

LABORATORY WORK NO. 31

SPECTROPHOTOMETRY

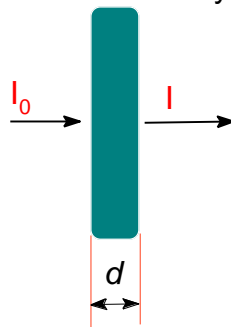
■ **PRINCIPLE:** Spectrophotometry is a very sensitive analytical method used for stating the concentration of inorganic substances, especially metals. Spectrophotometric measurement is based on the **Lambert-Beer's Law**.

The law states that the intensity of solution colouring (so called absorbance) is directly proportional to the molar concentration.

$$A = \varepsilon \cdot c \cdot d,$$

where A solution absorbance
 ε molar decadic absorbent coefficient [$\text{dm}^3 \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$]
 c solution concentration [$\text{mol} \cdot \text{dm}^{-3}$]
 d cuvette length [cm]

Průchod světla kyvetou



Sample absorbance $A = \log \frac{I_0}{I}$.

Passage of light through the cuvette

I_0 intensity of incoming beam
 I intensity of outgoing beam

One of the mostly used metals is Fe, it is an important biogenous element. The Fe statement is determined at the wave length of 495 nm.

TASK N.1: SPECTROPHOTOMETRIC DETERMINATION OF IRON

- **CHEMICALS:** sample of Fe^{3+} , 20% KSCN, 0,1 M-HCl, 30% H_2O_2 , $\text{NH}_4\text{Fe}(\text{SO}_4)_2$, distilled water
- **AIDS:** 7 pcs of 50 ml volumetric flasks, 6 pcs of 50 ml beakers, graduated pipette 5 ml, pipette 10 ml, weighing boat, SPEKOL or PF 10 spectrophotometer
- **PROCEDURE:**

Preparation of standard solution of Fe^{3+} (c = 0,05 mg/ml):

For the preparation of the standard solution of Fe^{3+} in concentration of 0,05 mg in 1ml, we weigh 0,005 g of $\text{NH}_4\text{Fe}(\text{SO}_4)_2$ and we dissolve it in appr. 50 ml of distilled water. We pour it into a 100 ml volumetric flask. We mix it and we add distilled water exactly up to the scale line.

Determination of calibration curve to define Fe^{3+} :

We measure out (with a pipette) 0 ml, 2 ml, 4 ml, 6 ml, 8 ml and 10 ml of a standard solution of Fe^{3+} into 6 beakers and we add 10 ml of 0,1 M-HCl (into each of them). Then we add 4 drops of 30% H_2O_2 into the beakers and we boil the content (for 1 min).

We pour the solutions into the 50 ml volumetric flasks, we add 10 ml of 20% KSCN and we fill the flasks with 0,1 M-HCl exactly up to the scale line. We close the flasks and shake. We leave the calibration solutions to rest for 5 minutes.

We set up the spectrophotometer for the solution with zero content of Fe^{3+} and we measure the absorbance for various prepared calibration solutions at wave length of 495 nm or 470 nm. We write down the absorbance and concentration values into the table:

Standard solution of Fe [ml]	A measured	c [mg/ml] calculated
0	-	-
2		
4		
6		
8		
10		

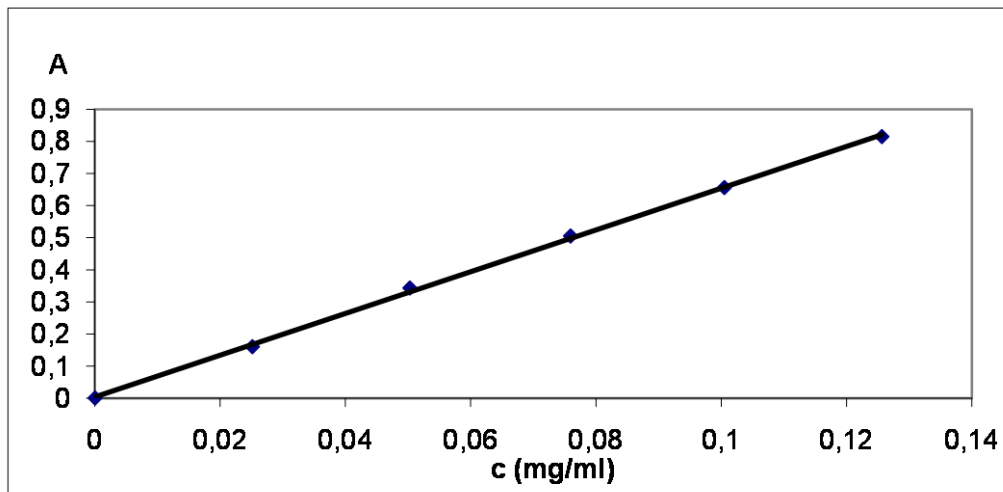
After measuring the absorbance values and calculating the concentration values, we draw a **calibration line**.

