Laboratory work № 5. The Photocolorimetric determination of Iron and zianocobalammine.

In photocolorimetry and spectrophotometry the following main methods of quantitative analysis are applied: calibration diagram method, the method of comparing, the method of addition, etc. The most suitable for the common analysis is the method **of calibration diagram.**

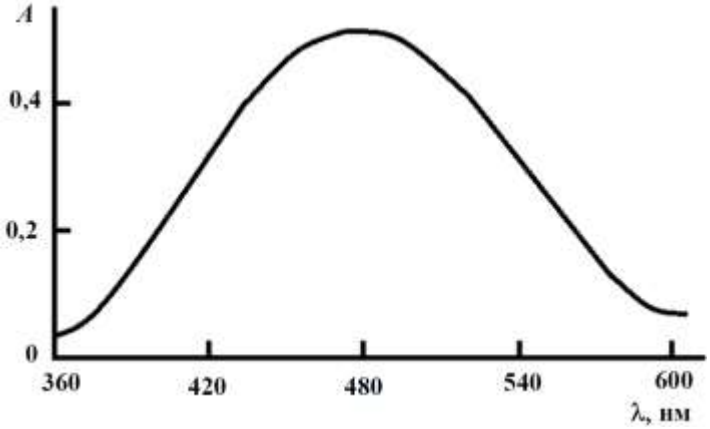
For this method one should:

1. Prepare 4-8 Standard (of known concentration) solutions of analysis material;
2. Measure optical densities of Standard solutions;
3. Plot the calibration diagram in coordinates „Concentration - optical density";
4. Measure optical densities of analysis solutions with the cuvettes of the same path length and at the same wavelength;
5. Find the concentration of the analyte from the calibration diagram.

According to the Buger-Lambert-Beer law, all points in the diagram should be in a line, crossing the O point of coordinates. In case the diagram is non-linear, for quantitative analysis it can be used only in the concentration interval with least deviation from the line. The concentration range of the calibration diagram should involve all concentrations of analysis solutions. Calibration diagram may be used only for the measurements with the same instrument.

<u>Part I.</u> The Photocolorimetric determination of Iron.

Fe^{3+} reacts with excess of thiocyanate (SCN^-) to give an intense red colour complex. Absorbance of this $[Fe(SCN)_6]^{3-}$ complex at λ_{max}= 480 nm is proportional to the concentration of Fe^{3+} ion in the sample.



Materials/Chemicals required:

- Standards Fe^{3+} solution of 100,0 mkg/mL,
- SCN^- solution (KCNS or NH4CNS), 10% solution.
- 50,00 mL volumetric flasks,
- 5 mL transfer pipette.

Task: To prepare colored Standard solutions for the calibration diagram from Fe^{3+} ion solution of the known concentration, with KCNS or NH_4CNS. To measure optical density of these solutions and plot the calibration diagram. To prepare colored analysis solution of Fe^{3+} ions and measure its optical density. From the calibration diagram, to estimate Fe^{3+} ion concentration in the solution.

Procedure:

1. Transfer to 6 volumetric flasks from standard solution (c = 0,01 mg Fe^{3+}/1ml) 6 aliquots of Fe^{3+} ion solution of known concentration as given in Table.

2. Add 5 ml solution of KCNS or NH_4CNS to each volumetric flask.
3. Dilute the eack volumetric flask by distilled water to the mark and mix thoroughly the stopped flask.
4. Measure optical density of these solutions by photoelectrocolorimeter. Use a blue filter for photoelectrocolorimeter (λ=490 nm).
5. Put down the values of optical density A to the Table.

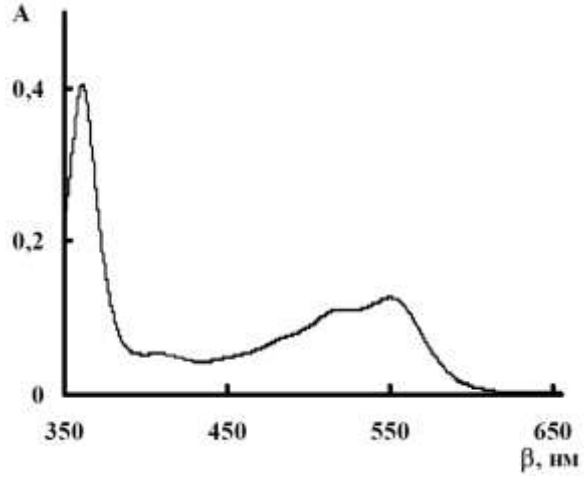
Table 1. Calibration diagram for quantitative determination of Fe^{3+}

Volumetric flask No	Fe^{3+} ion solution, ml	KCNS or NH_4CNS solution, ml	H_2O, ml	Fe^{3+} ion concentration, mkg/ml	Optical density A of the solution
1	0.50	5	44.5		
2	1.00	5	44		
3	1.50	5	43.5		
4	2.00	5	43		
5	2.50	5	42.5		
6	3.00	5	42		
X		5			

6. Plot the calibration diagram. In abscise axis put the concentration of Fe^{3+} ion solution (mkg/ml), in ordinate axis put the optical density A.
7. Transfer analysis solution to volumetric flask.
8. Add 5 ml solution of KCNS or NH_4CNS and dilute by distilled water to the mark and mix thoroughly the stopped flask.
9. Measure instrumentally the optical density A_x of the analysis solution.
8. Find from the equation of calibration diagram the concentration of Fe^{3+} ions in the analyze sample.

<u>Part II.</u> The Photocolorimetric determination of zianocobalammine.
Materials/Chemicals required:

- Standard solution of B_{12} vitamin 15.0 and 25.0 mkg/ml
- 25,00 mL volumetric flasks,



Procedure:

1. Transfer analyzed solution of zianocobalamine to volumetric flask V=25.00ml
2. Dilute the volumetric flask by distilled water to the mark and mix thoroughly the stopped flask.
3. Measure optical density of these solutions by photoelectrocolorimeter. Use a filter with λ=550 nm**.** $\mathbf{A_x}$**=_____**
4. Measured optical density of 2 standard solutions by photoelectrocolorimeter
   $\mathbf{A_1}$ **=_____and** $\mathbf{A_2}$**=_____.**
5. Calculate a mass of zianocobalamine in the sample.

$$m_x = \frac{15{,}0 \cdot (A_2 - A_x) + 25{,}0 \cdot (A_x - A_1)}{A_2 - A_1} \cdot 25{,}0.$$

\

The results you present to teacher.

Teacher's sign