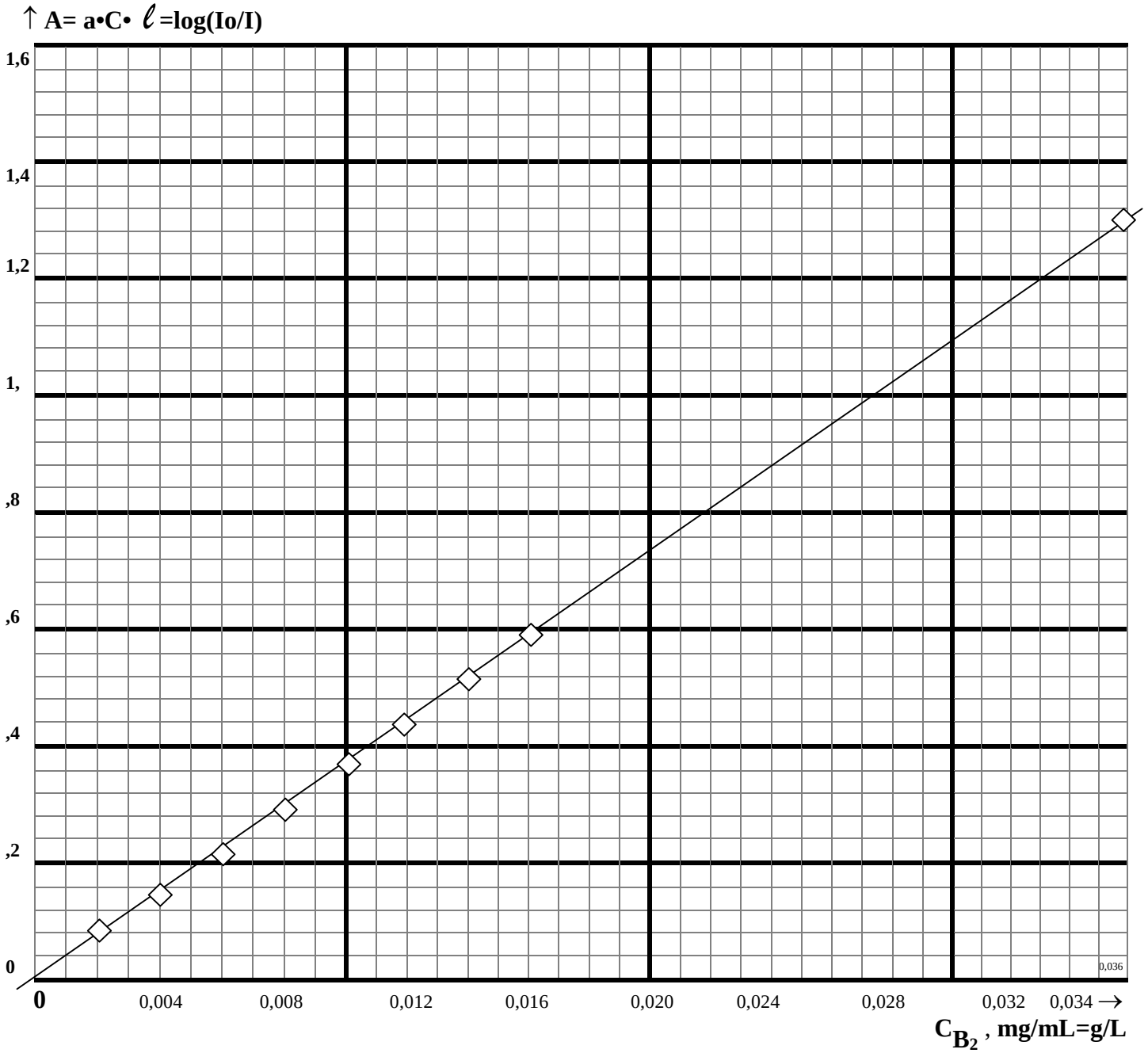
Reduction is hydrogen atoms addition: Reduction: **FMN+2 H => $\mathbf{FMNH_2}$**

Oxidation is hydrogen atoms removing: Oxidation: **$\mathbf{FMNH_2}$ => FMN + 2 H**

Water soluble two electrons carrier **$\mathbf{FMNH_2}$** vitamin $\mathbf{B_2}$.

Vitamin $\mathbf{B_2}$, which oxidized form **FMN** we analyze with spectrophotometer "Jenway" is: **driver** of oxidation-reduction reactions as water soluble two electrons carriers, which have been designated about cofactor or vitamin. Vitamins $\mathbf{B_2}$ and $\mathbf{B_3}$ as cofactors collaborate with first class E.1 enzymes having the common name **OxidoReductases**

Calibration graph place for reading data $\mathbf{A_x}$ versus C_{B_2}



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 wavelength ..360.....nm and..445.....nm

3. Ratio falling light intensity **Io** over throughout going light **I** as logarithm absorption measure is $\mathbf{A_x}$=.....

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

5. Riboflavin concentration in the sample **Nr**..... exhibits absorption $\mathbf{A_x}$=.....

6. Beer-Bugeers-Lambert's Law $\mathbf{A_x= a•C•\ell =log(Io/I)}$ according light absorption $\mathbf{A_x}$ is proportional to vitamin B_2 concentration $\mathbf{C_x}$=.....g/L
Riboflavin Vitamin B_2 molar concentration is $\mathbf{C_M}$=.....mol/L.

7. Calculated molar absorbtion coefficient at λ=445 nm is **a**=15499.....$M^{-1}cm^{-1}$