Detrmination of Fe in a sample:

We pipette 5 ml of an unknown sample of Fe into a 50 ml volumetric flask; we add 10ml of 20% KSCN. We fill the flask up to the scale line with 0,1 of M-HCl. We stir the solution and leave it 5 minutes to rest.

After pouring the sample into the cuvette and setting the apparatus to zero volume, we measure the absorbance of the sample at wave length of 495 nm.

Calibration line (sample):



- **CONCLUSION:** Evaluate the measurement results – draw a calibration line and read out the unknown concentration of Fe^{3+} in the sample from the graph in mg/ml. State the measured absorbance of the unknown sample.



STUDENT'S SHEET No. 31

SPECTROPHOTOMETRY

1. Find the right word:

- | | |
|----------------------|-------------------|
| a) cocnenrtaiotn | concentration |
| b) vewa lnghet | wave length |
| c) dssiofle | dissolve |
| d) sacle lnei | scale line |
| e) spcetcopohtoemtyr | spectrophotometry |
| f) cvuette | cuvette |

2. Translate the following word into English:

- a) absorbance
- b) biogenní
- c) ryska
- d) obsah, objem
- e) odečíst
- f) kalibrační křivka
- g) měřit

3. Which word is correct according to the definition?

- 1) the quantitative measurement of the reflection or transmission properties of a material as a function of wave length is
 - a) spectophonemtry
 - b) spectrophotometry
 - c) spektrofotometry
- 2) to mix with a liquid and become part of it
 - a) dismantle
 - b) disobey
 - c) dissolve
- 3) the form that some types of energy such as heat, sound, light etc. take as they move is
 - a) length
 - b) wave
 - c) beam
- 4) another word for optical density
 - a) absorbent
 - b) absorbance
 - c) abortance



4. Complete the crossword. Find the following words: dissolve, volume, set up, draw, sample, subtract, biogenous.

O	T	J	B	W	R	U	R	G	U	J	P	O
T	G	S	B	A	G	M	G	A	P	S	O	L
B	U	R	E	T	T	E	A	B	B	A	H	K
R	G	D	A	C	X	L	H	I	L	M	K	I
X	U	I	K	H	S	P	H	O	T	P	Z	J
E	I	S	E	G	S	P	M	G	R	L	A	R
Q	L	S	R	L	E	S	T	E	J	E	T	H
Y	V	O	L	U	M	E	I	N	N	P	V	G
H	N	L	U	S	M	T	N	O	M	I	D	F
N	E	V	Z	S	I	U	M	U	T	P	G	D
I	D	E	F	O	P	P	U	S	N	E	R	S
K	C	L	G	N	L	D	E	L	R	T	N	A
O	V	D	H	T	O	E	D	T	F	T	F	Y
L	R	E	T	E	S	T	T	D	R	A	W	Q
P	S	U	B	S	T	R	A	C	T	U	E	W
Z	W	A	T	E	R	B	A	T	H	U	G	E

5. Fill in the missing letters and translate the words:

- α) c-l-b-at-on l-n-
 β) s-ect--p-o-om-t-y
 γ) c-v-tt-
 δ) c-lc-l-t-
 ε) d-s-o-ve
 φ) me-s-r-



6. Describe the picture and the process pictured there:

